Serological biomarkers in the risk stratification of patients with suspected ACS and high sensitivity troponin T levels below the 99th percentile

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Medicine by

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August 2021

Declaration

I confirm that the work presented in this thesis is my own. Any contribution made by others is explicitly acknowledged.

J. D. Jones

August 2021

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Abstract

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Introduction

Coronary Heart Disease (CHD) kills almost 70,000 people each year in the UK and is responsible for considerable NHS resource expenditure. Accurate risk stratification could improve patient care and reduce NHS expenditure. The aim of this thesis is to assess the incremental role of biomarkers to risk scores in the risk stratification of patients who present with suspected Acute Coronary Syndrome (ACS) and have high sensitivity troponin T levels below the 99th percentile.

Methods

A prospective observational study in a single centre in a large university teaching hospital. Inclusion criteria consisted of chest pain that was suspected to be ACS with Hs-cTnT levels below the 99th percentile when measured between 6-12 hours post pain. Exclusion criteria included clear non-cardiac pain, chronic atrial fibrillation, heart failure and recent ACS. The biomarkers HFABP, GDF-15, NTproBNP, Galectin-3, HSCRP, Hs-cTnT and Hs-cTnI were measured, to determine if these markers could improve risk stratification in isolation or when combined with the HEART, TIMI and GRACE risk scores.

Results

487 patients were included with a median follow up of 5.83 years. 48 experienced MACE and 42 patients experienced all-cause mortality during follow up. There were high levels of cardiovascular risk factors with 85% of patients having at least 1 traditional cardiovascular risk factor (excluding gender) and nearly a third of patients (28%) having a history of angina. Over 40% of patients were discharged without a firm diagnosis. MACE occurred as an inpatient in 3% and at 8-weeks in 4% of patients. 7% of patients represented to the same department at 8-weeks.

The HEART and TIMI score perform well in the risk stratification of these patients with a C-Statistic of 0.76 and 0.74 respectively for 12-month MACE. The HEART score is more favourable at ruling out future early MACE. The high sensitivity troponin values, even below the 99th percentile provide incremental independent information beyond each risk score. GDF-15 may have a role in the risk stratification of patients, particularly in combination with the HEART and TIMI scores.

Conclusion

The HEART score performs well in the prediction and rule-out of MACE and the incorporation of high sensitivity troponin levels improves these scores. GDF-15 appears to be a promising biomarker that provides incremental information beyond risk scores.

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Contributions

The project was designed by my primary supervisor, Dr Aleem Khand, and sample size calculations were performed by an external statistician, Dr Isla Gemmel, prior to my involvement in the project.

I performed the analysis of the biomarker GDF-15, with the help of Mrs Jennifer Hawkes at The Clinical Sciences Laboratory, University of Liverpool. Analysis of HFABP was carried out by the Department of Clinical Biochemistry in the Countess of Chester Hospital NHS Foundation Trust. Analysis of NTproBNP, Galectin-3, Hs-cTnI and HSCRP was performed by Abbott Laboratories in Illinois.

Adjudication of the end point myocardial infarction was performed by experienced cardiologists Dr Rebecca Dobson, Dr Tom Heseltine and Dr Aleem Khand.

I was responsible for ethics submission, recruitment of patients, data collection and data interpretation. I performed all the statistical analysis on the data collected but had guidance from a statistician at the University of Liverpool, Mr Matthew Gornall.

Acknowledgements

Firstly, I wish to express my appreciation to University Hospital Aintree NHS Trust for providing funding for this research and the Biochemistry Department for their assistance in allowing me access to obtain samples.

I wish to thank my supervisors, Dr Aleem Khand, Prof Graham Kemp and Prof Rob Moots. Dr Aleem Khand developed this project and allowed me to carry things through. Prof Graham Kemp and Prof Rob Moots were there to help with guidance whenever I asked.

I would also like to express my deepest appreciation to Mrs Jennifer Hawkes and Dr Andrew Cross for help with the storage of biomarker samples and analysis of GDF-15, and to Dr Emma Lewis at the Countess of Chester Hospital who was able to carry out the analysis of HFABP.

I would like to thank Dr Rebecca Dobson and Dr Tom Heseltine with the assistance of adjudication of myocardial infarction, and, Matthew Gornall for taking the time to discuss different statistical aspects and techniques.

I would also extend my eternal gratitude to my family who have enabled me to get to where I am today. In particular, I would like praise my husband for his continued support over the years and help that ranged from looking after our children to give me time to carry this through to helping with advanced coding for the statistical analysis required in this project.

Finally, I apologise to my children, Bethany and Sophie, for not being around as much as I would have liked during their early years. I hope to make up for that now that this work is complete.

This thesis is dedicated to the memory of my sister

Abbreviations

ACS	Acute Coronary Syndrome
ADP	Adenosine BiPhosphate
AED	Accident and Emergency Department
AMI	Acute Myocardial Infarction
ANP	Atrial Natriuretic Peptide
ATP	Adenosine TriPhosphate
AUC	Area Under the Curve
BBB	Bundle Branch Block
BNP	Brain Natriuretic Peptide
CABG	Coronary Artery Bypass Grafting
CAD	Coronary Artery Disease
CCG	Clinical Commissioning Group
COPD	Chronic Obstructive Pulmonary Disease
CHD	Coronary Heart Disease
CRP	C Reactive Protein
CT	Computed Tomography
CTCA	Computed Tomography Cardiac Angiography
CVD	CardioVascular Disease
CXR	Chest X-ray
cTnI	Cardiac Troponin I
cTnT	Cardiac Troponin T
DM	Diabetes Mellitus
ECG	Electrocardiogram
ED	Emergency Department
ESC	European Society of Cardiology
ETT	Exercise Tolerance Test
GDF-15	Growth Differentiation Factor 15
HFABP	Heart Type Fatty Acid Binding Protein
HSCRP	High Sensitivity C Reactive Protein
Hs-cTnI	High Sensitivity Troponin I
Hs-cTnT	High Sensitivity Troponin T
IHD	Ischaemic Heart Disease

KM	Kaplan-Meier
LDL	Low Density Lipoprotein
LoB	Limit of Blank
LoD	Limit of Detection
LoQ	Limit of Quantification
LR	Likelihood ratio
MI	Myocardial Infarction
NHS	National Health Service
NPR	Negative Predicted Value
NRI	Net Reclassification Index
NSTEMI	Non-ST Elevation Myocardial Infarction
NTproBNP	NT pro Brain Natriuretic Peptide
OGD	Oesophago-gastro-duodenoscopy
OPD	Outpatient Department
PCI	Per Cutaneous Intervention
PHE	Public Health England
PIL	Patient Information Leaflet
PPV	Positive Predicted Value
ROC	Receiver Operator Curve
SBP	Systolic Blood Pressure
STD	Standard Deviation
STEMI	ST Elevation Myocardial Infarction
Tn	Troponin
UA	Unstable Angina
UHA	University Hospital Aintree NHS Foundation Trust
UK	United Kingdom
URL	Upper Reference Limit
WHO	World Health Organisation

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Preamble

This thesis examines if serological biomarkers can improve the risk stratification of patients with suspected Acute Coronary Syndrome (ACS) who have had Myocardial infarction (MI) excluded. It will test if these biomarkers provide incremental information beyond traditional risk scores and high sensitivity troponin levels. It will evaluate the prognostic role of two high sensitivity troponin assays, Roche Elecsys troponin T and Abbott Architect troponin I. The ability to accurately identify those at high risk of short-term Major Adverse Cardiac Event (MACE) could facilitate appropriate early treatment and cardiovascular investigation, whilst allowing early discharge and reducing unnecessary investigation in low-risk patients. Identifying patients at risk of longer-term MACE provides an opportunity to intervene with lifestyle advice and aggressive risk factor modification.

Background

Coronary Heart Disease (CHD) kills almost 70,000 people each year in the UK. That is equivalent to 1 death every 8 minutes.(1) Despite mortality rates more than halving over the last 50 years, CHD remains one of the biggest killers in the UK.(2) 1 in 4 premature deaths are caused by cardiovascular disease;(3) deaths that could have been prevented by tackling modifiable cardiovascular risk factors.(4) In addition, cardiovascular disease has considerable resource expenditure, costing the NHS a staggering £8,960,000,000 each year.(5) The last decade has seen an unprecedented decline in NHS funding growth. This austerity has placed NHS services under tremendous financial pressure, and we must find ways to relieve this pressure.

The fight against CHD

The World Health Organisation (WHO) and Public Health England (PHE) have ambitions of reducing the number of preventable deaths from Cardiovascular Disease (CVD); a key theme in these is addressing modifiable risk factors.(6,7) The WHO describes interventions that can help prevent and control CVD at a population-wide level and on an individual basis. Population-wide methods include restrictive laws governing tobacco and taxation on foods high in fat, sugar and salt. On an individual level, measures would involve targeting those at high risk of future cardiovascular disease; interventions could include primary and secondary preventative medication, lifestyle advice, and coronary revascularisation.

The difficulties faced at the front door

The most frequent manifestation of CVD to the Emergency Department (ED) is ACS. ACS is an umbrella term that encompasses Acute Myocardial Infarction (AMI) and Unstable Angina (UA). The fourth universal definition of AMI(8) relies on a dynamic change in the biomarker cardiac troponin. Management of AMI is established and typically includes medical treatment and invasive coronary angiography to help determine if coronary revascularisation is warranted. The diagnosis of UA is considerably more challenging, as this relies purely on the clinical history, and research has consistently shown that features of chest pain are particularly unreliable when it comes to diagnosing ACS in patients with undifferentiated chest pain.(9,10) Investigations in UA tend to be unremarkable, with normal troponin levels and ECG. These features of UA pose a particular challenge to clinicians as it is well known that atypical presentations are common and there is substantial fear of 'missing' an ACS, as this can have disastrous consequences, including death.(9,11,12)

Chest pain can result in 6% of Emergency Department (ED) visits and 27.4% hospital admissions.(13) Despite a reduction in the incidence of AMI over recent years, there has been an increase in the numbers of patients presenting to hospital with chest pain.(14,15) Early identification and treatment of ACS is associated with improved outcome.(16) Patients with Non-ST Elevation Myocardial Infarction (NSTEMI) and UA tend to have a lower quality of care than those with ST Elevation Myocardial Infarction (STEMI).(17) Nevertheless, most patients who present with chest pain do not have an underlying ACS. Yet, several studies still show an adverse cardiovascular outcome for patients labelled with non-specific chest pain or non-cardiac chest pain.(18–22)

Risk stratification

Improved risk stratification for patients with suspected ACS and normal troponin levels (<99th percentile) could help in several ways. Patients identified as high risk of short-term MACE could have early cardiovascular investigation. Those at an increased risk of long-term MACE could have targeted individual risk factor management and lifestyle advice. Patients accurately identified as low risk of short-term MACE could have their hospital discharge expedited with consideration of outpatient investigation. Patients accurately identified as low risk of long-term MACE could be discharged without further follow-up or cardiovascular investigations.

ACS and atherosclerosis

ACS is a life-threatening manifestation of atherosclerosis. Atherosclerosis is a chronic, relatively benign, inflammatory condition that may start in childhood. Although atherosclerosis is primarily a stable condition, it can undergo periods of instability, which may or may not manifest clinically.

The process of atherosclerosis begins with an insult, such as shear stress or chemical irritants, that results in endothelial dysfunction. This can lead to a build-up of lipoprotein particles in the intima, which become modified and contribute to foam cell formation and recruitment of leukocytes.(23) Inflammation is central to the progression of atherosclerosis with a cytokine-mediated attraction of monocytes into the plaque. Monocytes differentiate into macrophages, which take up low-density lipoproteins (LDL), leading to the formation of foam cells. These foam cells produce cytokines that propagate plaque development.(24,25) Atherosclerotic plaques may be present for decades and never cause any symptoms. However, plaques may also

undergo changes that lead to plaque rupture or erosion, leading to thrombi formation and ACS.

The pathophysiology of ACS

The pathophysiology of ACS is not fully understood. The main two pathologies involved in ACS are plaque rupture and plaque erosion.(26) Plaque rupture is an inflammatory process that occurs in around 70% of ACS; this happens when the thin fibrous cap overlying an atherosclerotic plaque is disrupted, and luminal blood is exposed to the thrombogenic material found in the necrotic core. There is an abundance of macrophages in plaque tissue of ACS over chronic stable angina.(27) Plaque rupture tends to be seen in males and those with hypercholesterolemia. Plaque erosion is the cause of approximately 30% of ACS and is caused by the loss of endothelial integrity. The mechanism of plaque erosion is thought to be due to loss of adhesion to the extracellular matrix and an increase in apoptosis of endothelial cells. There is an abundance of smooth muscle cells and proteoglycans in eroded plaques with fewer inflammatory cells.(28) The fibrous cap in endothelial erosion tends to be much thicker, and the necrotic core may be absent or be deeply embedded in the vessel wall.(26) Endothelial erosion is more prevalent in premenopausal women, and smoking is a substantial risk factor.(29,30) Both plaque rupture and erosion tend to occur at sites of non-significant luminal stenosis on coronary angiography.(31) Calcified nodules have also been demonstrated to be responsible for ACS in up to 2-7% of cases.(32)

The diagnosis of ACS

ACS is an umbrella term for AMI and UA. The Fourth Universal Definition of Myocardial Infarction(8) for type 1 AMI is dependent on a detection of a rise and fall

of the biomarker, troponin with at least one value above the 99th percentile (see Table 1).

Table 1. Fourth universal definition of myocardial infarction (2018)

Criteria for type I MI
A rise and/or fall of cardiac troponin with at least one value above the 99th percentile and one of the following: • Symptoms consistent with AMI • New ischaemic ECG changes, • Development of pathological Q waves, • Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with ischaemia • Identification of a coronary thrombus.

Fourth universal definition of myocardial infarction (2018)(8)

Troponin

Troponin is a fundamental component in the regulation of striated and cardiac muscle contraction. Muscle contraction occurs when the thick myosin filaments slide along the thin actin filaments shortening the sarcomere length; this process is regulated by the troponin/tropomyosin complex). Troponin is a complex comprising of three polypeptide chains: troponin T (cTnT), troponin I (cTnI) and troponin C (cTnC). The troponin complex is attached to tropomyosin. Tropomyosin wraps around the thin actin filaments and, in the relaxed state, blocks the myosin-binding sites (Figure 1). During muscle contraction (see Figure 2), calcium is released from the sarcoplasmic reticulum, which then binds to cTnC. This leads to a conformational change to the tropomyosin molecule, resulting in the exposure of myosin-binding sites on the actin filaments.

Figure 1. Thick and thin filaments

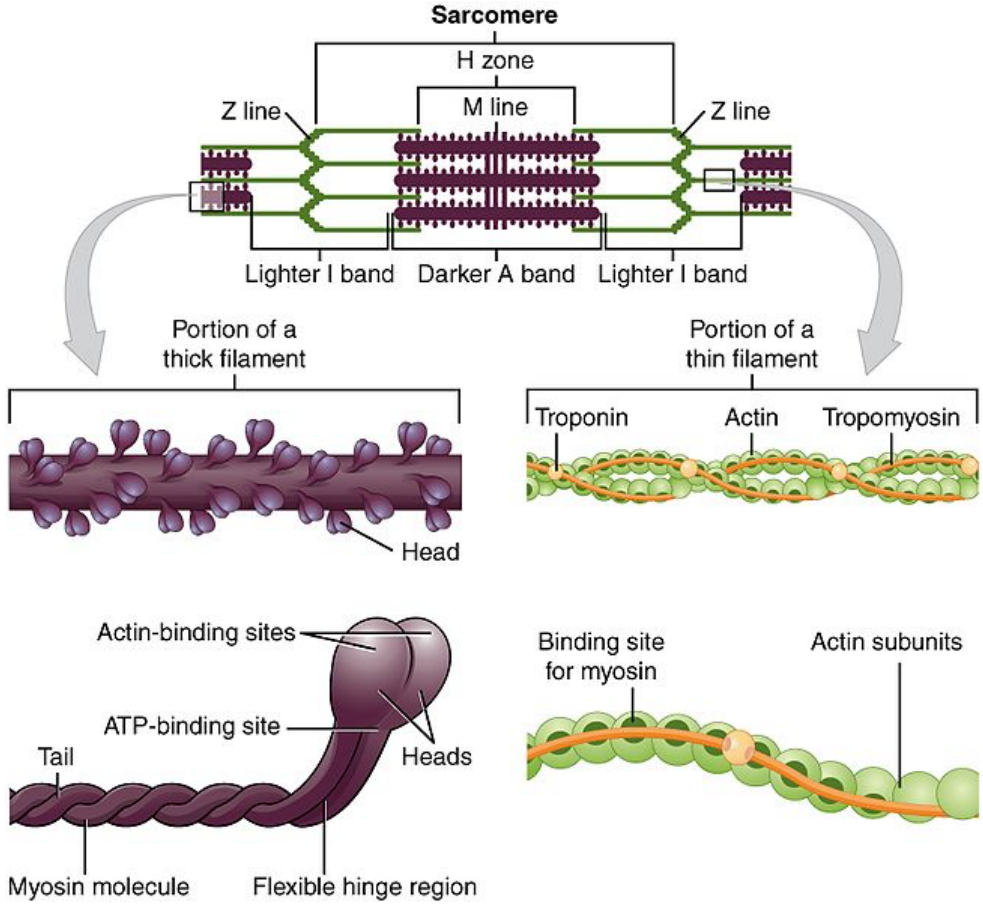
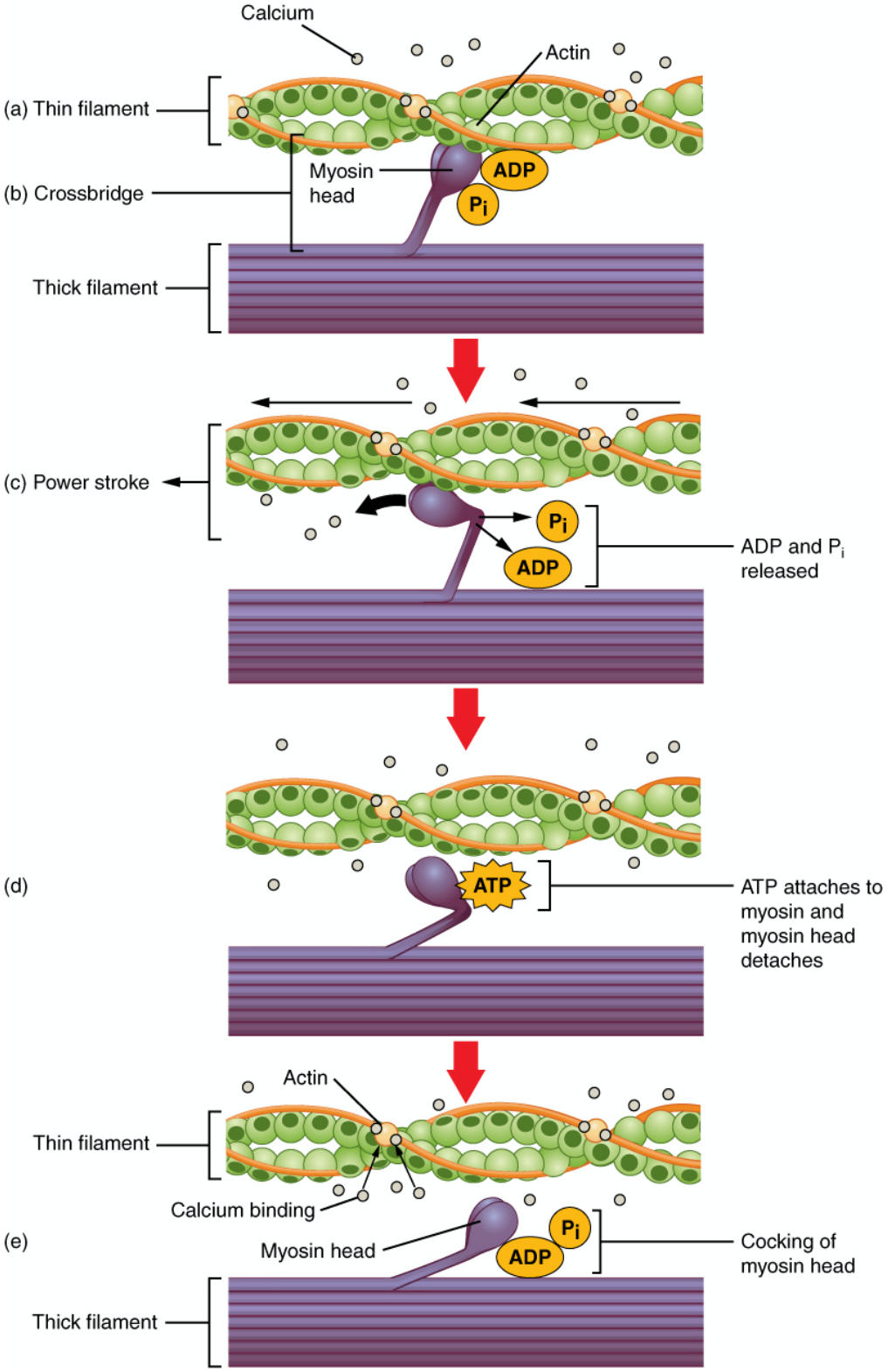


Diagram showing thick and thin filaments and troponin/actin position on thin filaments. Image reproduced under Creative Commons license.(33)

Before myosin cross-bridges are formed, the myosin head has stored energy and an ADP and phosphate molecule attached. The phosphate molecule is released when a bond is formed between the myosin head and the actin filament. The stored energy is then used to move the myosin head, causing the myosin and actin filaments to move along passing each other resulting in the shortening of the sarcomere length. The ADP is released as this motion occurs. Breaking the cross-bridge occurs when an ATP molecule then re-attaches to the myosin head, which is subsequently broken down to ADP and phosphate. The cycle repeats if further calcium molecules are available. Calcium returns to the sarcoplasmic reticulum for muscle relaxation.

Figure 2. Steps of muscle contraction



Steps of muscle contraction. a and b) Myosin heads are bound to actin by the formation of cross-bridges. c) Myosin cross-bridges move towards the centre of the sarcomere, known as the power stroke. d) The myosin heads bind ATP, and the cross-bridges detach from actin. e) Myosin heads hydrolyze ATP and return to baseline position, and become energised. Image reproduced under Creative Commons license.(34)

The cTnT and cTnI subunits in cardiac muscle have different amino acid sequences to their skeletal muscle counterparts. The majority of troponin resides within the contractile apparatus of the muscle; however, there is a small cytosolic pool of free cTnT and cTnI (approximately 6-7% and 3-5% respectively).(35–37)

Cardiac troponin T and I kinetics during myocardial infarction

Troponin levels are released during the first few hours following symptom onset of myocardial infarction and reaching peak concentration at around 24 hours - 48 hours, with some variations depending on the size of infarct and whether reperfusion has occurred (see Figure 3).

Figure 3. Profile of troponin release following myocardial infarction

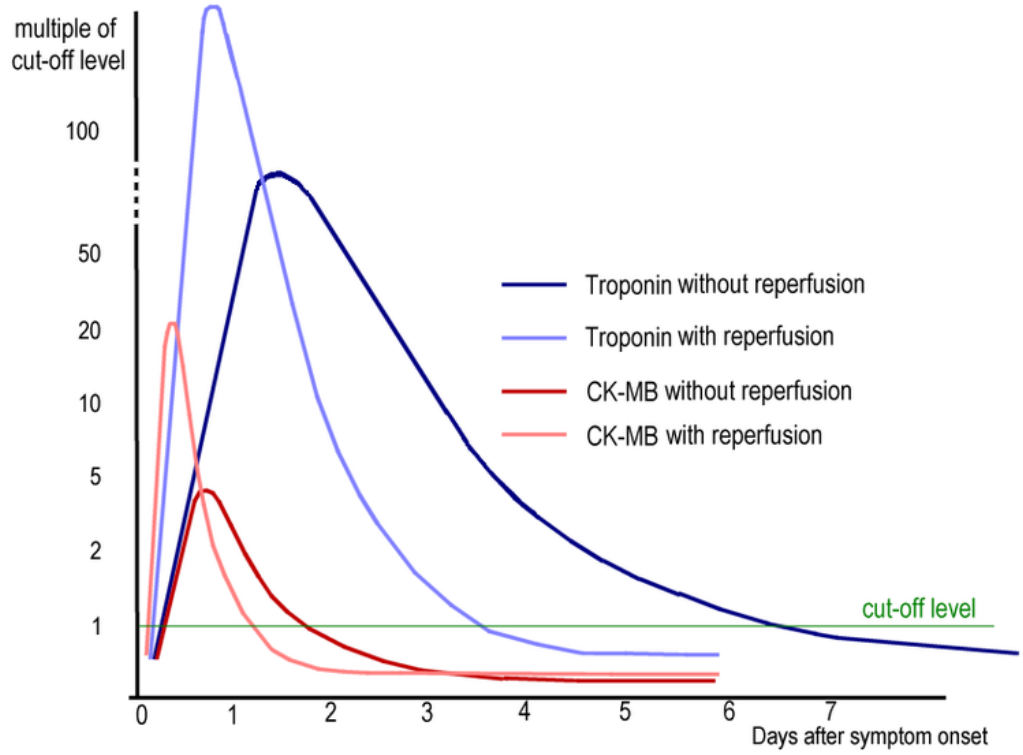


Diagram showing the profile of troponin release following myocardial infarction. The profile for CK-MB is included for reference. Image reproduced under Creative Commons license.(38)

The profiles of cTnT and cTnI post-myocardial infarction are similar regarding the first appearance, time to peak levels, and return to baseline, albeit that the cTnT rise and fall is slightly slower compared to cTnI.(39,40) The total time troponin remains elevated following acute myocardial infarction is mainly dependent on the infarct size. Levels of cTnI are higher than cTnT.(39,40) cTnT is released into the serum with a biphasic profile with an additional but smaller peak at around 3 - 4 days. This is thought to be due to the initial release of the cytosolic pool of free cTnT, which requires disruption to the myocardial cell membrane, and the later peak due to the slower degradation of bound cTnT following proteolytic degradation of myofibrils.(35,41) Another hypothesis is that it is due to the formation of immune reactive cTnT fragments. Successful early coronary reperfusion is associated with a steeper rise to the first peak, and the second peak is much smaller when compared with late perfusion.(42) It has been shown that the ratio of the second to the first peak of cTnT reflects the infarct area and area at risk.(42) Up to 10 different fragments (between 10-30kDa) of cTnT in serum have been identified in studies looking at the degradation of cTnT during acute myocardial infarction.(43) More specifically, the intact cTnT has only been identified in the first 12 hours following myocardial infarction; after this time, only fragments of cTnT are identified.(43) The cTnT assay measures both intact and fragmented cTnT. The half-life of cTnT is 120minutes.(35) cTnI levels at 72 hours can provide insight into the infarct size and the degree of microvascular obstruction.(44)

High sensitivity troponin assays

The development of high sensitivity troponin assays has enabled the detection of very low levels of circulating troponin levels with a high degree of precision. Precision

signifies how well an assay can provide the same result when the same sample is repeated on multiple occasions. The precision recommended for a high sensitivity troponin assay is a Coefficient of Variation (CoV) of less than 10% at the level of the 99th percentile. To fulfil the criteria for high sensitivity assays, the Limit of Detection (LoD) of the assays should facilitate measurable concentrations of troponin in at least 50% of healthy individuals. The ability to accurately detect low troponin levels permits the earlier identification of myocardial necrosis and can assist in the earlier rule out of AMI.(45)

Normal troponin levels in the high sensitivity troponin era

It has been well established that high troponin levels are associated with an adverse prognosis.(46) High sensitivity troponin assays have enabled detection of very low levels of circulating troponin, this has facilitated risk reclassification in some individuals from a low to a higher risk.(47,48) However, even with the advent of high sensitivity troponin, normal troponin levels can be associated with adverse outcomes.(49) The Heart and Soul study found that there were higher levels of Hs-cTnT in outpatients with coronary heart disease and was an independent predictor of cardiovascular events. (50). Hs-cTnT can be detected in the majority of people with stable CAD; plaque volume has been found to be positively associated with troponin levels. Higher troponin levels observed in patients with thin cap fibroatheroma. (51) HS trop I in stable symptomatic outpatients undergoing CTA for diagnosis suspected CAD, higher concentrations of Hs-cTnI were associated with increasing presence and severity of coronary atherosclerosis.(52) A study found that in patients who presented with suspected ACS and had troponin levels in the normal range, high trop I levels were associated with an increased probability of inducible myocardial ischaemia, but

interestingly, reversible ischaemia was still seen on exercise echocardiography in 18.8% of patients with undetectable Hs-cTnI vs 33.5% with detectable Hs-cTnI.(53) Any detectable level of high sensitivity is associated with an increased risk of death.(54).

Acute chest pain pathways incorporating high sensitivity troponin

High sensitivity troponins, introduced in clinical practice in 2010, have altered pathways of care for acute chest pain/suspected ACS.(55–57) There is increasing interest in very low levels of high sensitivity troponin and the delta change between serial troponins in the rule out of ACS and generally studies show good results, particularly at cut-off levels near the LoD and minimal delta change between samples. Both the ESC and NICE have produced guidance recommending high sensitivity troponin for the early rule-out of NSTEMI.(56,58) There is increasing evidence that these rule-out strategies are very successful in most patients; however, they are not 100% accurate. Moreover, some of these strategies have a missed AMI rate above a commonly accepted threshold of 0.5%.(59) Analysis comparing fourteen rule out scores(60) found the NICE algorithm had a missed MI rate of 0.7% when using the high sensitivity cTnI assays, albeit improved at 0.1% when using High sensitivity Troponin T (Hs-cTnT). The same study also found that the ESC 0/3h algorithms had a missed AMI rate of 0.6% and 1.1% for Hs-cTnT and High Sensitivity Troponin I (Hs-cTnI) respectively. A large collaborative meta-analysis(61) found that a single hs-cTnT below the LoD in combination with an ECG that was not ischaemic was able to rule out myocardial infarction successfully, with a pooled NPV involving 11 studies of 0.993. There has been some investigation as to whether CT angiography could have

a role in the management of acute chest pain, but this has not been found to improve clinical outcome and increased the hospital length of stay.(62)

Risk scores

There are many risk scores that have been developed and validated for risk stratification in patients with confirmed or suspected ACS. The HEART, TIMI and GRACE scores are popular scores, that have been developed in quite different ways. NICE guidelines advocate the use of the GRACE score, although research suggests this score may not perform well in the lower risk population.(63) There is no consensus as to which risk score should be used in those who are low risk or who have had MI excluded. The HEART score was developed by a clinician based on clinical experience to stratify patients who present to the ED with chest pain.(64) The initial trial was small (125 patients) and performed in a single centre in the Netherlands. The TIMI score(65) was developed from early TIMI randomized controlled trials (TIMI 11B and ESSENCE which were trials investigating low molecular weight heparin and unfractionated heparin in unstable coronary disease). The GRACE score was initially developed using international registry data with just over 11,000 patients with all subsets of ACS included and unstable angina being the most prevalent (38% final diagnosis).(66) Table 2 shows the difference in components for scores. Whilst both HEART and TIMI risk scores use risk factors for cardiovascular disease in their scoring system, the definitions of risk factors have some difference, the definition of risk factors can be seen in Table 3.

The TIMI, GRACE and HEART score were analysed in this study. These scores were chosen because they are popular scores that are already in use in the NHS and are recommended for use in national guidelines.

Table 2. Variables used in the TIMI, GRACE and HEART scores.

	HEART	TIMI	GRACE
Age	<45y 0 points 45-64y 1 point ≥65y 2 points	<65y 0 points ≥65y 1 point	0-100 points
Risk factors	No RFs 0 points 1 RF 1 point ≥ 2 RFs 2 points	≥ 3 RF 1 point	NA
ECG	No changes 0 points Repolarisation changes 1 point STD 2 points	STD 1 point	STD 0-28 points
Troponin	<URL 0 points 1-3xURL 1 point >3xURL 2 points	<URL 0 points >URL 1 point	> URL 0-14 points
History	Atypical 1 point Typical 2 points	Severe angina 1 point	NA
Medical history	NA	Known coronary stenosis 1 point	NA
Other	NA	Aspirin use 1 point	Cardiac arrest 0-39 Heart rate 0-46 SBP 0-58 Creatinine 0-28 Killip sign 0-59
Score range	0-10	0-7	0-372

Table showing components and contributions of each risk score and range of score total. RF risk factor. URL upper reference limit. STD ST depression. SBP systolic blood pressure.

Table 3. Definition of risk factors for TIMI and HEART score.

Risk factors for TIMI score	Risk factors for HEART score
• Hypertension • Hypercholesterolaemia • Diabetes • Family history of coronary artery disease • Current smoker	• Hypertension • Hypercholesterolaemia • Diabetes • Family history of parent or sibling under age 65 years • Current smoker • Obesity • Atherosclerotic disease (previous MI, PCI, CABG, CVA, TIA, peripheral arterial disease).

Table showing the risk factors that contribute to the TIMI and HEART score.

TIMI score

The TIMI score comprises seven variables and is easy to calculate. It was initially designed to predict 14-day mortality, new or recurrent MI and severe recurrent ischaemia prompting urgent revascularisation. A diagnosis of UA required ST depression or a history of coronary artery disease to be included in the initial trial. The model was found to be most accurate for the prediction of all-cause mortality, less so for combined or non-fatal endpoints (see Table 4). The score has subsequently been externally validated using high sensitivity troponin assays, including in more unselected chest pain groups.(67–69)

Table 4. C-statistic of TIMI score according to outcome.

Test group TIMI 11b	C-statistic
All-cause mortality, new or recurrent MI, severe recurrent ischaemia requiring urgent revascularization.	0.65
All-cause mortality	0.74
MI	0.66
Urgent revascularization	0.68
All-cause mortality or MI	0.63

C-statistic of initial trials assessing TIMI score.

GRACE score

The GRACE score uses 8 variables that focus on features seen on presentation. Calculation of the GRACE score is complex and is usually done using computer software. For a diagnosis of UA in the initial trials, patients were required to have symptoms consistent with coronary ischaemia, normal cardiac enzymes and either ECG changes (transient ST elevation or depression of greater or equal than 1mm, new T wave inversion of greater or equal than 1 mm or pseudo-normalization of previously inverted T waves), or a history of coronary disease. The score was initially designed to predict 6-month mortality. The c-statistics calculated in the original trial can be seen

in Table 5; this shows that the model, like the TIMI score, is more accurate at predicting mortality alone over the addition of myocardial infarction. Tang et al. (70) performed a retrospective observational study showing that GRACE score had a higher C-statistic for UA (0.91) subset of ACS as compared with NSTEMI (0.82) and all ACS (0.81) when looking at 6-month mortality; however, numbers for UA were small (247) and this was in the non-high sensitivity troponin era. A revised algorithm was recently developed substituting variables that might be unavailable to the clinicians, the GRACE score 2.0. In the TRACE-CORE(71) cohort, the C-statistic for GRACE 2.0 was 0.77, but 0.94 in those with STEMI and 0.78 in those with NSTEMI.

Table 5. C-statistic of GRACE score according to outcome.

For unstable angina / NSTEMI cohort	Derivation cohort C-statistic	Validation cohort C-statistic
6-month mortality	0.79	0.81
Death or non-fatal MI 6 months	0.70	0.73

C-statistic of initial trials assessing GRACE score.

HEART score

The HEART score uses 5 variables and is an easily calculable score. Endpoints in the initial trial included AMI, revascularisation, and mortality. A 'low risk' score of 0-3 points equated to a risk of 2.5%, 4-6 points correlated to 20.3%, and if the score was above 6 points, the risk was 72.7%. The score has since been validated by larger(72) and multicentre(73) studies. A score of 0-3 is defined as low risk. A systematic review and meta-analysis recently performed by Fernando et al.(74) was recently performed which assessed the predictive accuracy of the HEART score, which included 30 studies and 44,202 patients. Only four studies that remained in the final analysis used high sensitivity troponin, and only 1 study was in the UK. Previous work at Aintree

across the spectrum of ACS suggests that the HEART score performs well across the range of NSTEACS (C-statistic 0.910).

Comparison of risk scores

There are several studies comparing the performance of risk scores. In undifferentiated chest pain or those with suspected ACS and MI excluded, the HEART score tends to outperform the GRACE and TIMI score.(75–77) This is likely due to the GRACE and TIMI score using a number of variables that would most likely be normal in patients who have had MI excluded. Whilst the discriminative ability of risk scores is dependent on the high sensitivity troponin assay used, data suggests that risk scores in isolation would not be able to reliably identify those at low risk.(78–80)

Non necrosis biomarkers in chest pain with MI excluded

There are a number of studies investigating novel biomarkers in ACS, with many of these showing that biomarkers can provide prognostic information.(81,82) However, most studies do not assess the incremental value of biomarkers beyond accepted risk stratification methods. In this thesis, the following biomarkers: HFABP, GDF-15, NTproBNP, Galectin-3 and HS-CRP, are investigated to establish whether they provide independent prognostic information beyond the TIMI, HEART and GRACE score. These biomarkers were chosen to be studied as they have all been implicated to have a role in risk stratification or cardiovascular disease in some way and were able to be measured on the serum samples that were stored.

Heart type fatty acid binding protein

Heart type fatty acid binding protein (HFABP) is a small, 15kDa cytoplasmic protein primarily responsible for transporting long-chain fatty acids from the cell membrane

to the mitochondria, endoplasmic reticulum and other intracellular organelles.(83,84) HFABP is located mainly within cardiomyocytes, but lower levels can also be found in other tissues, including skeletal muscle, brain, intestines, lung, kidney and adrenals.(85) Given the relatively small molecular size of HFABP and the cytoplasmic location, it is released rapidly into the serum during myocardial infarction, as early as 30 minutes after symptoms, reaching peak levels at 4 - 6 hours and returning to baseline within 24 hours of the event.(86,87) This early release of HFABP prompted research into the diagnostic ability of HFABP in the setting of ACS, and it is a sensitive and early marker of myocardial infarction. HFABP has also been found to show direct evidence of myocardial ischaemia.(88,89) HFABP predicts long-term mortality in the community and after ACS.(90,91) Body et al.(92) developed the Manchester Acute Coronary Syndromes decision rule that incorporated HFABP, which was able to accurately identify patients at very low risk for MACE at 30 days which could potentially be safely discharged but a subsequent study by the same author assessed the risk score without the HFABP component and found that this provided a model with comparable sensitivity but greater specificity.(55). Viswanathan et al.(93) was one of the first large studies in the high sensitivity troponin era to assess the role of HFABP in ACS in patients with normal high sensitivity troponin levels; the study included 756 patients who presented with chest pain and had normal high sensitivity Advia TnI-Ultra troponin levels at 12 hours. Endpoints were death or readmission with MI over 12 months. This study found that HFABP was able to risk stratify these patients yet did not assess the prognostic information beyond standard clinical risk stratification. A previous systematic review(94) conducted by our research department and a more recent systematic review performed by an external

research unit(95,96) concluded that there was little evidence to support the use of HFABP in combination with high sensitivity troponin T.

Growth Differentiation Factor-15

GDF-15 is a distant member of the Transforming Growth Factor (TGF) superfamily. TGF constitutes a superfamily of cytokines that exert prominent actions in adult haemostasis and adaptation by regulating cell survival, proliferation, and differentiation. In animal models, GDF-15 is induced in response to ischaemia-reperfusion injury, pressure overload and heart failure, possibly via pro-inflammatory cytokine and oxidative stress-dependent signalling pathways.(97,98) GDF-15 has been found to be a promising biomarker in the prediction of heart failure and cardiac death in the general population.(99) A secondary analysis of the PLATO biomarker study(100) demonstrated that GDF-15 was a strong marker of all-cause mortality over 17,000 patients with ACS. There is evidence of an adverse outcome in patients with an elevated GDF-15 level in highly selected trials with NSTEMI and the non-selected chest pain population.(101–103) There is evidence that GDF-15 can provide incremental information beyond the GRACE score.(104,105) GDF-15 has also been found to be independently associated with cardiovascular events and all-cause mortality when adjusted for high sensitivity troponin.(106) Schaub et al.(107) reported that GDF-15 could predict all-cause mortality in unselected chest pain independently and with greater accuracy than high sensitivity troponin and BNP, however, did not aid the early diagnosis of myocardial infarction.(107) Whilst there are several studies that show a prognostic role of GDF-15 in ACS, these studies generally include fair proportions of patients with confirmed AMI.(107) There is little information about whether GDF-15 can provide incremental information beyond the HEART and TIMI score, particularly when MI has been excluded.

High sensitivity C Reactive protein

C-reactive protein (CRP) is an acute-phase reactant released in response to inflammation. CRP primarily exists in the plasma in its pentameric form, where five identical monomers are bonded non-covalently to give rise to the 115,135Da unit resembling the structure of a doughnut.(108) It is primarily synthesized in hepatocytes under the transcriptional regulation of cytokines including Interleukin-6 (IL-6), Interleukin-1 (IL-1) and Tumour Necrosis Factor-α (TNF- α).(109) Hepatic synthesis is driven at the transcriptional level by IL-6, expressed mainly by macrophages, T cells, and adipocytes. CRP may also be produced by other tissues, including smooth muscle cells, arteries, and the kidney. In healthy subjects, CRP levels are usually less than 2mg/litre(109) with median concentrations of 0.8mg/l. In the setting of ACS, levels of CRP increase.(110,111) There has been considerable debate over the role of CRP in cardiovascular disease, and in particular, whether CRP has a direct causal role in atherogenesis or whether CRP levels simply reflect the underlying process. Higher CRP levels in ACS are an independent predictor of mortality(112–114) heart failure(112,113) and future cardiovascular events,(115) and could add prognostic information to the TIMI risk score in patients with NSTEACS, but not the GRACE score.(116,117) Other studies show a limited role of high sensitivity CRP, particularly in those studies using high sensitivity troponin and those with MI excluded.(118–120)

Natriuretic peptides

Brain Natriuretic Peptide (BNP) is a hormone secreted along with the prohormone N-terminal BNP (NTproBNP) from cardiac myocytes in response to increased ventricular wall stress. BNP and NTproBNP are already used in screening and the prognosis of heart failure, as endorsed by NICE guidelines. After myocardial

infarction, BNP rises rapidly and peaks at 24 hours, and the peak levels are proportional to the infarct size.(121,122) Prior to the development of high sensitivity troponin assays, BNP was found to be elevated in ischaemia in the absence of necrosis.(123) NTproBNP has been found to be an independent predictor of outcome in ACS, including when assessed in patients with chest pain and the absence of ST elevation.(124,125) A sub-study of OPUS TIMI 16(126) demonstrated a relative risk of 7.9 for mortality between the lowest and highest quartile of BNP amongst patients who had confirmed unstable angina. Morrow et al. reported in the TACTICS-TIMI 18 trial(127) that BNP level was found to improve risk stratification in patients with confirmed ACS who had non-high sensitivity troponin levels in the normal range. There have been some studies looking at the role of BNP/NTproBNP beyond the GRACE and TIMI score, but was not found to be significant.(128,129) There is a paucity of data for the role of BNP/NTproBNP in patients who present with chest pain and have MI excluded, particularly beyond risk scores in the high sensitivity troponin era.

Galectin-3

Galectin-3 is a member of the galectin family, a family of proteins that can bind to carbohydrate beta-galactosidase. Galectin-3 is a protein that is approximately 30kDa in size and is elevated at 1 – 4 hours following acute myocardial infarction and by 4-5 days falls below baseline.(130–132) Galectin-3 is involved in a wide range of functions, including cell adhesion, cell activation and inflammation. Galectin-3 has also been identified to play critical roles in cardiovascular conditions, including cardiac remodelling and myocardial fibrosis.(133,134) Galectin-3 is associated with cardiovascular disease risk factors and a predictor of all-cause mortality in the general population.(135) Galectin-3 is an independent predictor of mortality and heart failure

following acute myocardial infarction.(136) Some researchers have speculated that Galectin-3 may be an initiator of plaque rupture.(137) Galectin-3, measured post-MI, has been found to yield a similar prognostic value to GRACE score in patients with confirmed MI.(138) Kittanakom et al.(139) studied Galectin-3 using two high sensitivity troponin assays (Abbott and Roche) in patients with chest pain, but this was with small numbers and a very short follow up (72 hours); this reported Galectin-3 was inferior to high sensitivity troponin in early risk prediction, and provided no additional information. Tian et al.(140) summarised in a meta-analysis that there limited evidence for Galectin-3 in the prediction of outcomes in AMI.

Statistical methods for analysing biomarkers

Many different statistical methods can be used in assessing the performance of a biomarker, and usually, a number of approaches are used.

Sensitivity, Specificity, PPV and NPV

Sensitivity and the Negative Predictive Value (NPV) are useful ways of assessing the ability of a test to rule-out a condition. Sensitivity is the ability of a test to identify a true positive based on the true positive rate. NPV is the probability that a patient does not have the condition if they have a negative test result. These two parameters are related. The Specificity and PPV are usually of more relevance when trying to rule-in a condition.

Equation 1

$$Sensitivity = True\ Positives/(True\ Positives + False\ Negatives)$$

Equation 2

$$Specificity = True\ Negatives/(True\ Negatives + False\ Positives)$$

Equation 3

$$Positive\ Predicted\ Value = True\ Positives/(True\ Positives + False\ Postives)$$

Equation 4

$$Negative\ Prediced\ Value = True\ Negatives/(False\ Negatives + True\ Negatives)$$

Table 6. 2x2 contingency table showing sensitivity, specificity, NPV and PPV

	MACE	No MACE	
Biomarker elevated	True positives (TP) (Correctly identified)	False positives (FP) (Type I error)	PPV =TP/(TP+FP)
Biomarker not elevated	False negatives (FN) (Type II error)	True negatives (TN) (Correctly identified)	NPV =TN/(TN+FN)
	Sensitivity =TP/(TP+FN)	Specificity =TN/(FP+TN)	

2x2 contingency table showing calculations for sensitivity, specificity, NPV and PPV.

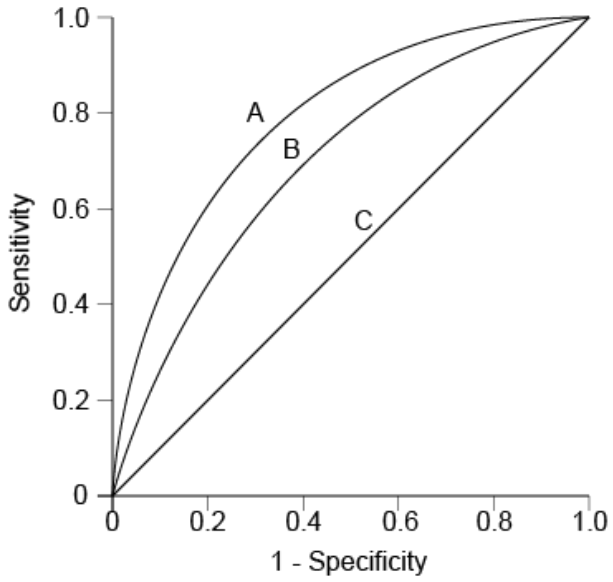
Receiver Operator Characteristic analysis

Receiver Operator Characteristic (ROC) analysis is a tool used to evaluate the performance of a diagnostic test over the range of values and the accuracy of a statistical model (such as logistic regression and Cox proportional hazards regression models) with a binary dependent variable. ROC curves are a plot of the True Positive Rate (Sensitivity) vs the False Positive Rate (1-Specificity), and the Area Under the Curve (AUC) provides the C-statistic. The C-statistic provides information on the model as a whole. A test with perfect discrimination between those with a condition and those without would have a C-statistic of 1. A C-statistic equal to 0.5 would suggest the test is no better than random chance.

Suggested C-statistic cut off values(141)

0.90 - 1.00: Excellent discrimination
0.80 - 0.90: Good discrimination
0.70 - 0.80: Fair discrimination
0.60 - 0.70: Poor discrimination
0.50 - 0.60: No discrimination

Figure 4. ROC curves for different strengths of models



Graph showing ROC curves for three models. Curve A, reaching closest to the upper left corner of the chart, has the best model discrimination. Curve B shows some discriminatory ability of the model but not as strong as A. Curve C plots a model with random chance.

Optimal cut-off value

The Generalized Youden's Index (J) is a common measure of the maximal potential effect of a variable when equal weighting is assigned to both sensitivity and specificity. Graphically, it is represented as the maximum vertical distance between the plotted curve and the diagonal line. It is represented by Equation 5. Another common measure is the point on the curve closest to the upper left corner, as shown in Equation 6. However, when investigating a continuous variable for accurately ruling out a condition, the top right-hand side of the graph provides the most useful

information.(142) An ideal rule-out test would have a curve that shows high sensitivity corresponding to a high NPV (see Figure 5). An ideal 'rule-in' test would have a curve that shows high specificity corresponding to a high PPV.

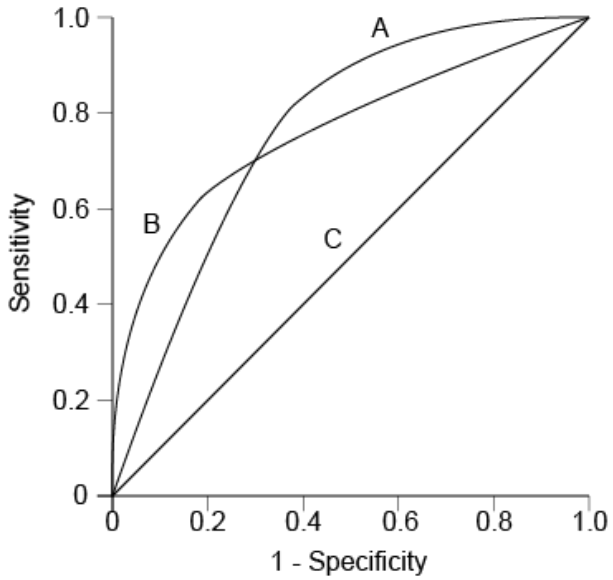
Equation 5

$$J = \text{Max}\,(\text{sensitivity} + \text{specificity}) - 1$$

Equation 6

$$\text{Distance} = \sqrt{(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2}$$

Figure 5. ROC curves showing favourable 'rule in' and 'rule out' tests



Curves A and B have an equal AUC; Curve A has a profile more favourable of a test that 'rules out' a condition, and Curve B has a profile more favourable of a test that 'rules in' a condition.

Kaplan-Meier analysis

KM analysis is a non-parametric assessment of time to event. It provides a plot of the probability of event-free survival over time in subject groups with different conditions or treatments. KM analysis can deal with censored data. The Mantel-Cox Log-Rank

is one method used to compare curves across follow-up, with statistical significance between curves being usually accepted when the p-value is <0.05.

Logistic regression

Logistic regression is a method of assessing variables and provided odds ratios when the dependent variable is binary. ROC curve analysis can be performed on logistic regression. Equation 7 shows the formula for logistic regression.

Equation 7

$$\text{Logistic Regression} = \ln\left(\frac{P(X)}{1-P(X)}\right) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_k X_k$$

β_0 = estimation of regression intercept
β_1 = estimation of regression slope
X = independent variable
ε = random error component

Net Reclassification Index (NRI)

The NRI was first developed by Pencina(143) for categorical variables, and a method for continuous variables (category-free) was created a few years later.(144) The NRI measures how well a new model can correctly reclassify patients over a previous model. The overall NRI is a sum of the net percentages of patients who have either been correctly reclassified (event NRI) or without (non-event NRI) the event in question. The maximum value of the overall NRI is 2; an NRI above 0.6 would be considered strong, 0.4 intermediate and 0.2 weak.(145) It is essential to also look at the values of the non-event NRI and event NRI individually.(146) The event NRI and non-event NRI can be interpreted as percentages, ranging between -100% and 100%. The formula for NRI is shown in Equation 8.

Equation 8

$$\text{NRI} = \text{P}(\text{up}|\text{event}) - \text{P}(\text{down}|\text{event}) + \text{P}(\text{down}|\text{nonevent}) - \text{P}(\text{up}|\text{nonevent})$$

Cox Proportional Hazards Regression

Cox Proportional Hazards Regression is a form of survival analysis that assesses event-free survival time on predictors. This form of regression provides Hazard Ratios (HR).

Equation 9

$$h_i(t) = Exp(\alpha + \beta_1 x_{i1} + \beta_2 x_{i2} + \cdots + \beta_k x_{ik})$$

$h_i(t)$ = hazard function for individual i
β = regression coefficients estimated from the data
x = covariates

The population of Liverpool

Finally, it is important to note that Liverpool is one of the most deprived regions in England. The local authority districts of Liverpool and Knowsley are in the top five districts in England with the largest proportion of highly deprived neighbourhoods. Liverpool has the highest Health Deprivation and Disability index, at a staggering 45.8% of neighbourhoods.(147) Cardiovascular disease kills 20% of people in Liverpool.(148) The Healthy Life Expectancy in Liverpool is six years lower than the average across England; there are higher rates of smoking (18.9% vs 15.5%), high alcohol consumption (29% vs 26%) and having a Body Mass Index above 25kg/m^2 (65% vs 61%). It has also been calculated that the avoidable admissions rate per 100,000 population in Liverpool is 1.112 whereas in England is 0.821. Liverpool Clinical Commissioning Group (CCG) proposed *One Liverpool*,(148) a strategic plan for improving health in the region. This includes improving the premature mortality

rate from cardiovascular disease and reducing unplanned avoidable admissions with cardiovascular disease. Epidemiology data shows that although mortality due to coronary heart disease has fallen over the last decade, those in the most deprived deciles have a worse prognosis than those in the least deprived.(149) It has also previously been demonstrated that unemployed patients or live in deprived areas have a worse outcome following PCI.(150)

Gaps in the literature

There are many studies looking into the management of patients with ACS, although there are some areas in which the evidence is lacking. There is little data on the incremental information biomarkers provide beyond standard clinical risk methods in the high sensitivity troponin era, particularly when troponin levels are normal.

This thesis aims to bridge some of the gaps in the current literature. Chapter 3 will report the demographics of the study cohort, how these patients are managed and associated MACE rates. Chapter 4 will look at the risk scores TIMI, HEART and GRACE scores and evaluate the performance of risk score in patients who present with ACS who have had MI excluded. Chapter 5 examines levels of novel biomarkers and the prognostic information these provide. Chapter 6 is a preliminary analysis looking at the role of biomarkers in the rule out of early MACE. Chapter 7 analyses the prediction of MACE using established risk scores and whether serological biomarkers can provide additional information beyond these scores.

This thesis describes how serological biomarkers could help manage patients who present to the Emergency Department with chest pain and have a ‘normal’ high sensitivity troponin level. It will assess how these biomarkers could improve the short and long-term prediction of MACE. The ability to accurately identify those at high

risk of short-term MACE could facilitate early appropriate cardiovascular investigation and treatment of these patients whilst allowing early discharge and reducing unnecessary investigation in low-risk patients. The ability to accurately identify those at risk of long-term MACE provides an opportunity to intervene for lifestyle advice and aggressive risk factor modification to improve their long-term prognosis.

Main research question

Can non-necrosis biomarkers provide incremental prognostic information to established risk scores and high sensitivity troponin levels?

Aims and objectives

To investigate the demographics, management and outcome of patients who present with chest pain and have high sensitivity troponin T levels below the 99th percentile.

To determine the risk score that performs best in these patients.

To establish the prognostic role of serological biomarkers in patients who present with chest pain and have MI excluded.

To examine whether the incorporation of biomarkers to risk scores can improve risk stratification.

CHAPTER 2. GENERAL METHODS

Study design

A prospective observational study looking at the diagnosis, management and risk stratification of patients who present with suspected ACS who have Hs-cTnT levels below the 99th percentile, and, the additional role beyond risk scores that non-necrosis biomarkers may play.

Centre of recruitment

University Hospital Aintree NHS Foundation Trust is a large teaching hospital providing acute healthcare to a population of 330,000. There is a catchment area of 33-square miles, covering the areas of North Liverpool, South Sefton and Kirkby, which, include some of the most socially deprived communities in the country. There is a large Emergency Department (ED) which treats over 86,000 patients a year. There are 712 inpatient beds. At the time of recruitment, within the cardiology unit there is a 52-bedded ward and an 8-bedded Coronary Care Unit (CCU). The Heart Assessment Centre (HAC) is an 18-bedded inpatient ward aimed to rapidly assess, diagnose, and treat patients with potential cardiac pathology, including low to medium risk ACS. The department has a strong imaging profile, which includes a stress echocardiography, CT coronary imaging and a cardiac MRI service. There is a cardiac catheterisation laboratory for diagnostic angiography during normal working hours.

Invasive treatment of ACS in the region

UHA does not perform coronary intervention. Liverpool Heart and Chest Hospital (LHCH) is the regional tertiary cardiac centre and the only unit covering Merseyside, the Wirral and parts of Cheshire that performs PCI and CABG. If a patient with cardiac chest pain is identified as having ST elevation or a presumed new LBBB on their ECG, the PPCI pathway should be activated and the patient would be taken, or transferred,

to LHCH for urgent coronary angiography +/- coronary intervention. When a patient presents to a local hospital with an NSTEMI, initial medical treatment will usually be provided by that hospital and a referral made to LHCH, with a view to transferring the patient for inpatient coronary angiography/coronary intervention. Patients who have unstable angina may also be referred via this method, particularly if they have convincing on-going symptoms or dynamic ECG changes.

Participants

Adult patients who provided written informed consent to take part in the study, after presenting to University Hospital Aintree with chest pain who were found to have Hs-cTnT levels below the 99th percentile when taken between 6-12 hours post chest pain. Patients were excluded if they had a history of chronic atrial fibrillation or chronic heart failure as biomarker levels are likely to be affected by these underlying conditions.(151–153)

Patient inclusion criteria:

- Age 18 years and above.
- Presenting with chest pain that suspected to be ACS by reviewing doctor.
- Hs-cTnT ≤14ng/l between 6–12 hours of chest pain.
- Consent for inclusion in the study and consent for follow up.

Patient exclusion criteria:

- Known history of chronic heart failure
- Chronic atrial fibrillation.
- Recent ACS in preceding 6 weeks.
- Prisoners

- Pregnancy
- Non-English speakers

Patient identification and recruitment

All patients who present as an emergency to University Hospital Aintree NHS Foundation Trust are logged on to the hospital computer system Sigma, along with the presenting complaint. During times of recruitment, this list was reviewed to identify patients who may fulfil inclusion criteria. Electronic notes were also reviewed to look for established exclusion criteria. If the troponin result was within the normal range, the patient's admission case notes were reviewed to ensure suitability. A discussion was then made with the patient to review the clinical presentation and recruited to the study, if appropriate. Patient recruitment was performed by a single researcher (JJ) that primarily worked during daytime and evening hours and mostly over weekdays, although weekends were also used to recruit. The facility where serum samples were stored was closed between 10pm and 7am, therefore no patients who had troponin samples analysed during this time were recruited. It would have been optimal to recruit consecutive patients 24/7, but it is not anticipated that this would have led to significant bias.

Standard clinical care

Patients presenting to the ED with chest pain are triaged by an ED nurse. An initial 12 lead ECG is taken at this point. Blood samples for troponin levels were taken at least 6 hours after the onset of pain and in some patients with high clinical suspicion would have had these repeated at 12 hours. Patients self-presenting to ED or via the paramedics would be reviewed doctors in the ED; if admission was felt necessary,

patients would have been referred to the Medical Assessment Unit (MAU), Coronary Care Unit (CCU) or Heart Emergency Centre (HEC). Patients referred via the GP may have bypassed the ED step and been directly admitted to HEC. This study did not interfere with standard hospital care. All clinical decisions were made by the patient's clinician who were blinded to biomarker results. Internal pathways and protocols exist for the medical management of ACS in UHA, but these are not strictly enforced.

Electrocardiogram

It is hospital protocol to perform a 12-lead ECG in patients who present with chest pain on admission. Further ECGs are performed depending on the request of the clinician looking after the patient and the clinical situation. Only ECGs performed in hospital were interpreted. These ECGs were reviewed by a single experienced observer (JJ) and the following information was recorded: cardiac rhythm, ventricular rate, QRS duration, ECG criteria for left ventricular hypertrophy, ST-T wave changes and the presence of ventricular ectopy. Cardiac rate, QRS and QTc interval were reported on the automated ECG analysis software, these figures were checked by the observer and if they were felt inaccurate - measurements were made manually. Left ventricular hypertrophy was defined as fulfilling either Sokolow-Lyon or Cornell voltage criteria, measured manually. ST segment deviation, T wave changes and presence of ectopy and Q waves were determined manually.

Definition of ECG abnormalities

ST segment depression

ST segment shift in any lead below the isoelectric line to the nearest 0.5mm.

T wave changes

T waves were classed as either normal, flat, inverted or deeply inverted in any lead other than AVR and lead III. Inverted T waves were defined as a negative deviation from the isoelectric line. Deep T waves were defined as the nadir of the T wave measuring 3mm from the isoelectric line.

Left ventricular hypertrophy definition

Left ventricular hypertrophy was defined using the Sokolow-Lyon index and Cornell voltage criteria.

Sokolow-Lyon index

- S in V1 plus R in V5 or V6 ≥ 35mm
- R in AVL ≥ 11mm

Cornell voltage criterion

- R wave in aVL and S in V3 >28mm in men or >20mm in women

Abnormal Q waves

A normal Q wave was defined as narrow <0.04s and should not exceed 25% of the amplitude of the following r wave, though exceptions exist in leads III, aVL and aVF. An abnormal Q wave was defined as the presence of Q waves that did not fit the aforementioned criteria for a normal Q wave.

General Laboratory procedures

Post phlebotomy, blood samples are taken to the biochemistry laboratory in a timely fashion. All troponin samples are marked 'urgent' and analysis for these samples were

prioritised over routine clinical samples. Samples were centrifuged for 10 minutes. Troponin samples were analysed as part of clinical care in the UHA biochemistry laboratory. For patients recruited to the biomarker study, the remainder of serum was divided, depending on the quantity of serum remaining, between 3 and 5 storage containers 'Elkay 1.5mL Printed tube with skirted base' with 'Elkay screw cap with O ring' and stored at -80 degrees centigrade.

High sensitivity Troponin T analysis

Initial troponin samples were analysed on a Roche Elecsys 2010 Immunoassay Analyser. In November 2011 there was an upgrade in the analysing equipment in the biochemistry laboratory and subsequently troponin samples were analysed on a Roche Cobas 8000 e602 Module Analyser. All troponin assays were measured using Roche Cobas Troponin T immunoassay, prior to 2012 the Roche Cobas Troponin T HS electrochemiluminescence 'ELICIA' immunoassay STAT assay was used, which was then substituted for the Roche Cobas Troponin T HS electrochemiluminescence ELICIA immunoassay so that Troponin T could be analysed at the same time as other standard biochemical tests (renal function and electrolytes, liver function tests, bone profile, lipids) in order for a faster turn-around time. During recruitment, Roche Diagnostics issued a technical bulletin reporting a downward shift in the standardisation against the master lot. This led to an underestimation of Hs-cTnT levels, particularly in those with very low concentrations. Affected batches 16019701 and 16370401 were in use at the time of recruitment and for Hs-cTnT values measured on this, adjustments were made in keeping with manufacturer guidelines. The normal range for Hs-cTnT is ≤14ng/l. The limit of blank is <3ng/l and LOD and 5ng/l. The

coefficient of variation was 10% at a level of 13ng/l. For statistical analysis, levels less than the limit of blank were reported as 2ng/l.

Haemolysis

If high sensitivity troponin samples were haemolysed, these samples were repeated as per routine clinical care and research samples were only taken on non-haemolysed serum samples.

Baseline characteristics

Baseline characteristics were obtained from patient questioning and their medical notes. These included age, gender, time of chest pain, time of arrival, time of discharge, if discharged directly from ED, haemoglobin level, platelet count, white cell count, packed cell volume, creatinine, troponin, blood pressure, heart rate, respiratory rate, past medical history, existing medication and acute medical treatment.

Clinical scoring systems

GRACE, HEART and TIMI risk scores, the Charlson Comorbidity Index, and, NICE Chest Pain pre-test probability of coronary artery disease, were determined by a single physician (JJ). There was a 'Chest pain proforma clerking document' produced for the emergency department to streamline the clerking procedure and assist with management of patients. This included a 'tick-box' history section with commonly used parameters to describe pain in keeping with the HEART score pain descriptors. If these were completed then they would have been used for calculation of the risk score, but history was confirmed by patient interview. Whilst the GRACE score was in use in the department at the time of recruitment, this score was primarily used for those with established myocardial infarction and was infrequently completed for those

patients who had normal high sensitivity troponin levels. Therefore, all scores were calculated by the author using case notes and patient interview and determined at the time of recruitment, thereby reducing bias as outcome events would have not been undertaken at this time.

GRACE

The absolute GRACE Score was calculated for each patient using the online GRACE Score calculator found at the Uniform Resource Locator (URL): https://www.outcomes-umassmed.org/grace/acs_risk/acs_risk_content.html

HEART SCORE

The HEART Score was determined by using the online HEART Score calculator found at the URL: https://www.mdcalc.com/heart-score-major-cardiac-events

The history graded as ‘Highly suspicious’ if all features of pain were in the high-risk features, 'slightly suspicious' if no high-risk features were present and 'moderately suspicious' if features from both groups were present. If a patient only had 1 feature from the high-risk category and no features from the low-risk features of chest pain then the pain was graded as ‘moderately suspicious’. ECG changes were counted, repolarization abnormalities if they had T wave inversion.

TIMI score

TIMI Score for Unstable Angina / NSTEMI was calculated using the online software found at the URL: https://www.mdcalc.com/timi-risk-score-ua-nstemi

Charlson Comorbidity Index

The Charlson Comorbidity Index (CCI)(154) was calculated using the online Charlson Comorbidity Index calculator found at the Uniform Resource Locator (URL): https://www.mdcalc.com/charlson-comorbidity-index-cci#evidence

Table 7. Risk according to features of chest pain.

High risk features of chest pain	Low risk features of chest pain
Tight / heaviness / pressing/ crushing / squeezing pain Retrosternal discomfort Nausea and/or vomiting Perspiration Improved with nitrates Exacerbated by cold/ emotion / exercise	Well localized Sharp Reproducible with palpation Seconds in duration Worse on movement Positional Pleuritic Related to food

Table showing the features of chest pain categorised into high or low risk features, in keeping with HEART score typicality of chest pain.

Novel biomarker analysis

Novel biomarkers were analysed using the serum sample alloquots stored at -80 degrees centigrade. GDF-15 was measured in-house in the University of Liverpool's Clinical Sciences Laboratory. HFABP was analysed in the Countess of Chester Hospital Laboratory. The other novel biomarkers Galectin-3, HS-CRP, NTproBNP were measured by Abbott's Laboratories in Illinois, USA. Specialist courier services

were used to transport the samples by air on dry ice. Sample temperature was monitored throughout and were repeatedly topped up with dry ice.

Heart type fatty acid binding protein

HFABP was measured using the HFABP CE marked automated Immunoturbidimetric immunoassay from Randox (Randox Laboratories Ltd, Crumlin, UK). This was analysed using a AU5800 Beckman Coulter Analyser. The analytical range is between 3.49-120ng/ml. The coefficient of variation at the Level of Quantification (LoQ) of 3.19µg/l is 10.4%, with a Level of Blank (LoB) of 0.455µg/l and Level of Detection (LoD) of 0.765µg/l.(155) HFABP was analysed as per the manufacturer guidelines.

GDF-15

GDF-15 was measured via a quantitative sandwich enzyme immunoassay technique using the Quantikine® ELISA Human GDF-15 kit from R&D systems. The assay range was 23.4pg/ml– 1,500pg/mL. The coefficient of variation at 225pg/mL was 6%. Samples were defrosted to room temperature prior to analysis.

GDF-15 was analysed as per the manufacturer guidelines:

- 100µL of assay diluent was added to each well.
- 50µL of standard or sample was added to each well and incubated for 2 hours at room temperature on a horizontal orbital microplate shaker.
- Each well was then aspirated and washed four times using the wash buffer.
- 200µL of GDF-15 conjugate was added to each well and incubated at room temperature for a further 1 hour.
- Samples were again aspirated and washed.

- 200µL of substrate solution was added to each well and incubated for 30 minutes at room temperature protected from light.
- 50µL of stop solution was added to each well.
- The plate was then inserted into the Tecan GENios Plus microplate reader set at 450nm.

The average of duplicate readings for each standard, control and sample were calculated and the average zero standard optical density was subtracted. XFLUOR4 software was used to create a four-parameter logistic curve-fit.

Galectin-3

Galectin-3 was measured using the Abbott Architect Galaectin-3 chemiluminescent microparticle immunoassay and performed according to manufacturer guidelines at an Abbott Laboratory. This assay is designed to have an imprecision of ≤10% total coefficient of variation at concentrations ranging from 4.0 to 114.0ng/ml. The LoB was 1.0ng/mL and LoD was 1.1ng/ml.

High sensitivity C reactive protein

HS-CRP was measured using the Abbott CRP Vario latex immunoassay for use with ARCHITECT. The LoB 0.04mg/l, LoD 0.13mg/l and LoQ 0.30mg/l. The coefficient of variation is less than 20% at the level of quantitation.

N terminal brain natriuretic peptide

NT-proBNP was measured using the Abbott Architect Alere NT-proBNP chemiluminescent microparticle immunoassay and performed according to manufacturer guidelines at an Abbott laboratory. The measurable range is between 20

and 35,000pg/ml. The LoB 8pg/ml, LoD 20pg/ml and LoQ 48pg/ml. The coefficient of variation is 20% at the LoQ.

Troponin I Architect

High sensitivity Troponin I was measured using the Abbott Architect STAT High Sensitive Troponin I chemiluminescent microparticle immunoassay in accordance with manufacturers guidelines at an Abbott laboratory. The precision at the 99th percentile of 26.2pg/ml is 4.0%.

Primary study end points

The primary end point was MACE. MACE was defined as a composite of cardiovascular mortality, adjudicated non-fatal myocardial infarction and unstable angina requiring revascularization. Cardiovascular mortality and adjudicated non-fatal myocardial infarction are fairly hard endpoints that are relevant to the study question. Unstable angina requiring revascularisation is a softer endpoint, but this end point was again felt to be relevant to the study question. Secondary end points included repeat attendance to UHA with chest pain, any coronary revascularisation and all-cause mortality were also analysed.

A number of methods were used to identify patients who had experienced an end point. Electronic medical records (SIGMA) at University Hospital Aintree were searched specifically for further AED presentations, hospital admissions, and death; if present, notes were reviewed for presence of recorded symptoms, signs or diagnosis compatible with an endpoint. Mortality is logged on SIGMA for all in-hospital deaths and is updated via the NHS Spine. A 6-month gap between end of follow up period and identifying mortality was left to ensure time for data to be uploaded onto electronic

patient record. Biochemistry results at University Hospital Aintree were also reviewed to determine if further troponin levels had been measured.

The Patient Administration System (PAS) at the regional tertiary cardiology centre, Liverpool Heart and Chest Hospital (LHCH), where CABG and both elective and emergency angioplasty (including PPCI) is carried out. This identified patients who have had an encounter at this hospital. If present, LHCH electronic medical records were then reviewed. If patients were logged on the PAS, then coding information was also viewed on this system. The angiography and angioplasty reporting system (TOMCAT) at LHCH were reviewed to determine if a patient had been logged as having a procedure, primarily those undergoing angioplasty. The angiography imaging software (Xcelera) was also reviewed to see which patients may have undergone angiography or angioplasty at LHCH. Picture Archiving and Communication System (PACS) was used to identify patients who had undergone a chest x-ray, which is routine in chest pain, myocardial infarction and CABG and the reason sought. Clinical Radiology Imaging system (CRIS) was reviewed to see which patients may have been referred for a radiological procedure following admission, including chest x-rays and angiography. CRIS is linked between hospitals in the region, so activity in local hospitals including The Royal Liverpool Hospital, Whiston Hospital, Southport Hospital, Arrowe Park Hospital, Liverpool Heart and Chest Hospital, and University Hospital Aintree. For any patients who required chest x-ray the indication was reviewed for any admissions that might be compatible with chest pain or an end point. The Confidentiality Advisory Group provisionally supported the application for Hospital Episode Statistics/linked Office of National Statistics mortality data. This was dependent on a further application to the Research and Ethics Committee. The results of this were outside of the timeframe of this thesis.

Non-fatal myocardial infarction

Any patient who was found to have a subsequent troponin elevation or coronary revascularisation was considered to have potential for achieving the end point myocardial infarction. An adjudication pack was produced for all patients who had a troponin elevation providing details of the admission (age, gender, admission observations, blood results, ECGs, relevant excerpts of clinical history and past medical history, relevant imaging up to 6 weeks following admission, and discharge details). Adjudication was then performed by an independent cardiologist (AK/RD/TH) who were blinded to the novel biomarker results. Details of the universal definition were provided in the pack and they were asked if they felt this presentation was due to a Type 1 Myocardial Infarction, Type 2 Myocardial Infarction, acute myocardial injury, chronic myocardial injury or other. An option for requiring more information was given. Patients who presented as part of the PPCI pathway activation who went onto have emergency coronary angiography and revascularisation were considered as having an AMI. The admission details for any patient who had undergone revascularisation, or any patients transferred for revascularisation were reviewed to look for the potential of MACE.

Unstable angina and revascularization

The end point unstable angina and revascularization was defined as any patient, with myocardial infarction excluded or normal troponin levels, who required inpatient coronary revascularization, by means of percutaneous intervention or coronary artery bypass grafting, after presenting with symptoms felt to be cardiac in nature by the treating physicians. Patients who had 'early' or expedited outpatient revascularization

after presenting were recorded, but not considered a primary outcome event; as this was felt a softer endpoint.

Mortality

The cause of death was determined in one of two methods, review of hospital records and official death certification. Hospital records of all patients who had died were reviewed. For patients who were in hospital at the time of death and the cause of death was clear from diagnostic information and had been documented in the notes, this cause was used. If a patient was under the care of a palliative care team with a clear terminal condition (such as metastatic malignancy) in the 6 weeks prior to death, the cause of death was attributed to that condition. If no information was available from hospital notes, copies of the official death certificates were retrieved from the General Register Office. All-cause mortality was defined as any patient who had died within the follow up period of any cause. Cardiovascular mortality was defined as having a cause of death in section I(a) on the official death certificate due to coronary artery disease, myocardial infarction, and heart failure due to coronary heart disease.

Figure 6. Flow chart for diagnosis when troponin levels are elevated

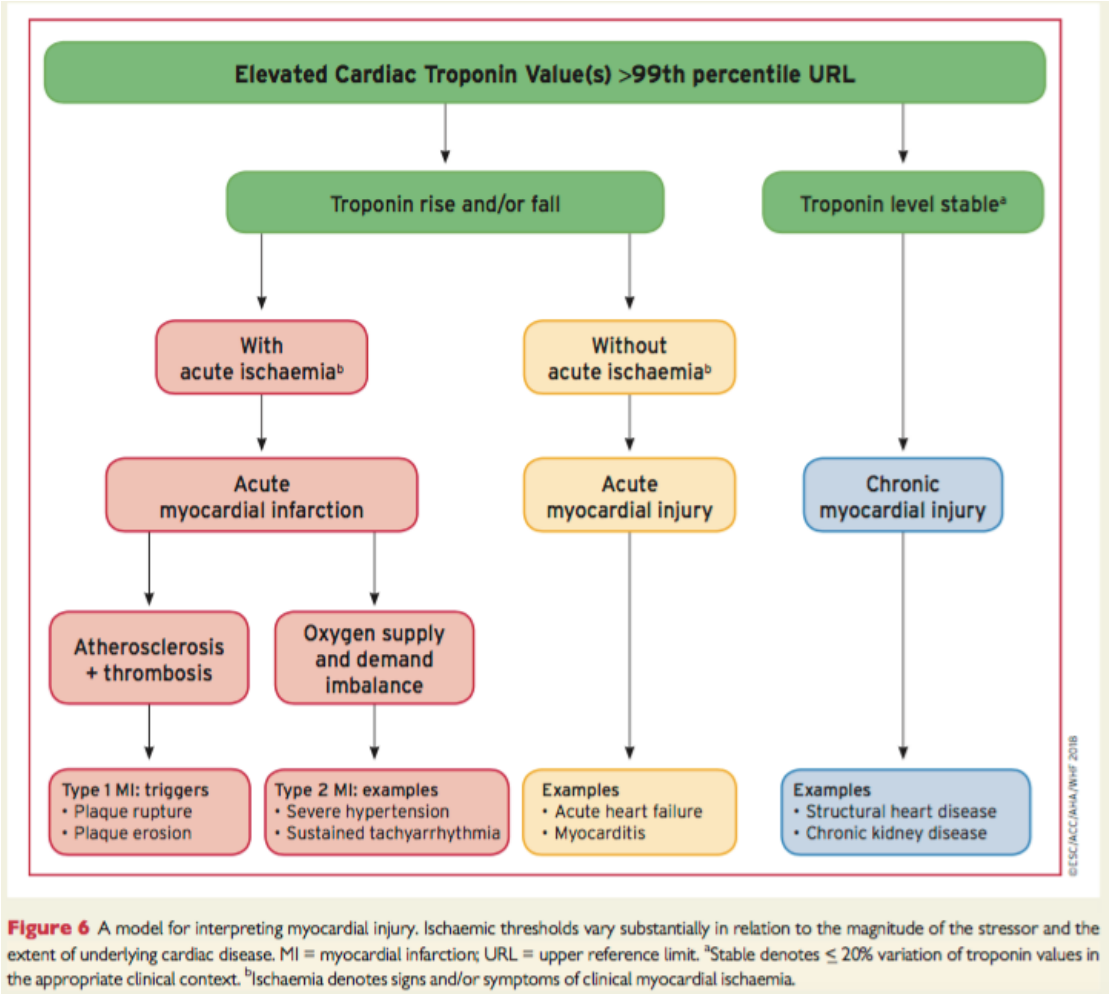


Figure 6 A model for interpreting myocardial injury. Ischaemic thresholds vary substantially in relation to the magnitude of the stressor and the extent of underlying cardiac disease. MI = myocardial infarction; URL = upper reference limit. [a]Stable denotes ≤ 20% variation of troponin values in the appropriate clinical context. [b]Ischaemia denotes signs and/or symptoms of clinical myocardial ischaemia.

Thygesen et al. Fourth universal definition of myocardial infarction (2018).

Representation with chest pain

A record was also kept of patients who had presented to UHA who had a further presentation to UHA with chest pain, regardless of the aetiology.

Sample size calculations

To fulfil the 'rule of thumb' of covariate analysis in logistic regression of 1:10 ratio between covariates and number of endpoints would mean the enrolment of approximately 500 acute chest pain admissions with a median follow-up of approximately 2 years (10 co-variates in the logistic regression analysis). This would

equate to an estimated event rate of death or nonfatal acute coronary system in 100 patients. Assuming that 15% of our sample will have at least one positive biomarker and using a significance level of 0.05, a sample of 500 patients will give us 84% power to detect a relative risk of 2.3 and 91% power to detect a relative risk of 2.5.

Timeframe of project

Patient recruitment took place between January 2011 and February 2013. Follow up took place in July 2018 and looked for events up until the end of 2017.

Time points analysed

A number of time points were assessed during analysis depending on the outcome being assessed. Rule out strategies were assessed using outcome at 8 weeks and 6 months; these time points were chosen to maximise events whilst trying to retain clinically relevant time frames. Logistic regression was performed using 3 year outcome data, as this reflected the prespecified study protocol. Cox-Proportional Hazards Regression was performed on long term data, as the length of follow up was greater than expected and the non-cardiovascular mortality was higher than anticipated, and this method takes into account censoring.

Statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics Version 27, R, Microsoft® Excel for MAC Version 16.49 and easyROC.(156) Statistical methods are described in detail in each results chapter. Descriptive statistics were performed using Microsoft® Excel for MAC Version 16.49 and IBM® SPSS® Statistics Version 27. Several methods of analysis were used to assess each of the biomarkers in isolation and in combination with other risk stratification methods, including the HEART, TIMI

and GRACE scores. ROC analysis using IBM® SPSS® Statistics Version 27 and easyROC(156) software which is based on R programming language was used to assess the overall model fit of risk scores and biomarker levels, and to establish the optimal cut-off value defined as a NPV of 99.5%. Sensitivity was calculated using easyROC(156) for several different strategies for predicting short-term MACE and the distribution of biomarkers between those who experienced MACE and those with event-free survival were reviewed. Univariable and multivariable logistic regression and Cox Proportional Hazards Regression was performed using IBM® SPSS® Statistics Version 27 to determine significant predictors of MACE. ROC analysis would be calculated to assess logistic regression models using IBM® SPSS® Statistics Version 27 to determine the C-statistic with confidence intervals for each model. The ROC curves produced by different models would be compared visually using the classification plots and C-statistics. The Net Reclassification Index (NRI) was computed using R for the addition of biomarkers to the risk scores HEART, TIMI and GRACE scores. Survival analysis was performed using Kaplain-Meier and Cox Proportional Hazards Regression analysis using IBM® SPSS® Statistics Version 27. ROC analysis would also be calculated to assess Cox regression models using IBM® SPSS® Statistics Version 27 to determine the C-statistic with confidence intervals for each model. The statistical analysis was performed by myself with no influence from industry that was involved in biomarker analysis.

Ethical considerations

Ethical approval was obtained after review by Greater Manchester East Research and Ethics Committee 8. See Appendix for Ethics submission documentation, patient information leaflets, consent forms and approval certificate. GCP was obtained by the

primary researcher (JJ) and Principal Investigator (AK). Informed consent was obtained for each participant. All serum was stored in aliquots with a unique identifier number for the study. Minor amendments were made to the Patient Information Leaflet (PIL) to shorten the document and make the consent process easier. University Hospital Aintree NHS Foundation Trust. The study was registered on ClinicalTrials.gov registration number NCT03628586.

Funding

Funding for this study was provided in house by University Hospital Aintree.

CHAPTER 3. DESCRIPTIVE STATISTICS OF THE INVESTIGATIVE COHORT.

Introduction

Approximately 700,000 people in England and Wales present to hospital with chest pain each year, but less than 15% of these have sustained a myocardial infarction.(13) There is emerging evidence that patients who have high sensitivity troponin levels below the 99th percentile are not automatically free from risk.(157,158) The aim of this chapter is to gain a greater understanding of the demographics and outcomes of the patients who present to hospital with suspected ACS and have MI excluded. The baseline characteristics of the patients recruited to the study, clinical features, medical history, management, and outcomes will be reported.

Methods

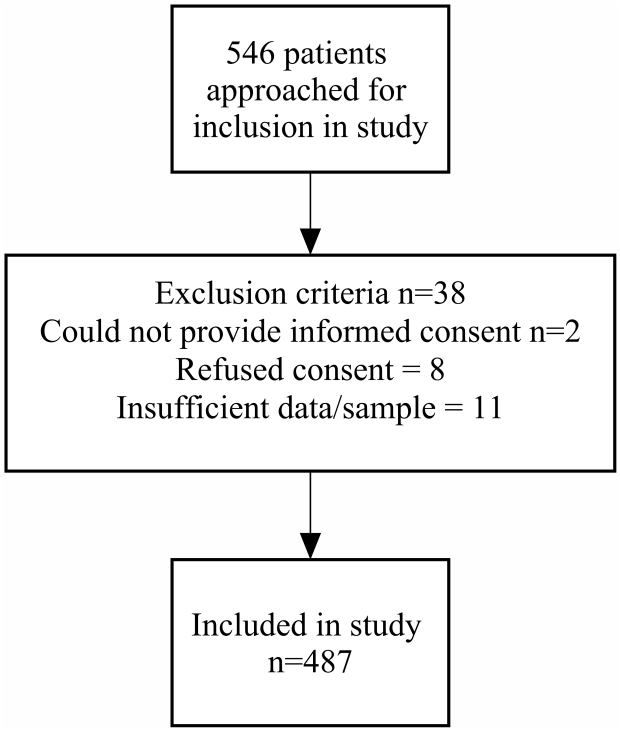
The methods of this study have been described fully in the General Methods Chapter. In brief, participants were consenting adult patients who presented with chest pain and had normal Hs-cTnT levels taken between 6-12 hours post chest pain. Patients were excluded if they refused consent, had chronic atrial fibrillation, recent ACS or non-cardiac pain apparent from the outset. Patient recruitment took place between January 2011 and February 2013. Demographic information, medical therapy, past medical history, and details of symptoms were determined at the time of consent from patient interview and medical notes. Chest pain character was recorded from patient notes and patient interview. The suspicion of chest pain according to the HEART score(159) was assessed, with pain that was strongly suspicious consisting of pain with two traditional typical features of ischaemia and the absence of any atypical features, moderate suspicion was defined as pain with both typical and atypical features, and slight suspicion when there was the absence of any typical features. Traditional typical features were defined as radiation to the jaw/left shoulder/arms, duration of 5-10 minutes, initiated by exercise/cold/emotion, perspiration, nausea or vomiting, or relief with nitrates. Traditional atypical features were defined as well-localised, sharp, reproducible with palpation or pleuritic. Combinations of features thought to be more strongly consistent with a diagnosis of unstable angina were defined as the presence of chest tightness (or synonyms chest pressure, heaviness, or restriction) and the number of additional typical features in the presence of no atypical features. The Charlson Comorbidity Index (CCI)(154) was calculated for each individual. ECGs were recorded by a single experienced reviewer (JJ) in keeping with the Minnesota Code Classification System for Electrocardiographic Findings. The results of any

other cardiac investigations were obtained from electronic medical records and logged in keeping with the final generated clinical report. The discharge diagnosis was recorded as specified in the clinical notes on discharge after relevant inpatient investigations had been completed. The primary outcome variable of interest was a MACE, defined as unstable angina requiring revascularisation, non-fatal type I myocardial infarction and cardiovascular mortality. Variables were analysed according to the entire cohort and the subgroups of those who experienced MACE. Statistical analysis in this chapter was performed using Microsoft Excel and IBM SPSS Statistics Version 25.

Results

487 patients were included in the analysis. Median duration from admission to time of follow up was 5.83 year, with a maximum duration of 6.97 years from recruitment date. 750 presentations were screened for inclusion. Hs-cTnT above the 99th percentile was the primary reason for non-recruitment. 546 patients were approached for consideration of inclusion. 38 patients had met the exclusion criteria of atrial fibrillation or recent ACS; 2 patients could not provide consent as 1 had significant learning difficulties and 1 did not speak English; 8 patients refused participation after being approached and 11 patients had insufficient serum samples. See Figure 7 for flow chart of recruitment. There was complete data for the variables assessed in this chapter.

Figure 7. Flow chart for recruitment



Flow chart showing total number of patients approached for inclusion in study and total number showing each outcome and final number of patients included in study.

Baseline characteristics

Baseline characteristics are reported in Table 8. The median age was 56 (IQR 49-66). 56% of patients were male. 98.4% patients were Caucasian, 1.85% Asian and 0.21% Black. All patients were haemodynamically stable, and there were no patients who needed inotropic/mechanical cardiovascular support or a referral to Critical Care. 191 (39.2%) patients self-presented to the AED, 128 (26.3%) patients were admitted via a General Practitioner or Walk-In Centre, 153 (31.6%) patients were brought in by ambulance after calling 999, and 15 (3.1%) patients were admitted from the outpatient clinic.

Chest pain features

The characteristics of chest pain were assessed according to a number of single descriptors, typicality of pain in keeping with the HEART score and, NICE definition of angina, as shown in Table 9. This shows that most patients have chest tightness as a descriptive feature, with nearly a fifth who gain relief with Glycerine Trinitrate (GTN). Just over a third have the presence an atypical feature of chest pain. Most patients do not fulfil the NICE criteria of angina. Yet, 94% of patients do have a moderate or high suspicion history of ischaemic pain according to the HEART score.

Cardiovascular history and risk factors

There were high rates of cardiovascular risk factors (Table 8) with nearly two thirds (64.7%) of patients with hypertension or hypercholesterolaemia. There were also high rates of smoking, with 31.2% of patients being a current smoker. Just under a quarter (22.2%) had a first-degree family member with a history of ischaemic heart disease. 16.2% were diabetic. There were 27.5% of patients who reported a past medical

history of angina and 20.9% had experienced a previous myocardial infarction. 13.8% had undergone percutaneous revascularisation and 5.1% had Coronary Artery Bypass Grafting (CABG).

Medical history

The most prevalent condition in the non-coronary past medical history was COPD (19%). COPD was strongly associated with MACE at 12 months (see Table 8). The comorbidity of the cohort was assessed using the Charlson Comorbidity Index (CCI).(154) The frequency of the total score can be seen in Figure 5. Most patients had a score between 0-3.

Medical therapy

Nearly half (46%) of patients were taking a statin before admission, reflecting the high rates of risk factors (see Table 8). Just over a third (35%) took aspirin, and just under a third (33%) were taking an ACE inhibitor. Only 12% were taking a thienopyridine. Those who experienced MACE were more likely to be on pre-admission cardiovascular medication.

Table 8. Baseline characteristics of patients recruited and according to outcome

	Total 487	12-month MACE 22	Long-term MACE 48
Demographics			
Age	56 [11.8]	61 [12.1]	61 [12.2]
Male	273 (56)	14 (63)	31 (64.6)
Risk factors			
Smoker	152 (31.2)	8 (36.3)	16 (30)
Diabetes	79 (16.2)	9 (40.9)	16 (30)
HTN	232 (47.6)	14 (63.6)	33 (68.8)
Hchol	239 (49.1)	16 (72.7)	30 (62.5)
FHx	108 (22.2)	6 (27.3)	15 (32.3)
CV history			
Angina	134 (27.5)	13 (59.1)	32 (60.0)
MI	102 (20.9)	6 (27.3)	21 (43.8)
PCI	67 (13.8)	6 (27.3)	19 (39.6)
CABG	25 (5.1)	0	2 (4.2)
PMH			
COPD	93 (19.1)	7 (31.8)	13 (27.1)
PVD	11 (2.3)	1 (4.5)	2 (4.2)
Baseline meds			
Aspirin	172 (35.3)	17 (77.3)	33 (68.8)
Clopidogrel	58 (11.9)	3 (13.6)	11 (22.2)
Betablocker	116 (23.8)	10 (45.5)	22 (45.8)
ACE	161 (33.1)	14 (63.6)	30 (62.5)
Statin	222 (45.6)	14 (63.6)	34 (70.8)

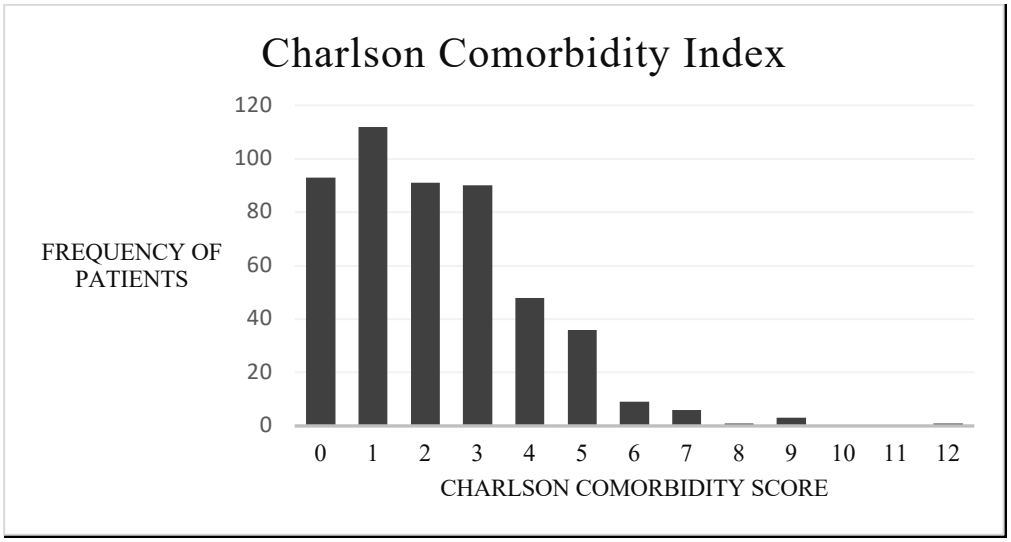
Age is presented as the median with STD in squared parenthesis. Binary variables are displayed as absolute number with percentage of group in rounded parenthesis. HTN hypertension, Hchol Hypercholesterolaemia, FHx family history, MI Myocardial infarction, PCI percutaneous Intervention, CABG coronary artery bypass grafting, PMH past medical history, COPD Chronic Obstructive Pulmonary Disease, PVD Peripheral Vascular Disease, ACE Angiotensin 2 receptor blocker.

Table 9. Chest pain features in cohort and according to MACE

	Total 487	12-month MACE	Long-term MACE
Single descriptors of chest pain			
Atypical features	178 (36.6%)	4 (2.2%)	11 (6.2%)
Stress induced	49 (10.1%)	4 (8.2%)	6 (12.2%)
Relief with GTN	86 (17.7%)	5 (5.8%)	14 (16.3%)
Chest tightness	286 (58.7%)	12 (4.2%)	31 (10.8%)
NICE definitions of angina			
Non-anginal	378 (77.6%)	12 (3.2%)	27 (7.1%)
Atypical angina	80 (16.4%)	4 (5%)	13 (16.3%)
Typical angina	29 (6.0%)	6 (20.7%)	8 (27.6%)
HEART score suspicion of ischaemic chest pain			
Slight suspicion	30 (6.2%)	1 (3.3%)	3 (10%)
Moderate suspicion	215 (44.2%)	5 (2.3%)	12 (5.6%)
High suspicion	242 (49.7%)	16 (6.6%)	33 (13.6%)

Table showing absolute number of patients with each chest pain category and number of patients who experienced MACE at 12-months and during long term follow up. Stress induced = physical or emotional stress.

Figure 8. Bar chart showing frequency of Charlson Comorbidity Score



Frequency of patients according to Charlson Comorbidity Score.

Investigations

279 (57.3%) patients did not have any investigations beyond a standard 12 lead ECG, routine haematology and biochemistry, echocardiography, and a chest radiograph. 178 (36.6%) patients went on to have an investigation for a coronary cause. See Appendix for flow charts of coronary investigation.

Troponin levels

The median index Hs-cTnT level was 4µg/l (IQR 3–7 µg/l). 277 (56.9%) patients had a second Hs-cTnT level requested during the index admission by the clinical team, the median level of this second Hs-cTnT sample was 4µg/l (IQR 2-8). The median delta between the first and second troponin level was 11µg/l (IQR 0-33), and the median duration between these samples was 6.3 hours (IQR 5.1–9.6). Twelve (4.3%) of the 277 patients who had a repeat Hs-cTnT sample had an elevation of Hs-cTnT beyond the 99th percentile. Six (50%) of these patients with an elevated Hs-cTnT on the second sample were adjudicated to have experienced a type I myocardial infarction by a reviewer (RD) blinded to the non-necrosis biomarker results.

ECG

The majority of patients (70.8%) had an ECG that was within normal limits. Most patients were in sinus rhythm, with a small proportion (3.6%) having new-onset atrial fibrillation. T wave inversion occurred in 98 (20.1%) patients, which was deep inversion 2mm or greater in 8 (1.6%) patients. 28 (5.7%) patients had ST segment depression, twenty (4.1%) had a QRS duration of over 120ms and 41 (8.4%) patients had abnormal Q waves.

Table 10. ECG changes in cohort and according to MACE

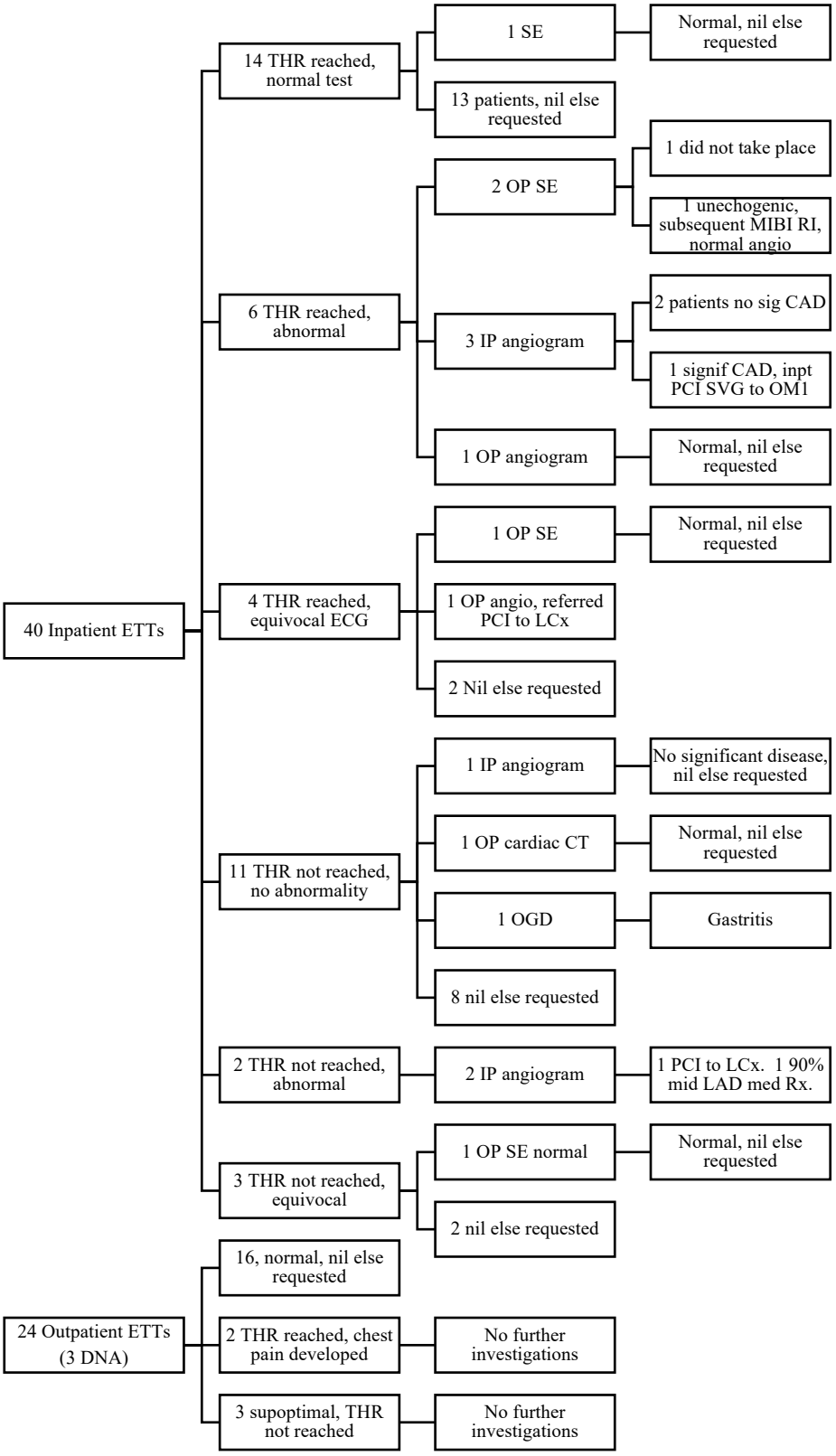
	Total 487 N (%)	12-month MACE 22 N (%)	Long-term MACE 48 N (%)
ECG			
New atrial fibrillation	18 (3.6%)	1 (5.5%)	2 (11.1%)
T wave inversion (any)	98 (20.1%)	7 (7.1%)	13 (13.3%)
T wave >2mm	8 (1.6%)	1 (12.5%)	2 (25%)
ST depression	28 (5.7%)	4 (18.0%)	8 (16.2%)
BBB	20 (4.1%)	1 (5%)	4 (20%)
Q waves	41 (8.4%)	2 (4.9%)	8 (19.5%)

Table showing number of patients with associated ECG changes according to the entire cohort and those who experienced MACE at differing time points. Values are absolute number of patients in each category with percentage of cohort in rounded parenthesis. BBB Bundle Branch Block.

Exercise tolerance tests

Exercise tolerance tests occurred as a first-line investigation in 63 (12.9%) patients, 39 (8.0%) of these as an inpatient investigation and 24 (4.9%) as an outpatient (see Figure 9 and Table 12). One exercise tolerance test was performed as a second-line investigation as an inpatient. There were three (0.6%) patients who had an ETT requested, but they did not attend for this. For those who exercised, the intensity varied between 3.3 and 15.3 METS. Fifteen (3.1%) patients went on to have further investigation; Six (1.2%) had an inpatient coronary angiogram, two (0.4%) had outpatient coronary angiograms, five (1.0%) outpatient stress echocardiogram, one (0.2%) outpatient CT and one (0.2%) OGD. Figure 10 illustrates these investigations and results. PCI was undertaken in two (0.4%) patients because of the ETT and subsequent investigations.

Figure 9. Diagram of outcome of ETTs.

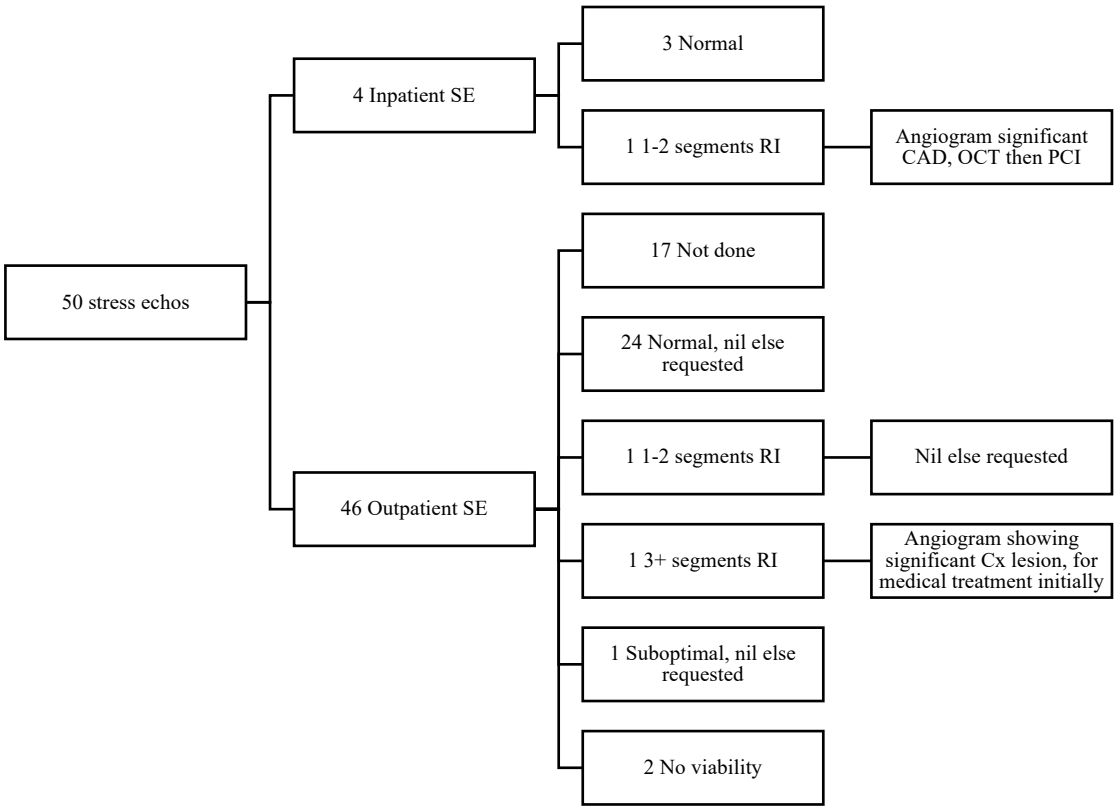


THR Target Heart Rate, SE stress Echo, RI Reversible Ischaemia, CAD Coronary artery disease, MIBI Myocardial Perfusion Scan, CT Computed Tomography, PCI Percutaneous Intervention, OGD Oesephagogastroduodenoscopy, med Rx medical treatment, IP Inpatient, LAD Left Anterior Descending Coronary Artery.

Stress echocardiography

Stress echocardiography was performed in 50 (10.3%) patients as a first-line investigation, four as an inpatient and 46 as an outpatient (See Figure 10 and Table 12). Twelve (2.5%) patients had an outpatient stress echo as an outpatient as a second-line investigation. 2 (0.4%) patients had an outpatient stress echo as a third-line investigation. The outpatient stress echocardiogram did not occur in 37% of the patients who had this requested, for reasons unclear.

Figure 10. Diagram of outcome of stress echocardiograms.

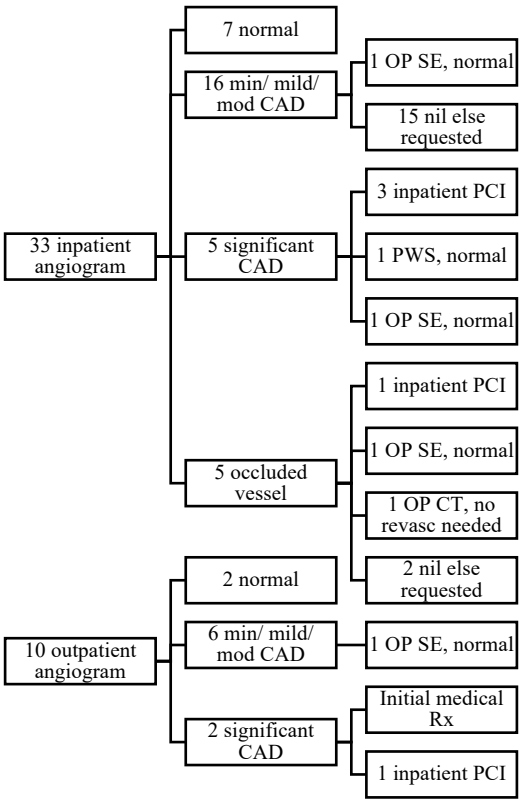


SE stress echo, RI Reversible ischaemia, CAD Coronary artery disease, OCT optical coherence tomography, PCI Percutaneous Intervention, Cx Circumflex.

Cardiac catheterisation

There were 43 (8.8%) patients who underwent cardiac catheterisation as a first-line investigation, 33 (6.8%) as an inpatient and 10 (2.1%) as an outpatient (see Figure 11 and Table 12). In total, 63 patients underwent invasive cardiac catheterisation, 49 (10.1%) as an inpatient and 14 (2.9%) as an outpatient. There were 10 (2.1%) inpatients who underwent cardiac catheterisation as a second-line investigation; 6 (1.2%) having had an ETT, 1 (0.2%) inpatient stress echo, 1 (0.2%) CT/VQ scan and 2 (0.4%) relying on cardiac investigations (stress echo) that were completed just prior to admission.

Figure 11. Diagram of outcome of cardiac catheterisations.



CAD Coronary Artery Disease. SE Stress echo. OP outpatient. PCI percutaneous Coronary Intervention. Coronary artery stenosis grading: Minimum 1-24%, Mild defined as 25-49%, moderate 50-69%, severe 70-99% or left main stem >50% or 3-vessel disease ≥70%. PWS pressure wire study.

Non-cardiovascular investigations

There were twelve oesophago-gastro-duodenoscopies (OGDs) requested (see Table 12); two of these were during the inpatient index admission. The two inpatient OGDs demonstrated abnormalities, with one having gastritis and one with gastric antral vascular ectasia. There were ten outpatient OGDs requested; this demonstrated gastritis in five patients, two cases of hiatus hernia, one ulcer, one was normal, and one patient did not attend the appointment. There were eight patients who went on to have a CTPA or VQ scan; two of these reported a pulmonary embolism. One patient who had an OGD went on to experience MACE at 127 days; this was an outpatient OGD which was reported as gastritis.

Length of stay

During the index admission, the length of stay ranged up to 23 days, with a mean and median duration of two days (48.2 hours). 395 (81.1%) patients were admitted to hospital from AED. 95 (19.5%) patients were discharged directly from AED. Most patients were discharged to their home address, but nine (1.8%) were transferred directly to the local tertiary centre as part of the ACS Percutaneous Intervention Pathway.

Follow-up appointments

131 (26.9%) patients did not have any further follow-up appointments arranged. 47 (9.7%) patients had a follow-up appointment booked in a general medical clinic. 212 (43.5%) patients had a cardiology outpatient appointment organised.

Discharge diagnosis

A staggering 40% of patients were either labelled as 'atypical chest pain' or the cause of chest pain was not specified (see Table 11). There were 78 patients (15.92%) who were thought to have a primary discharge diagnosis of ischaemic cardiac chest pain. Other cardiovascular diagnosis included 10 (2.1%) with pericarditis and 21 (4.3%) with arrhythmia-related chest pain. The most common non-cardiovascular diagnoses were muscular, gastrointestinal, or due to a lower respiratory tract infection.

Table 11. Discharge diagnosis and associated outcome

Primary diagnosis	Total cohort	12-month MACE Total 22	Long term MACE Total 48
Atypical/ not specified	196 (40.3%)	6 (3.1%)	14 (7.1%)
Ischaemic	78 (16.0%)	12 (15.4%)	19 (24.4%)
Pericarditis	10 (2.1%)	0	0
Arrhythmia	21 (4.3%)	1 (4.8%)	4 (19.0%)
Other cardiac	1 (0.2%)	0	0
Muscular	80 (16.4%)	2 (2.5%)	7 (8.75%)
Pulmonary emboli	2 (0.4%)	0	0
Gastrointestinal	58 (11.9%)	0	2 (3.4%)
LRTI	28 (5.7%)	1 (3.6%)	2 (7.2%)
Pleurisy	4 (0.8%)	0	0
Anxiety	4 (0.8%)	0	0
Asthma	3 (0.6%)	0	0
NIECOPD	2 (0.4%)	0	0
Anaemia	1 (0.2%)	0	0
Cancer	1 (0.2%)	0	0

Table showing number of patients with associated with discharge diagnosis according to the entire cohort and those who experienced MACE at differing time points. Values are absolute number of patients in each category with percentage of cohort in rounded parenthesis. LRTI Lower Respiratory Tract Infection, NIECOPD Non-Infective Exacerbation of Chronic Obstructive Pulmonary Disease.

Table 12. Investigations requested across the cohort

Type of investigation	First line Investigation	Second line Investigation	Overall
Inpatient ETT	39 (8.0%)	1 (0.2%)	40 (8.2%)
Outpatient ETT	24 (4.9%)	0	24 (4.9%)
Inpatient angiogram	33 (6.8%)	10 (2.0%)	43 (8.8%)
Outpatient angiogram	11 (2.2%)	6 (1.2%)	17 (3.5%)
Inpatient SE	4 (0.8%)	0	4 (0.8%)
Outpatient SE	46 (9.4%)	12 (2.5%)	59 (12.1%)
Inpatient CT	2 (0.2%)	0	2 (0.4%)
OP CT	10 (2.1%)	2 (0.4%)	12 (2.5%)
CTPA / VQ	9 (1.8%)	1 (0.2%)	10 (2.0%)
MIBI	5 (1.0%)	1 (0.2%)	8 (1.6%)
OGD/pH studies	10 (2.1%)	1 (0.2%)	12 (2.5%)
Cath LHCH	4 (0.8%)	0	4 (0.8%)
Pressure wire	0	2 (0.4%)	2 (0.4%)
Other imaging	4 (0.8%)	1 (0.2%)	5 (1.0%)
Previous test	14 (2.9%)	-	14 (2.9%)

Table showing number of patients according to investigation performed across the entire cohort and those who experienced MACE at differing time points. Values are absolute number of patients in each category with percentage of cohort in rounded parenthesis. ETT Exercise Tolerance Test. SE Stress Echo. CT computed Tomography. OP Outpatient. CTPA CT Pulmonary Angiogram. VQ Ventilation Perfusion scan. OGD Oesophagogastroduodenoscopy. LHCH Liverpool Heart and Chest Hospital.

Ischaemic diagnosis

16% of patients were diagnosed with ischaemic pain on discharge. 63 (12.9%) of these patients had a history that was highly suspicious of cardiac pain in keeping with the HEART score definition; fourteen patients (2.9%) had moderately suspicious pain, and 1 (0.2%) had mildly suspicious pain. Twenty-one patients had no functional or cardiac radiological investigations. Seven patients had an inpatient ETT, and three had an outpatient ETT. Sixteen (3.3%) patients had an inpatient coronary angiogram, and

eight (1.6%) had an outpatient coronary angiogram. One (0.2%) patient had an inpatient stress echocardiogram, and ten (2.1%) patients had an outpatient stress echocardiogram. One (0.2%) patient had an OGD. Seven patients had a recent cardiac investigation performed.

Revascularisation

Thirteen patients (2.67%) had revascularisation because of the admission. Three (0.6%) inpatient PCI, six (1.2%) outpatient PCI, three (0.6%) inpatient CABG and one (0.2%) outpatient CABG.

MACE

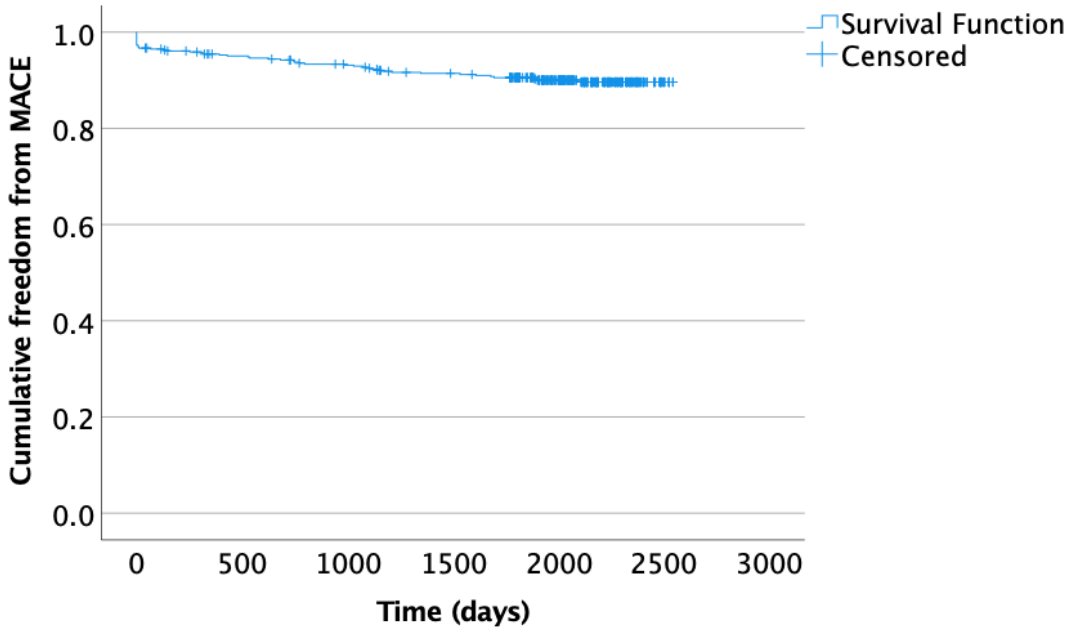
Event rates for different outcomes and time points are reported in Table 13. There were thirteen (2.67%) patients who had a MACE during their index admission; six (1.23%) of these were due to myocardial infarction, and seven (1.4%) of these due to inpatient revascularisation. The 8-week and 12-month MACE rate was 3.29% and 4.52%, respectively. Adjudicated MI was diagnosed at 1.4% and 2.46% at 8-weeks and 1-year, respectively. By contrast, 7.4% and 21.1% of the cohort represented with chest pain to the ER at 8-weeks and 12-months, respectively. Two (0.41%) patients had all-cause mortality at 8-weeks and 11 (2.26%) patients by 12-months. The event rate from cardiovascular death was exceptionally low, with no patients experiencing a cardiovascular death within four years of follow-up. Figure 12 shows a KM curve demonstrating MACE over time with non-cardiovascular mortality censored.

Table 13. Table showing outcome at varying time points

	Inpt	30-day	8-week	6-mo	12-mo	24-mo	36-mo	48-mo	Long-term
Mortality	0	0	2 (0.41)	5 (1.03)	11 (2.26)	14 (2.87)	18 (3.70)	26 (5.34)	42 (8.62)
UA/ revasc	7 (1.44)	9 (1.85)	9 (1.85)	11 (2.26)	11 (2.26)	14 (2.87)	14 (2.87)	14 (2.87)	16 (3.29)
MI	6 (1.23)	7 (1.44)	7 (1.44)	9 (1.85)	12 (2.46)	15 (3.08)	23 (4.72)	29 (5.95)	29 (5.95)
CV death	0	0	0	0	0	0	0	0	4 (0.82)
MACE	13 (2.67)	16 (3.29)	16 (3.29)	19 (3.90)	22 (4.52)	28 (5.75)	36 (7.39)	41 (8.42)	48 (9.86)
Any revasc	7 (1.44)	15 (3.08)	16 (3.29)	25 (5.13)	30 (6.16)	34 (6.98)	37 (7.60)	40 (8.21)	44 (9.03)
Chest pain	NA	27 (5.54)	36 (7.39)	69 (14.17)	103 (21.15)	133 (27.31)	155 (31.83)	189 (38.81)	210 (43.12)

Table showing outcome at different time points according to type of adverse outcome. Data is expressed in total number experiencing the outcome with percentage of total cohort in parentheses.

Figure 12. Graph showing MACE over time



KM curve showing cumulative freedom from MACE over time with non-cardiovascular mortality censored.

Discussion

There were extremely high rates of hospital admission, with only 19.5% of patients being discharged directly from AED. The average hospital stay for patients was 48 hours. Considering these patients have an excellent prognosis, there is clear room for improvement in expediting discharge. Despite the lengthy hospital stay, 40% of patients were not given a clear underlying cause for their chest pain, with no specified cause or 'atypical chest pain' reported. In patients with atypical chest pain, a fair proportion (7%) go on to have long term MACE. Only 16% of patients were given a discharge diagnosis of ischaemic pain. The most common non-cardiac cause of chest pain was muscular. Some features, such as pre-admission treatment with aspirin or ST depression, were much more likely to be present in those who experienced MACE. Still, these on their own did not provide enough information to categorise patients into risk groups. The testing yield of patients is low. Of the 50 stress echocardiograms performed, there was one subsequent revascularisation. Out of the 43 diagnostic coronary angiograms performed, there was 5 (11.6%) subsequent revascularisations. 42 out of 64 (65.6%) patients who had an ETT reached their target heart rate, 13 out of 64 (20.3%) patients went onto have a further cardiovascular investigation. Overall, three patients (4.7%) who had an ETT subsequently underwent coronary revascularisation. Despite the low pick-up rate of cardiovascular disease from testing, patients still go on to experience MACE.

This cohort justified the label of suspected ACS based on chest pain characteristics suspicious of ischaemia. There are high rates of cardiovascular risk factors in this

cohort, with 84.8% having at least one traditional modifiable cardiovascular risk factor and 120 patients (24.6%) having had a previous MI or coronary revascularisation.

Although these patients had an initial troponin below the 99th percentile, there is a small but significant risk of MI or UA requiring urgent coronary revascularisation by 12-months.

Whilst cardiovascular death rates are very low even with long-term follow up, 1.2% of patients had an AMI in their index admission, and 3.3% of patients experienced MACE by 8-weeks. The MACE event rates justify attempts at further risk stratification. The most striking finding was significant representation to the same department with further chest pain, with 21.2% and 38.8% of the cohort re-presenting at one and four years respectively, despite MACE occurring in 4.52% and 8.42% at these time points.

In subsequent chapters, multi-variate predictors of MACE and whether any additional biomarker provided incremental risk stratification beyond traditional risk scores such as HEART, GRACE and TIMI scores or high sensitivity troponins will be reviewed.

These cardiovascular past medical history was considerably higher than some other studies, a large multicentre Scottish study of over 32,000 consecutive patients with normal high sensitivity troponin levels had roughly half the cardiovascular risk factors and previous events (MI in 8%, DM in 6% and PCI in 7%).(160) The risk factor profile in this study was more similar to other studies performed in the North West of England.(92)

Conclusion

Patients who present with chest pain and have Hs-cTnT levels below the 99th percentile should not automatically be regarded as low risk. Likewise, most patients do not experience MACE. There are high rates of cardiovascular risk factors and previous cardiovascular events in this population, making it more challenging to differentiate. The next chapter will investigate the risk stratification using the established risk scores HEART, TIMI and GRACE.

CHAPTER 4. RISK STRATIFICATION USING ESTABLISHED RISK SCORES.

Background

It is evident from the previous results chapter that patients who present with suspected ACS who have Hs-cTnT levels below the 99th percentile have a good prognosis. Still, they are not risk-free, with approximately 5% MACE at 12-months. The ability to accurately risk-stratify could safely expedite discharge in most patients and identify those who need further investigation and treatment, considerably improving resource utilisation and reducing future MACE.

The HEART, TIMI and GRACE scores are popular validated tools used to risk-stratify patients with ACS. Both NICE and ESC guidelines have specifically endorsed the use of the GRACE score. Previous data from our centre suggests that the HEART score performs better for early discharge of patients, analysis that was done using pre-specified risk score cut-off ranges in patients with UA and NSTEMI.(161)

This chapter aims to evaluate the established risk scores GRACE, TIMI and HEART in patients who present with suspected ACS and have Hs-cTnT levels below the 99th percentile.

Methods

The HEART, TIMI and GRACE scores were calculated by a single investigator (JJ) as described in the General Methods Chapter. Risk scores were assessed according to recognised low, medium and high-risk categories. HEART score categories were defined as low-risk for a score between 0-3, medium-risk for 4-6 and high-risk for 7-8.(72) TIMI score categories were defined as low-risk for a score between 0-1, medium-risk for 2-3 and high-risk for 4-7.(162) GRACE score categories were initially defined as low-risk for a score between 0-88, medium-risk for 89-118 and high-risk for 119-263,(163) but this resulted in a very low number of patients and no MACE in the 'high-risk' group. Alternative GRACE groupings were produced by subdividing the cohort into three groups of equal size, which resulted in low-risk being defined as a score between 0-61, medium-risk for 62-80 and high-risk for above 80.

Kaplan-Meier (KM) survival analysis was performed for the HEART, TIMI and GRACE risk scores. An event was defined as a MACE which included unstable angina requiring revascularisation, myocardial infarction, and cardiovascular death. Right-censoring occurred due to non-cardiovascular mortality and the end of follow-up. For the fulfilment of KM assumptions, groups were reviewed to ensure a similar percentage of censored cases. KM curves were produced using cumulative survival curves. Statistically significant differences in MACE between groups were analysed using the Mantel-Cox log-rank test. MACE was also calculated by raw risk score for HEART and TIMI score and in small groups of 10 units for GRACE score. Particular interest in the 8-week follow-up for MACE was made, it is acknowledged that 30-day MACE is a popular time frame for assessing outcome in rule out strategies, however

this study was not powered to look at MACE at this early time point, therefore to reduce the chance of type II error, an event rate of 8-weeks was chosen. Additionally, it was felt that freedom from MACE within this period was likely to represent the safety of early discharge from hospital with potential for early outpatient investigation. Receiver Operator Characteristic (ROC) curves were produced along with calculating the area under the curve, 95% confidence interval and level of significance of each risk score.

Results

The GRACE, HEART and TIMI score was calculated for all participants. The median duration of follow-up, as previous specified, was 5.83 years (range 4.84 – 6.97 years).

Outcomes stratified by risk score

The HEART, TIMI and GRACE risk scores were assessed according to the raw score and MACE at 8-weeks, 6-months and 12-months (See Table 14, Table 15 and Table 16). The lowest HEART score that included a patient who experienced a 6-month MACE was 3. A HEART score of ≤3 represented 179 patients (36.8% of the cohort), and one patient (0.56%) experienced MACE at six months. A HEART score of ≤4 characterised 300 patients (61.6% of the cohort), and five patients (1.67%) experienced MACE at 6-months. There were patients with a TIMI score of 0 who went onto have a MACE at 8-weeks; 174 patients (35.7% of the cohort) had a TIMI score of 0, and 3 of these patients (1.72%) experienced a MACE by 6-months. The lowest GRACE score in which a patient experienced an 8-week MACE was 42. A score of <43 would represent 39 patients (8.0% of the cohort), with one patient (2.6%) experiencing a MACE at 6-months. This breakdown of scores and MACE suggest that the HEART score has the greatest ability to discriminate those who are at low risk of future events.

Table 14. HEART score and MACE

HEART score	Total 487	MACE 6-months	MACE 12-month	MACE long term
0	5	0	0	0
1	22	0	0	1
2	45	0	0	0
3	107	1 (0.93)	1 (0.93)	5 (4.67)
4	121	4 (3.31)	5 (4.13)	9 (7.44)
5	106	4 (3.77)	5 (4.72)	12 (11.32)
6	61	7 (11.48)	8 (13.11)	15 (24.59)
7	18	3 (16.67)	3 (16.67)	6 (33.33)
8	2	0	0	0

Table 15. TIMI score and MACE

TIMI score	Total 487	MACE 6-months	MACE 12-month	MACE long term
0	174	3 (1.72)	3 (1.72)	8 (4.60)
1	126	2 (1.59)	2 (1.59)	4 (3.17)
2	93	2 (2.15)	4 (4.30)	11 (11.83)
3	58	5 (8.62)	5 (8.62)	13 (22.41)
4	32	5 (15.63)	6 (18.75)	9 (28.13)
5	3	1 (33.33)	1 (33.33)	2 (66.66)
6	1	1 (100.00)	1 (100.00)	1 (100.00)

Table 16. GRACE score and MACE

GRACE score	Total 487	MACE 6-months	MACE 12-month	MACE long term
<30	3	0	0	0
30-39	21	0	0	1 (4.76)
40-49	46	2 (4.35)	2 (4.35)	3 (6.52)
50-59	81	2 (2.47)	2 (2.47)	6 (7.41)
60-69	84	4 (4.76)	4 (4.76)	8 (9.52)
70-79	72	1 (1.39)	1 (1.39)	5 (6.94)
80-89	63	3 (4.76)	4 (6.35)	7 (11.11)
90-99	45	2 (4.44)	3 (6.67)	9 (20.00)
100-109	36	3 (8.33)	3 (8.33)	5 (13.89)
110-119	18	1 (5.56)	1 (5.56)	1 (5.56)
120-129	7	0	0	1 (14.29)
130-139	4	1 (25.00)	1 (25.00)	1 (25.00)
140-149	3	0	1 (33.33)	1 (33.33)
>150	2	0	0	0

Tables showing risk score, number of patients and number experiencing MACE according to risk score, percentage experiencing MACE for given risk score or category in rounded parenthesis.

Receiver operator curve analysis

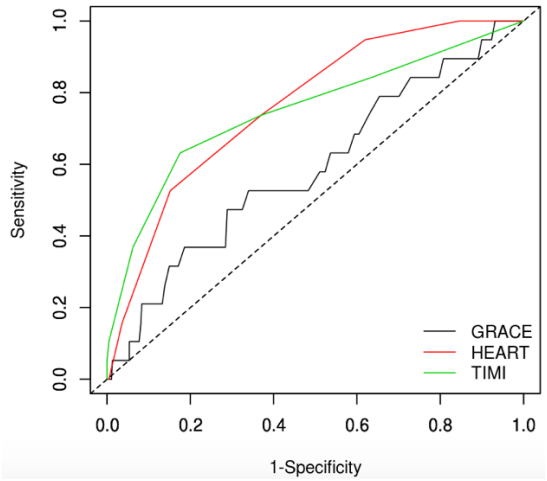
ROC curve analysis was performed on each risk score for MACE at different durations of follow-up (see Table 17 and Figure 13 to Figure 15). The HEART and TIMI score generally performed well in predicting MACE over differing time points of follow-up. The GRACE score performed poorly, with a lack of statistical significance for short-term MACE at 8-weeks to 12 months. Longer-term MACE led to some improvement in discriminatory ability with statistical significance, but the overall C-statistic was still lacking. The visual appearance of the ROC curves can be seen in Figure 13 to Figure 15, with HEART and TIMI score having a favourable trace and GRACE score being close to the reference line. The HEART score has a more favourable trace as a 'rule out' test, having a curve that adheres more closely to the maximum sensitivity at the upper right-hand corner of the graph.

Table 17. C-statistics for risk scores at different times points

	MACE 8 weeks	MACE 6-month	MACE 12-month	MACE long term
HEART	0.756 (0.650-0.862) P<0.001	0.765 (0.667-0.862) P<0.001	0.759 (0.669-0.849) P<0.001	0.725 (0.652-0.798) P<0.001
TIMI	0.742 (0.593-0.891) P=0.001	0.749 (0.618-0.881) p<0.001	0.759 (0.643-0.875) P<0.001	0.733 (0.652-0.814) P<0.001
GRACE	0.592 (0.445-0.740) P=0.209	0.589 (0.455-0.724) P=0.192	0.625 (0.502-0.749) P=0.051	0.598 (0.513-0.683) P=0.02

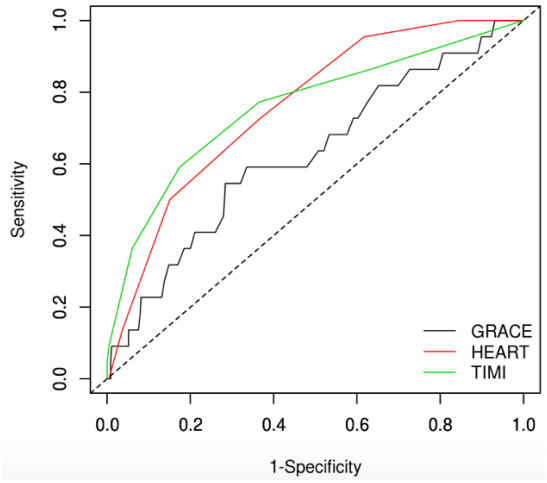
Table showing C-Statistic, 95% confidence intervals and significance level for each risk score at different time points of follow up.

Figure 13. ROC curves of risk scores MACE at 6-months



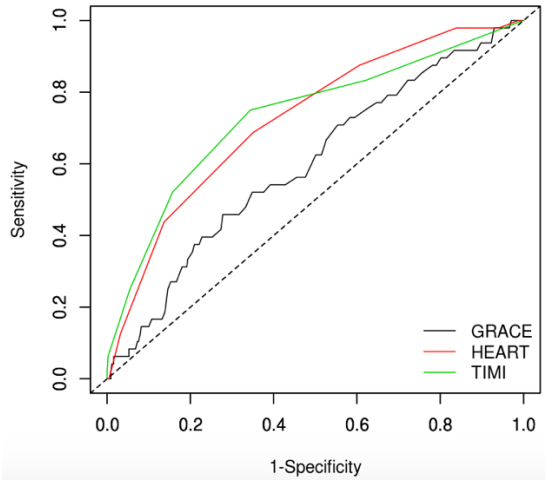
ROC curves showing each risk score with MACE at 6 months.

Figure 14. ROC curves of risk scores with MACE at 12 months



ROC curves showing each risk score with MACE at 12 months

Figure 15. ROC curves of risk scores with MACE at long term follow up



ROC curves showing each risk score with MACE at long term follow up.

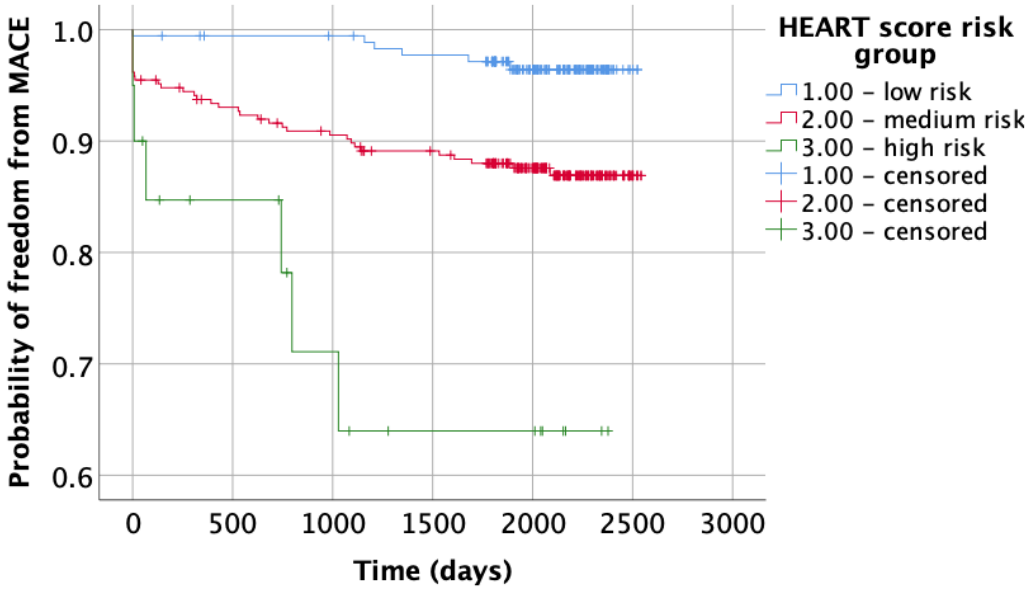
Kaplan-Meier analysis

HEART score

The HEART score ranged from 0-8 with a median score of 4. When using generally accepted cut-offs for risk, the number of events in each group increased when the risk tertile increased. There were 6/179 (3.35%) MACE events over long term follow up in the low-risk group, 36/288 (12.5%) in the medium-risk group and 6/20 (30%) in the high-risk group. There was a similar percentage of censored cases in the groups generated by the tertiles. Visually there was good discrimination between curves for tertiles (See Figure 16). A Bonferroni correction was made with statistical significance accepted at the $p < 0.0167$ level. The survival distributions between groups were statistically significantly different, $\chi 2(2)=25.364$, $p<0.001$. Pairwise log-rank comparisons demonstrated a statistically significant difference in outcome distributions between each set of groups (see below). This suggests that the HEART score can risk-stratify patients.

Low risk vs medium risk	$\chi^2(1)=11.427$	$(p=0.001)$
Low risk vs high risk	$\chi^2(1)=37.464$	$(p<0.001)$
Medium risk vs high risk	$\chi^2(1)=7.629$	$(p=0.006)$

Figure 16. Kaplan-Meier curve according to HEART risk score group



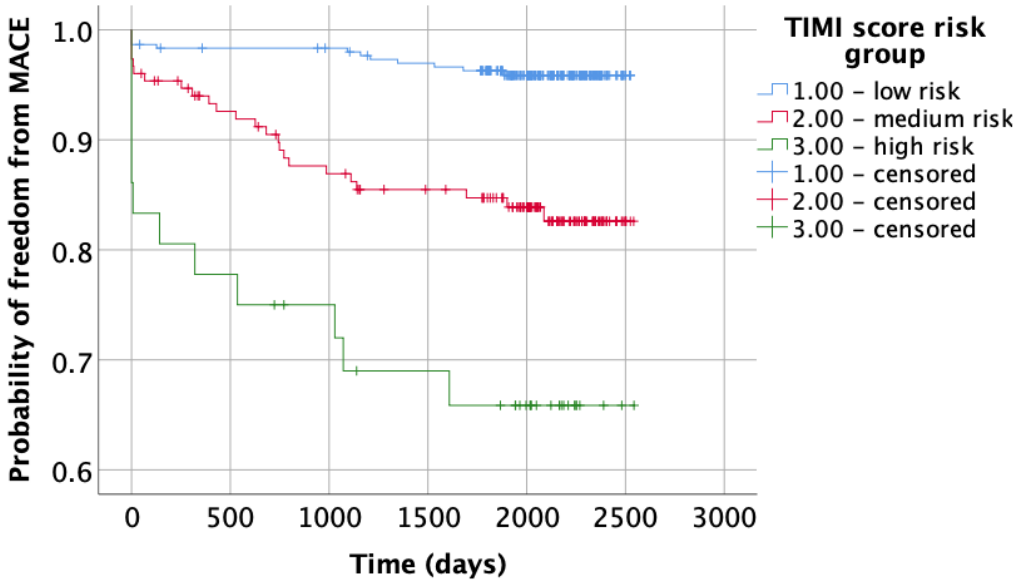
KM curves showing probability of freedom from MACE according to individual risk score category according to tertiles. False origin of y-axis set to near 0.6 to allow better visualisation of divergence of curves.

TIMI score

The TIMI score ranged from 0-6 with a median score of 1. When using generally accepted cut-offs for risk, the number of events in each group increased when the risk tertile increased. In the low-risk group, there was 12/300 (4%) long-term MACE, 24/151 (15.9%) in the medium-risk group and 12/36 (33.33%) in the high-risk group. There was a similar percentage of censored cases in the groups generated by the tertiles and the median. On the visual assessment of KM curves created by the tertiles, there was good discrimination of curves (See Figure 17). A Bonferroni correction was made with statistical significance accepted at the $p < 0.0167$ level. The survival distributions pooled over strata were statistically significant different $\chi2(2)=68.232$, $p<0.001$. Log-rank pairwise testing over strata found statistical significance between each set of risk groups (see below), suggesting the TIMI score can risk-stratify patients.

Low risk vs medium risk	$\chi^2(1)=34.077$	($p<0.001$)
Low risk vs high risk	$\chi^2(1)=59.077$	($p<0.001$)
Medium risk vs high risk	$\chi^2(1)=8.550$	($p=0.003$)

Figure 17. Kaplan-Meier curve according to TIMI score risk group



KM curves showing probability of freedom from MACE according to individual risk score category according to tertiles. False origin of y-axis set to near 0.6 to allow better visualisation of divergence of curves.

GRACE score

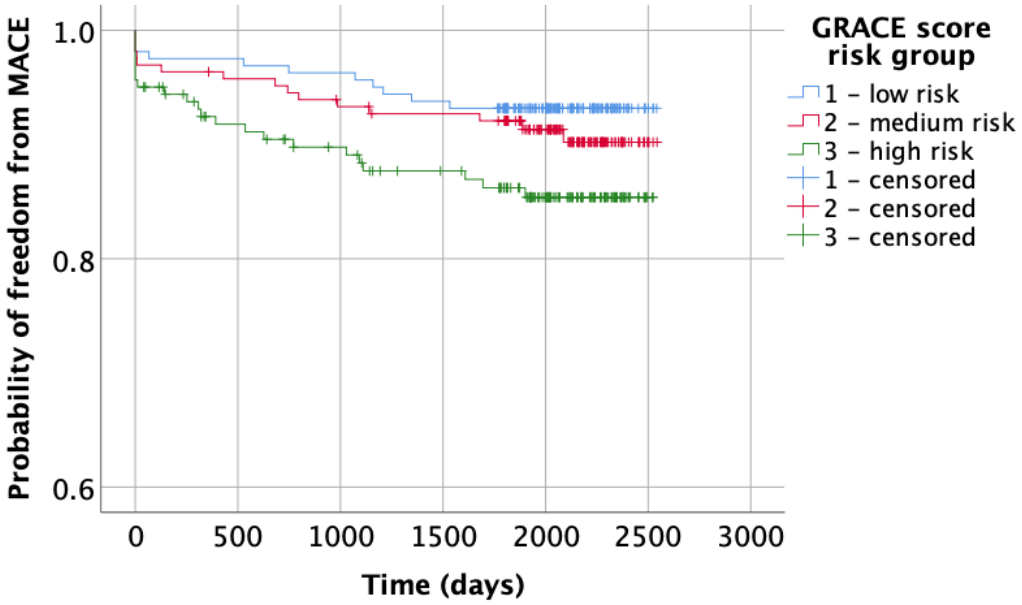
The GRACE score ranged between 27 and 177, with a median score of 70. For an 8-week MACE, there were 3/161 (1.86%) events in the low-risk tertile, 5/165 (3.03%) in the medium-risk tertile and 8/161 (4.97%) in the high-risk tertile. Visually, the KM curves demonstrated divergence in survival according to tertile, but the curves for low and medium-risk appeared close (See Figure 18). A Bonferroni correction was made with statistical significance accepted at the $p<0.0167$ level. The survival distributions

for the three groups were not statistically significantly different, $\chi 2(2)=5.463$, $p=0.065$. Pairwise log-rank (Mantel-Cox) comparisons were conducted to determine which groups had different outcome distributions. The difference in outcome distribution was not statistically significant for any set of risk groups.

Low risk vs medium risk	$\chi^2(1)=0.585$	$(p=0.444)$
Low risk vs high risk	$\chi^2(1)=4.972$	$(p=0.026)$
Medium risk vs high risk	$\chi^2(1)=2.228$	$(p=0.136)$

When the GRACE score was assessed according to median value and suggested groups in the literature, there was still no statistical significance between groups according to log-rank testing ($\chi^2(1)=3.188$, $p=0.074$ and $\chi^2(2)=1.542$, $p=0.214$, respectively). This suggests that the GRACE score has some discriminative ability between those with a low score vs those with a high score but is not particularly good at discriminating across all groups regardless of how assessed.

Figure 18. Kaplan-Meier curve according to GRACE score risk group



KM curves showing probability of freedom from MACE according to individual risk score category according to tertiles. False origin of y-axis set to near 0.6 to allow better visualisation of divergence of curves.

Discussion

The HEART and TIMI show a better overall model fit with comparable C-statistics of 0.756 and 0.742, respectively, compared with the C-statistic of the GRACE score of 0.692 for 8-week MACE. The HEART score was able to identify the most significant proportion of the cohort at a low risk with a low subsequent MACE rate. 14.8% of the cohort had a HEART score of ≤2, and no patients with this score experienced an 8-week or 12-month MACE. 36.8% of the cohort had a HEART score of ≤3, and only 1 (0.56%) of these experienced a MACE at 8-weeks. A HEART score of ≤4 increased the proportion of patients considerably (61.6%), and four patients (1.33%) experienced a MACE at 8-weeks, which may be considered too high an event rate to base expedited discharge on. A TIMI score of 0 represented 35.7% of the cohort, but there was a higher event rate of 1.72% patients. The lowest GRACE score in which a patient experienced an 8-week MACE was 42; a score of ≤42 only represented 8% of the cohort, with 2.6% of these experiencing a MACE at 8-weeks. The HEART and TIMI score are superior to the GRACE score in differentiating low, medium, and high-risk when assessed using KM analysis. Both HEART and TIMI score have statistically different KM curves when analysed over pairwise comparison using log-rank testing. In contrast, the GRACE score lacks statistical significance between groups regardless of the cut-off assessed. It is acknowledged that there is a limitation in a single investigator calculating risk scores, these were calculated at a time when future MACE was unknown which will reduce any bias.

Conclusion

The HEART score is better at accurately identifying the greatest number of patients at low risk and should be preferentially used over the GRACE and TIMI score to identify those patients truly at low risk who may be eligible for discharge. The GRACE score performs poorly regardless of the method investigated and should not be used in patients without elevation in Hs-cTnT.

CHAPTER 5. EXPLORATORY ANALYSIS OF SEROLOGICAL BIOMARKERS IN SUSPECTED ACS WITH MI EXCLUDED.

Introduction

There are numerous studies looking at the role of novel biomarkers in ACS. Yet, those who have an absence of troponin elevation, particularly in the high-sensitivity troponin era are least well-studied. Troponin levels have consistently been shown to provide reliable diagnostic and prognostic information in ACS. Currently, guidelines do not endorse the use of non-troponin biomarkers for diagnosis or risk stratification in ACS. Suspected ACS without elevation in Hs-cTnT is a heterogeneous entity with a variable clinical course, although most patients have an excellent prognosis. Accurate identification of those patients who are low risk could facilitate expedited discharge from hospital and improve resource utilisation. Precise identification of those who are high risk could allow appropriate investigation, management, and follow-up. This chapter aims to evaluate a number of biomarkers that relate to common substrates for atherosclerosis or are manifest in myocardial ischaemia, comprising of HFABP, GDF-15, NTproBNP, HSCRP, Galectin-3, Hs-cTnI and Hs-cTnT. The distribution of biomarker levels and features of the cohort when stratified by the tertiles for each biomarker is described. The tertiles for each biomarker were calculated according to cut-offs that gave rise to three groups of equal size. The outcomes for each of these biomarker groups is also reported. There is emerging data to show that gender specific reference values may improve the ability of a high sensitivity troponin test.(164) This is particularly noticeable for high sensitivity troponin I, over high sensitivity troponin T. This study did not use gender specified cut-offs for high sensitivity troponin T, as at the time of recruitment these had not been established and the gender cut-off for male is higher than the recruitment cut-off.

Methods

The methods of this study have been described fully in the General Methods Chapter. Levels of HFABP, GDF-15, NTproBNP, Galectin-3, HSCRP, Hs-cTnI and Hs-cTnT in patients with suspected ACS who had MI excluded were analysed. The normality of biomarkers was assessed by visual inspection of their histograms/normal Q-Q plots and the Shapiro-Wilk's test (see Appendix). Each biomarker was assessed according to groupings created using the tertiles, giving rise to low, medium, and high categories. Kaplan-Meier (KM) survival analysis was performed for each biomarker, with curves produced according to the rank of biomarker level and an event being defined as the occurrence of MACE. Right-censoring occurred due to non-cardiovascular mortality and the end of follow-up. To fulfil KM assumptions, groups were reviewed to ensure there was a similar percentage of censored cases in each group. KM curves were produced using cumulative survival curves. Statistically significant differences in MACE between groups were analysed using the Mantel-Cox log-rank test. The relative risk of having a biomarker level in the medium or high group was calculated. Receiver Operator Characteristic (ROC) curves were produced along with calculating the area under the curve, 95% confidence interval and level of significance of each novel biomarker.

Results

487 patients had values measured for the biomarkers Hs-cTnT, HFABP and GDF-15. Due to a limitation in serum samples remaining for analysis, Hs-cTnI was measured in 479 patients (98.36%), NTproBNP in 471 (96.71%), HSCRP in 445 (91.38%) and Galectin-3 in 386 (79.26%). Table 18 shows the range and median value of each novel biomarker.

Table 18. Range and distribution of biomarker levels

Biomarker	Minimum	Maximum	Median	IQ range
HFABP (ng/ml)	1.23	33.63	4.25	3.12-7.04
GDF-15 (pg/ml)	53.79	5872.10	281.82	184.527-494.537
NTproBNP (mg/dl)	0.2	5433.7	110.7	50.5-252.45
HS-CRP (ng/ml)	0.01	29.85	0.23	0.10-0.66
Galectin-3 (pg/ml)	5	33.1	14	11.5-17.175
Hs-cTnT (ng/l)	<3	14	4.2	3.2-7.6
Hs-cTnI (pg/ml)	0.4	53.8	3.2	2.2-5.5

Table showing range, median value, and interquartile range of each biomarker.

The distribution of all biomarker levels failed to reach the assumption of normality when assessed by visual inspection of histograms and normal Q-Q plots (See Appendix) and the Shapiro-Wilk test; therefore, all biomarker data was treated as non-parametric with regards to statistical analysis.

HFABP

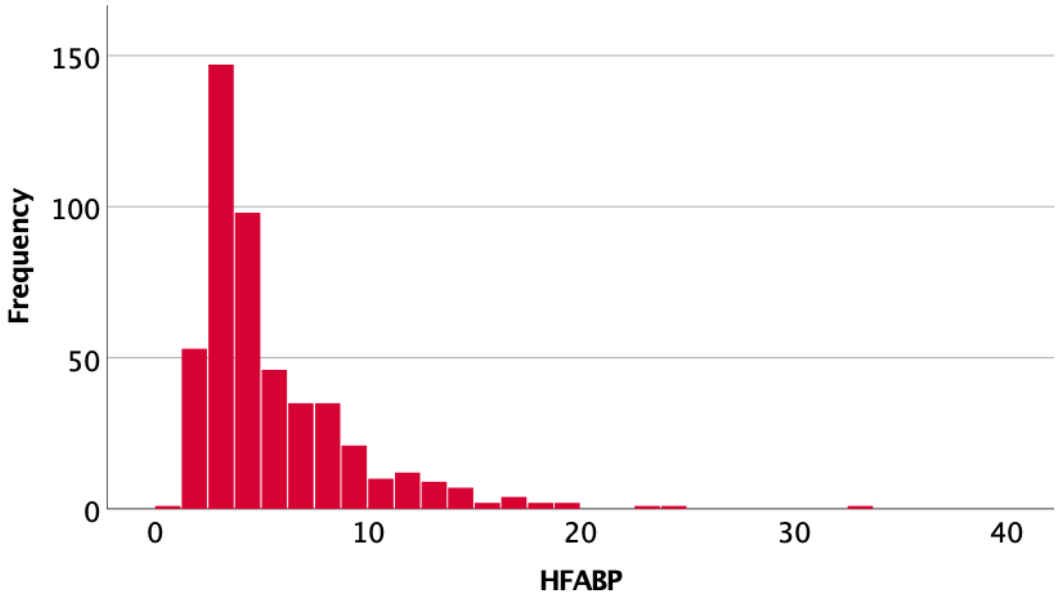
HFABP was measured in all 487 study participants; 22 experienced MACE at 12 months and 48 experienced MACE over a median follow up of 5.8 years. The tertiles for HFABP were defined as 3.44ng/ml and 5.63ng/ml. Table 19 shows the difference in proportion of patients with risk factors according to biomarker tertile. There is an increasing trend of those with diabetes and previous MI with increasing tertile of HFABP. KM curves generated by the tertiles of HFABP level (Figure 20) demonstrated poor discrimination of MACE, with the intersection of all three curves. Each grouped level of HFABP converges to a probability between 0.87 and 0.91 for long term follow up.

Table 19. Patient characteristics according to HFABP level

	HFABP		
Variable	Low (161)	Medium (164)	High (162)
Age >75 years	7 (4.3%)	16 (9.8%)	11 (6.8%)
Male	76 (47.2%)	102 (62.2%)	95 (58.6%)
Diabetes	17 (10.6%)	22 (13.4%)	40 (24.7%)
Smoker	48 (29.8%)	54 (32.9%)	50 (30.9%)
Hypertension	61 (37.9%)	88 (53.7%)	83 (51.2%)
Hypercholesterolaemia	68 (42.2%)	88 (53.7%)	83 (51.2%)
ST depression	15 (9.3%)	7 (4.3%)	6 (3.7%)
Previous MI	21 (13.0%)	39 (23.8%)	42 (25.9%)
Hs-cTnT high	34 (19.8%)	65 (37.8%)	73 (42.4%)
HEART score high	7 (4.3%)	8 (4.9%)	5 (3.1%)
TIMI score high	8 (5.0%)	15 (9.1%)	13 (8.0%)
GRACE score high	38 (23.6%)	64 (39.0%)	59 (36.4%)
Inpatient MACE	4 (2.5%)	5 (3.0%)	4 (2.5%)
12-month MACE	10 (6.2%)	6 (3.7%)	6 (3.7%)
3-year MACE	12 (7.5%)	13 (7.9%)	11 (6.8%)
Long term MACE	18 (11.2%)	14 (8.5%)	16 (9.9%)

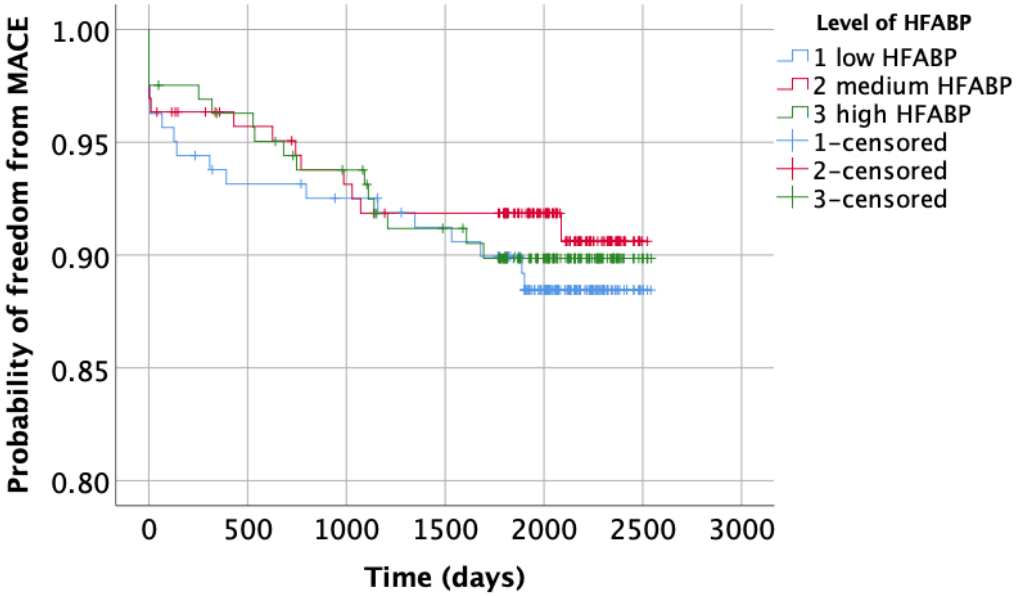
Table showing proportion of baseline characteristics, risk score, troponin levels and MACE according to biomarker level according to tertiles. Total number is displayed in each category along with percentage of group in rounded parenthesis.

Figure 19. Histogram showing distribution of HFABP level



Histogram showing distribution of biomarker level across the cohort.

Figure 20. KM curves according to HFABP level



KM curves showing probability of freedom from MACE according to biomarker levels when categorised by tertiles. False origin of y-axis set to near 0.8 to allow better visualisation of divergence of curves.

GDF-15

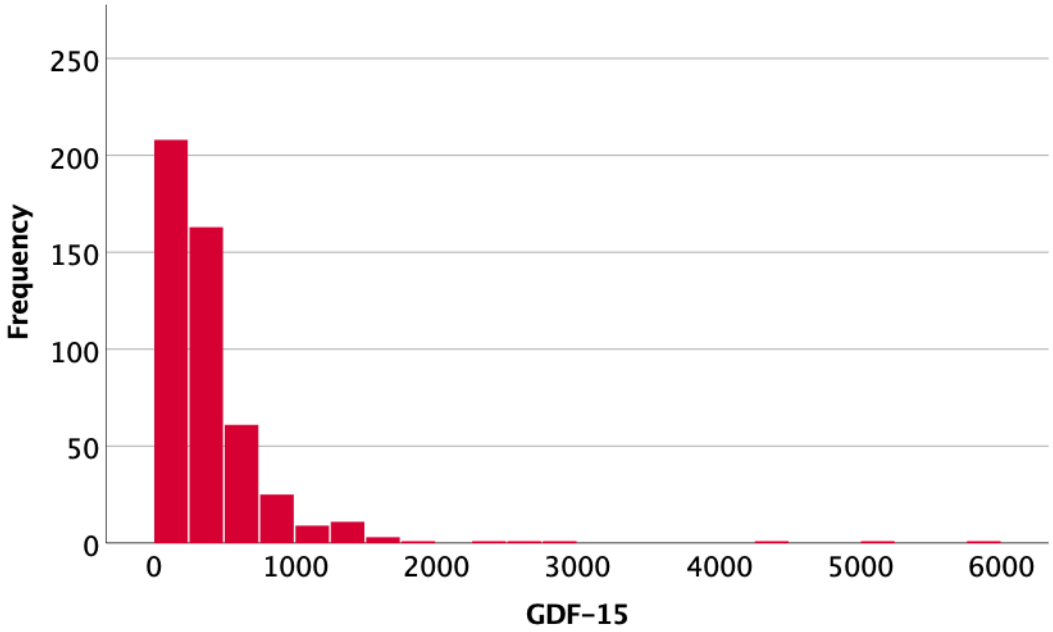
GDF-15 was measured in all 487 study participants; 22 experienced MACE at 12 months and 48 experienced MACE over long term follow up (median 5.8 years). The tertiles for GDF-15 were defined as 213.76pg/ml and 394.02pg/ml. Table 20 shows the difference in proportion of patients with risk factors according to biomarker tertile. There is an increasing trend of those with diabetes, hypercholesterolaemia, ST depression, previous MI with increasing tertile of GDF-15. There are an increasing number of patients with a high HEART, TIMI and GRACE score with increasing tertile of GDF-15. KM curves generated by the tertiles of GDF-15 (see Figure 22) show that low levels of GDF-15 are associated with a lower probability of MACE. Medium and high levels of GDF-15 initially show poor differentiation of MACE with the intersection of the curves but appear to diverge well for predicting MACE over longer-term follow-up.

Table 20. Patient characteristics according to GDF-15 level

	GDF-15		
	Low (162)	Medium (163)	High (162)
Age >75 years	2 (1.2%)	15 (9.2%)	17 (10.5%)
Male	90 (55.6%)	92 (56.4%)	91 (56.2%)
Diabetes	8 (4.9%)	32 (19.6%)	39 (24.1%)
Smoker	42 (25.9%)	57 (35.0%)	53 (32.7%)
Hypertension	55 (34.0%)	87 (53.4%)	90 (55.6%)
Hypercholesterolaemia	66 (40.7%)	90 (55.2%)	83 (51.2%)
ST depression	6 (3.7%)	8 (4.9%)	14 (8.6%)
Previous MI	19 (11.7%)	33 (20.2%)	50 (30.9%)
Hs-cTnT high	32 (18.6%)	61 (35.5%)	79 (45.9%)
HEART score high	3 (1.9%)	7 (4.3%)	10 (6.2%)
TIMI score high	7 (4.3%)	11 (6.7%)	18 (11.1%)
GRACE score high	23 (14.2%)	58 (35.6%)	80 (49.4%)
Inpatient MACE	1 (0.6%)	8 (4.9%)	4 (2.5%)
12-month MACE	3 (1.9%)	11 (6.7%)	8 (5%)
3-year MACE	3 (1.9%)	15 (9.2%)	18 (11.1%)
Long term MACE	5 (3.1%)	17 (10.4%)	26 (16.0%)

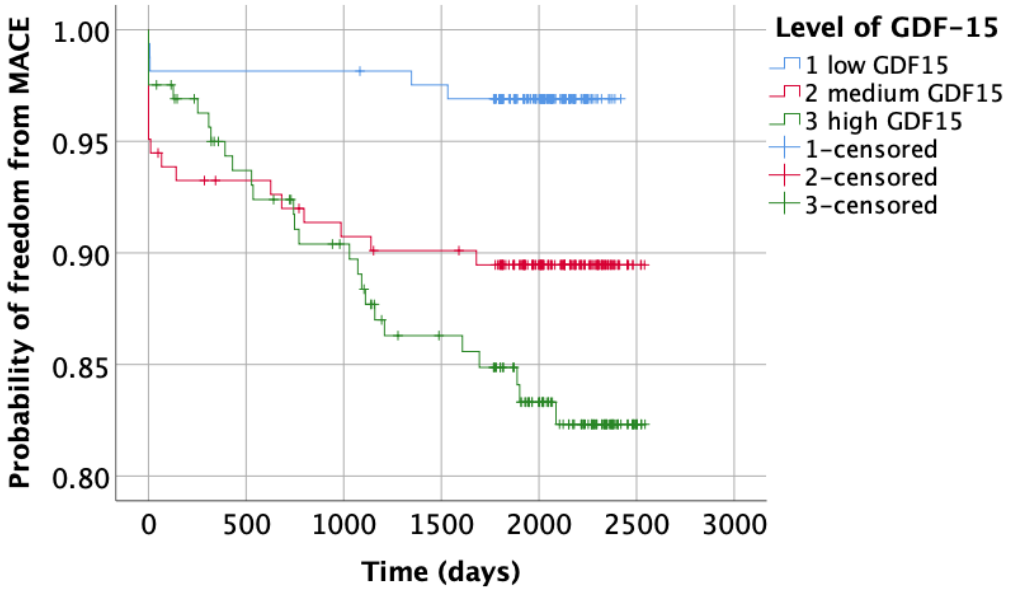
Table showing proportion of baseline characteristics, risk score, troponin levels and MACE according to biomarker level according to tertiles. Total number is displayed in each category along with percentage of group in rounded parenthesis.

Figure 21. Histogram showing distribution of GDF-15 level



Histogram showing distribution of biomarker level across the cohort.

Figure 22. KM curves according to GDF-15 level



KM curves showing probability of freedom from MACE according to biomarker levels when categorised by tertiles. False origin of y-axis set to near 0.8 to allow better visualisation of divergence of curves.

NTproBNP

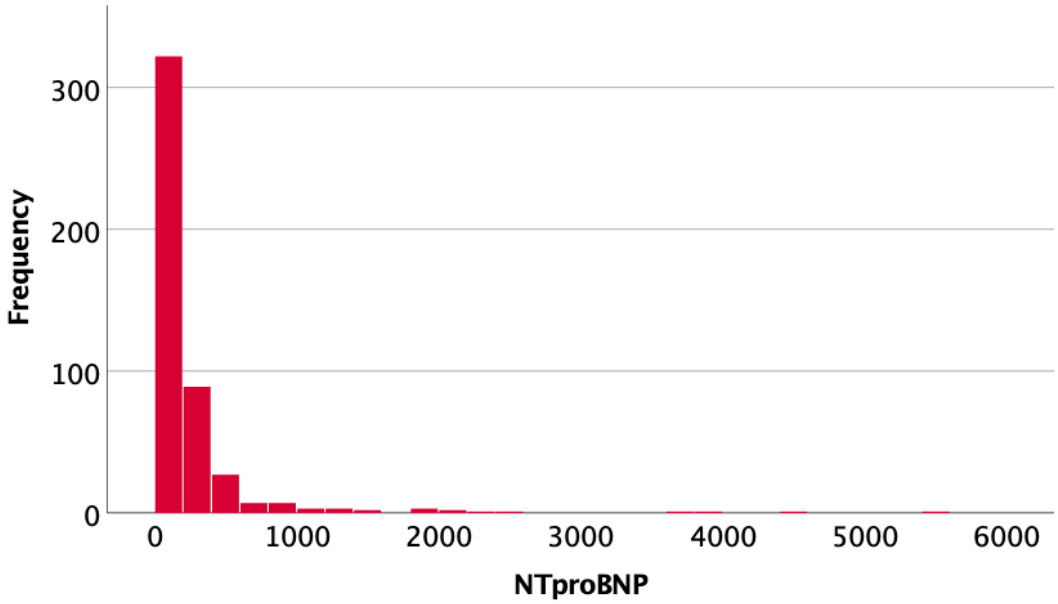
NTproBNP was measured in 471 patients; 21 experienced MACE at 12 months and 47 experienced MACE over a median follow up of 5.8 years. The tertiles for NTproBNP were defined as 68.02pg/ml and 190.54pg/ml for NTproBNP. Table 21 shows the difference in proportion of patients with risk factors according to biomarker tertile. There is an increasing trend of those with female sex, hypercholesterolaemia, previous MI, high Hs-cTnT levels with increasing tertile of NTproBNP. There are an increasing number of patients with a high TIMI and GRACE score with increasing tertile of NTproBNP. KM curves generated by the tertiles of NTproBNP (Figure 24) initially show poor differentiation of MACE with the intersection of curves. With longer-term follow up the curves diverge according to the level of NTproBNP.

Table 21. Patient characteristics according to NTproBNP level

	NTproBNP		
Variable	Low (157)	Medium (157)	High (157)
Age >75 years	1 (0.6%)	6 (3.8%)	27 (17.2%)
Male	107 (68.2%)	80 (51.0%)	71 (45.2%)
Diabetes	28 (17.8%)	25 (15.9%)	26 (16.6%)
Smoker	58 (36.9%)	43 (27.4%)	48 (30.6%)
Hypertension	67 (42.7%)	73 (46.5%)	88 (56.1%)
Hypercholesterolaemia	69 (43.9%)	79 (50.3%)	87 (55.4%)
ST depression	9 (5.7%)	6 (3.8%)	13 (8.3%)
Previous MI	24 (15.3%)	26 (16.6%)	51 (32.5%)
Hs-cTnT high	31 (18.0%)	57 (33.1%)	84 (48.8%)
HEART score high	8 (5.1%)	4 (2.5%)	8 (5.1%)
TIMI score high	5 (3.2%)	14 (8.9%)	17 (10.8%)
GRACE score high	22 (14.0%)	49 (31.2%)	89 (56.7%)
Inpatient MACE	1 (0.6%)	7 (4.5%)	4 (2.5%)
12-month MACE	5 (3.2%)	8 (5.1%)	8 (5.1%)
3-year MACE	9 (5.7%)	11 (7.0%)	15 (9.6%)
Long term MACE	11 (7.0%)	15 (9.6%)	21 (13.4%)

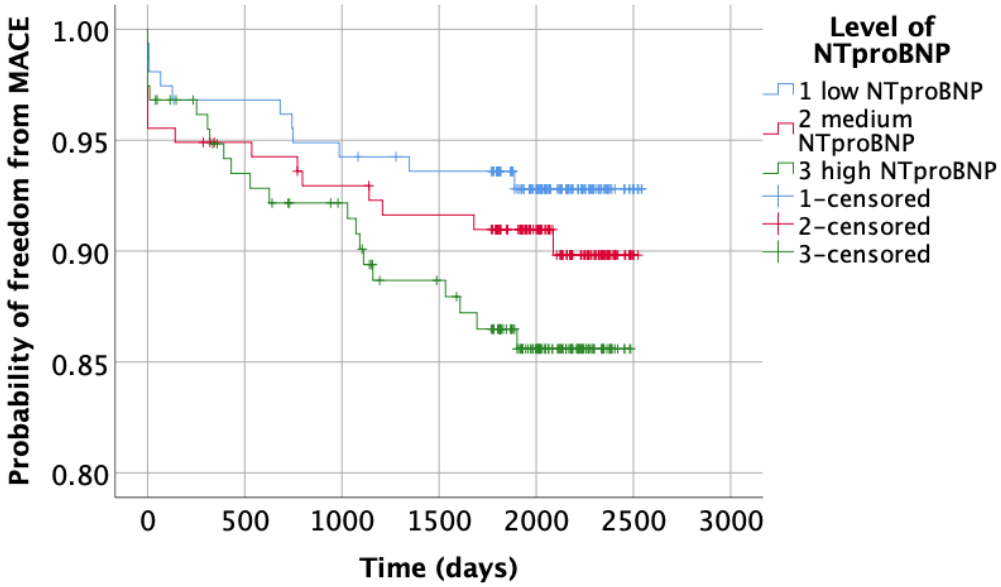
Table showing proportion of baseline characteristics, risk score, troponin levels and MACE according to biomarker level according to tertiles. Total number is displayed in each category along with percentage of group in rounded parenthesis.

Figure 23. Histogram showing distribution of NTproBNP level



Histogram showing distribution of biomarker level across the cohort.

Figure 24. KM curves according to NTproBNP level



KM curves showing probability of freedom from MACE according to biomarker levels when categorised by tertiles. False origin of y-axis set to near 0.8 to allow better visualisation of divergence of curves.

HS-CRP

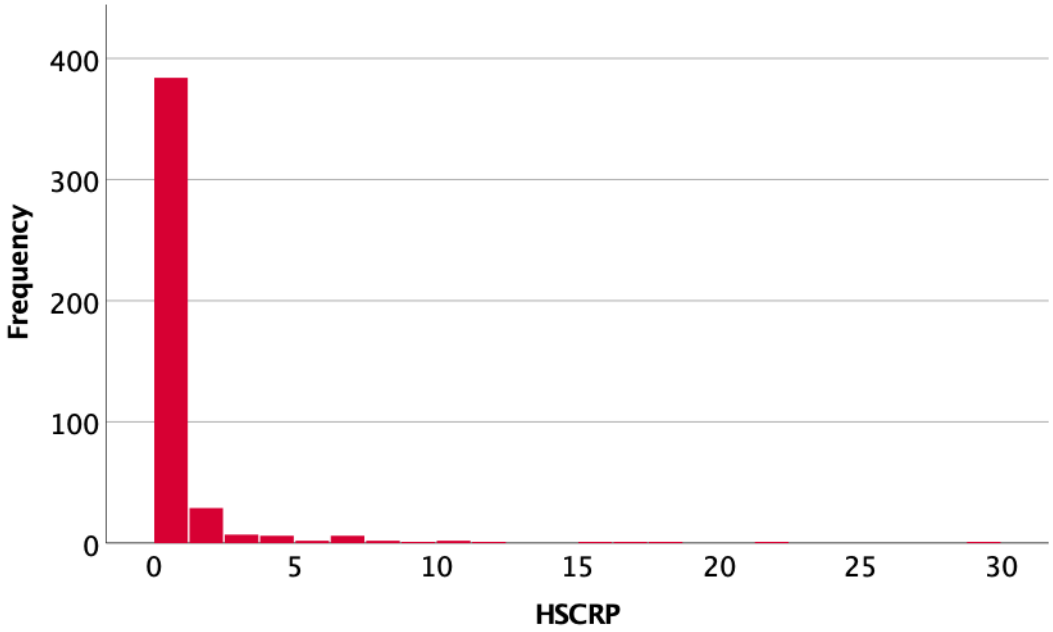
HS-CRP was measured in 445 patients; 19 had experienced MACE at 12 months and 45 at a median of 5.8 years follow up. The tertiles for HS-CRP were defined as 0.14mg/dl and 0.47mg/dl. Table 22 shows the difference in proportion of patients with risk factors according to biomarker tertile. There is an increasing trend of those with diabetes and smoking with increasing tertile of HS-CRP. KM curves generated by the tertiles of HS-CRP (Figure 26) show poor differentiation of MACE according to the level of HS-CRP with curves following the same trajectory and frequently intersecting throughout follow-up.

Table 22. Patient characteristics according to HSCRP level

	HSCRP		
	Low (146)	Medium (150)	High (149)
Age >75 years	17 (4.8%)	8 (10.6%)	4 (2.7%)
Male	83 (56.8%)	83 (55.3%)	85 (57.0%)
Diabetes	19 (13.0%)	26 (17.3%)	28 (18.8%)
Smoker	43 (29.5%)	45 (30.0%)	48 (32.2%)
Hypertension	67 (45.9%)	75 (50.0%)	69 (46.3%)
Hypercholesterolaemia	81 (55.0%)	72 (48.0%)	67 (45.0%)
ST depression	10 (6.8%)	4 (2.7%)	11 (7.4%)
Previous MI	34 (23.3%)	25 (16.7%)	35 (23.5%)
Hs-cTnT high	48 (32.0%)	56 (37.3%)	46 (30.7%)
HEART score high	6 (4.1%)	9 (6.0%)	4 (2.7%)
TIMI score high	14 (9.6%)	8 (5.3%)	10 (6.7%)
GRACE score high	50 (34.2%)	54 (36.0%)	44 (29.5%)
Inpatient MACE	4 (2.7%)	3 (2.0%)	3 (2.0%)
12-month MACE	9 (6.2%)	4 (2.7%)	6 (4.0%)
3-year MACE	10 (6.8%)	11 (7.3%)	12 (8.1%)
Long term MACE	16 (11.0%)	13 (8.7%)	16 (10.7%)

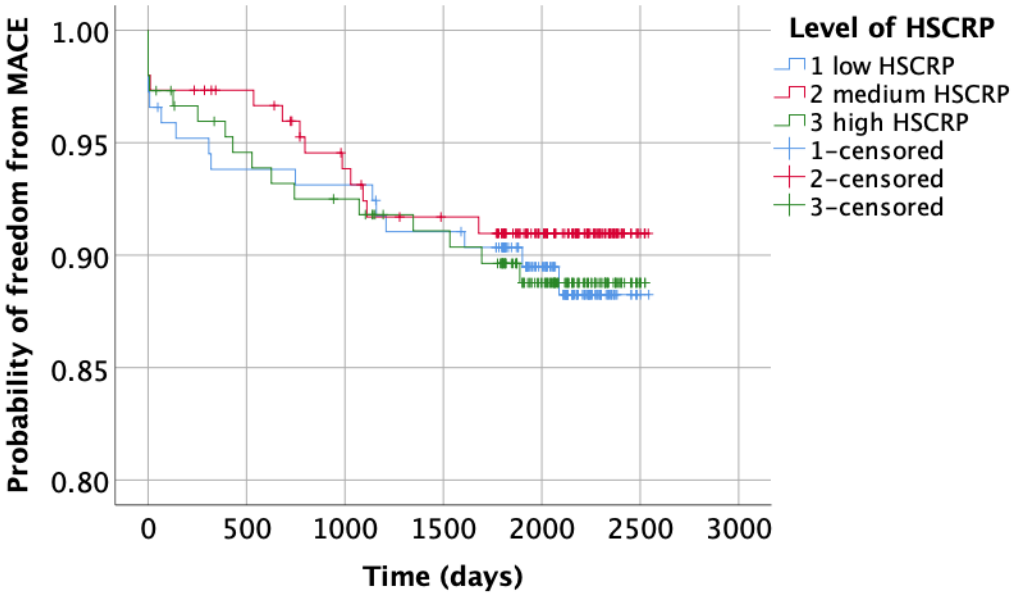
Table showing proportion of baseline characteristics, risk score, troponin levels and MACE according to biomarker level according to tertiles. Total number is displayed in each category along with percentage of group in rounded parenthesis.

Figure 25. Histogram showing distribution of HSCRP level



Histogram showing distribution of biomarker level across the cohort.

Figure 26. KM curves according to HSCRP level



KM curves showing probability of freedom from MACE according to biomarker levels when categorised by tertiles. False origin of y-axis set to near 0.8 to allow better visualisation of divergence of curves.

Galectin-3

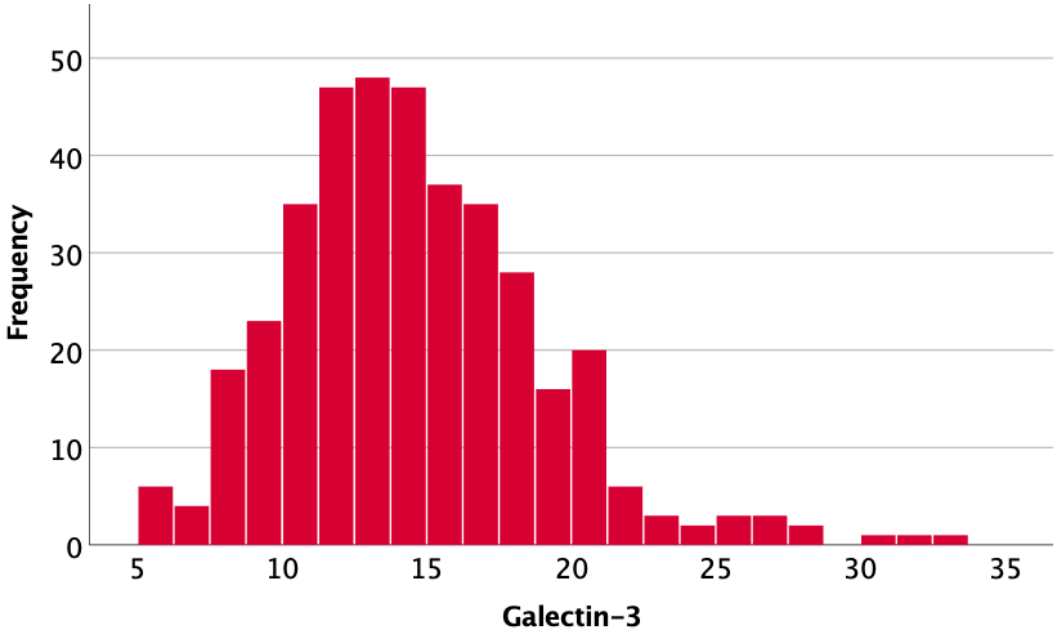
Galectin-3 was measured in 386 patients. There were 15 MACE events at 12 months and 37 MACE events over a median of 5.8 years. The tertiles of Galectin-3 were defined as 12.30ng/ml and 16.00ng/ml. Table 23 shows the difference in proportion of patients with risk factors according to biomarker tertile. There is an increasing trend of those with diabetes, those with a high Hs-cTnT, GRACE risk with increasing tertile of Galectin-3. KM curves generated by the tertiles of Galectin-3 (Figure 28) show divergence of the curves generated by low and high levels of Galectin-3, although the curves generated by medium levels initially tracks and intersects the low-level curve before tending towards the high-level curve.

Table 23. Patient characteristics according to Galectin-3 level

	Galectin-3 levels		
	Low (128)	Medium (129)	High (129)
Age >75 years	5 (3.9%)	5 (3.9%)	17 (13.2%)
Male	87 (68%)	67 (51.9%)	60 (%)
Diabetes	17 (13.3%)	18 (14%)	28 (21.7%)
Smoker	39 (30.5%)	47(36.4%)	38 (29.5%)
Hypertension	60 (46.9%)	56 (43.4%)	68 (52.7%)
Hypercholesterolaemia	59 (46.1%)	54 (41.9%)	68 (52.7%)
ST depression	10 (7.8%)	6 (4.7%)	8 (6.2%)
Previous MI	25 (19.5%)	21 (16.3%)	32 (24.8%)
Hs-cTnT high	37 (27.4%)	40 (29.6%)	58 (43.0%)
HEART score high	6 (4.7%)	3 (2.3%)	5 (3.9%)
TIMI score high	8 (6.3%)	8 (6.2%)	10 (7.8%)
GRACE score high	24 (18.8%)	38 (29.5%)	61 (47.3%)
Inpatient MACE	2 (1.6%)	3 (2.3%)	4 (3.1%)
12-month MACE	5 (3.9%)	4 (3.1%)	6 (4.7%)
3-year MACE	7 (5.5%)	9 (7.0%)	12 (9.3%)
Long term MACE	10 (7.8%)	13 (10.1%)	14 (10.9%)

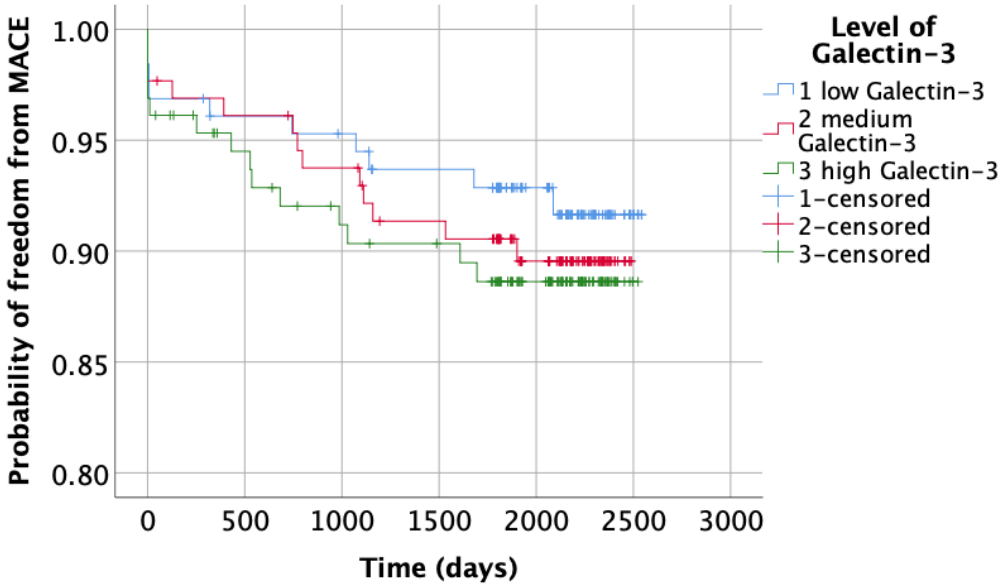
Table showing proportion of baseline characteristics, risk score, troponin levels and MACE according to biomarker level according to tertiles. Total number is displayed in each category along with percentage of group in rounded parenthesis.

Figure 27. Histogram showing distribution of Galectin-3 level



Histogram showing distribution of biomarker level across the cohort.

Figure 28. KM curves according to Galectin-3 level



KM curves showing probability of freedom from MACE according to biomarker levels when categorised by tertiles. False origin of y-axis set to near 0.8 to allow better visualisation of divergence of curves.

Hs-cTnI

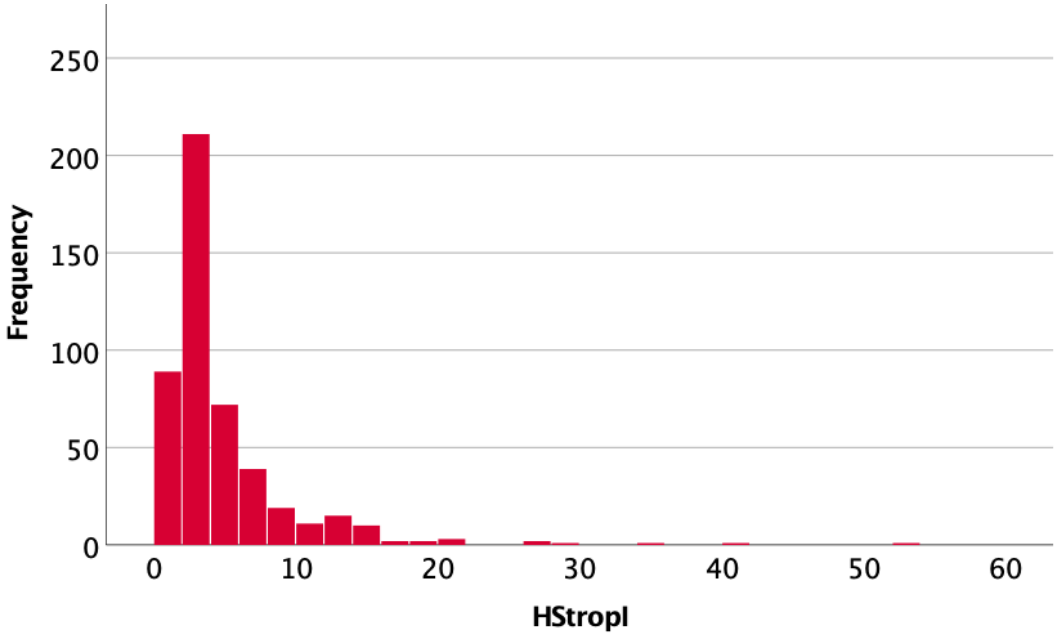
Hs-cTnI was measured in 479 patients. There were 22 MACE events at 12 months and 48 events over a median of 5.8 years. The tertiles for Hs-cTnI were defined as 2.60pg/ml and 4.30pg/ml. Table 24 shows the difference in proportion of patients with risk factors according to biomarker tertile. There is an increasing trend of those with hypercholesterolaemia, high Hs-cTnT levels, and high levels of GRACE score with increasing tertile of Hs-cTnI. KM curves generated by the tertiles of Hs-cTnI (Figure 29) show that high levels of Hs-cTnI are associated with a higher probability of MACE than the cohort. Low and medium levels of Hs-cTnI consistently show poor differentiation of MACE with curves running a similar trajectory with multiple intersections of the curves.

Table 24. Patient characteristics according to Hs-cTnI level

	Hs-cTnI		
	Low (159)	Medium (158)	High (162)
Age >75 years	5 (3.1%)	8 (5.1%)	21 (13.0%)
Male	72 (45.3%)	96 (60.8%)	97 (59.9%)
Diabetes	24 (15.1%)	22 (13.9%)	33 (20.4%)
Smoker	63 (39.6%)	44 (27.8%)	41 (25.3%)
Hypertension	58 (36.5%)	66 (41.8%)	106 (65.4%)
Hypercholesterolaemia	67 (42.1%)	74 (46.8%)	96 (59.3%)
ST depression	10 (6.3%)	5 (3.2%)	12 (7.4%)
Previous MI	27 (17.0%)	25 (15.8%)	49 (30.2%)
Hs-cTnT high	21 (12.2%)	46 (26.7%)	105 (61.1%)
HEART score high	6 (3.8%)	5 (3.2%)	8 (4.9%)
TIMI score high	7 (4.4%)	7 (4.4%)	21 (13.0%)
GRACE score high	39 (24.5%)	47 (29.7%)	75 (46.3%)
Inpatient MACE	1 (0.6%)	2 (1.3%)	10 (6.2%)
12-month MACE	4 (2.5%)	4 (2.5%)	14 (8.6%)
3-year MACE	7 (4.4%)	10 (6.3%)	19 (11.7%)
Long term MACE	12 (7.5%)	12 (7.6%)	24 (14.8%)

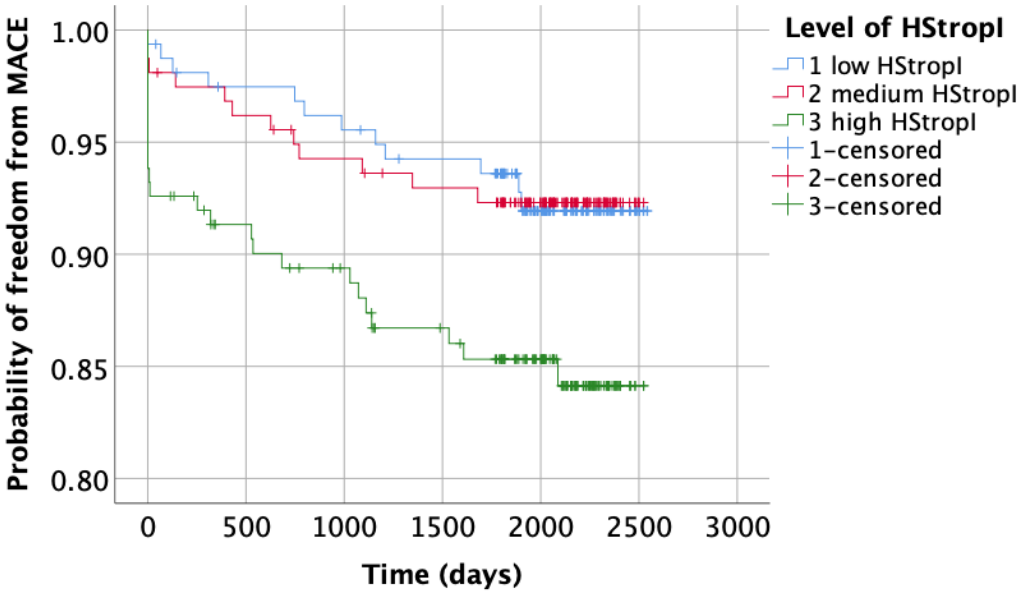
Table showing proportion of baseline characteristics, risk score, Hs-cTnT levels and MACE according to biomarker level according to tertiles. Total number is displayed in each category along with percentage of group in rounded parenthesis.

Figure 29. Histogram showing distribution of Hs-cTnI level



Histogram showing distribution of biomarker level across the cohort.

Figure 30. KM curves according to Hs-cTnI level



KM curves showing probability of freedom from MACE according to biomarker levels when categorised by tertiles. False origin of y-axis set to near 0.8 to allow better visualisation of divergence of curves.

Table 25. Patients who had normal Hs-cTnT and elevation in Hs-cTnI

Patient	A	B	C	D	E	F
Hs-cTnT ng/l	14	10	11	8	9	6
Hs-cTnI pg/ml	34.1	53.8	26.0	41.4	15.8	21.6
Gender 1=female	0	1	0	1	0	0
HEART RFs	5	3	1	3	3	3
HEART score	6	6	6	5	6	6
TIMI score	4	3	1	2	4	1
Discharge diagnosis	Atypical CP	Ischaemic CP	Arrhythmia	Atypical CP	Ischaemic CP	Atypical CP
Time to MACE (d)	-	8	-	-	0	-

Patients included who had elevation in Hs-cTnI beyond the gender-specific cut-offs for the 99th percentile (15.6ng/L for females, 34.2ng/L for males). HEART RF=total number of risk factors as used in HEART score. CP=chest pain. HEART and TIMI scores are displayed as absolute score.

There were 6 patients who had normal Hs-cTnT levels but had an elevated Hs-cTnI above the 99th percentile for gender-specific cut-off values. The associated Hs-cTnT levels and other important findings are displayed in Table 25. Of these patients, two experienced the MACE end-point unstable angina requiring revascularisation; one as an inpatient and one at day 8 post discharge. No patients experienced MI or mortality. These patients did have high HEART scores between 5-6. The TIMI score was variable between 1-4. There were no ST changes in these patients. Patient A underwent elective revascularisation at 229 days. Patient D underwent an inpatient cardiac catheterisation, and this was reported as normal.

Hs-cTnT

Hs-cTnT was measured in all study participants as part of routine clinical care. There were 22 MACE events at 12 months and 48 MACE events over a median follow up of 5.8 years. Adjustments were made in keeping with the manufacturer guidance in 196 patients (67%) due to the use of assay batches affected by a downward shift in the standardisation against the master lot, as described in the methods section. The tertiles for Hs-cTnT were defined as 3.2ng/l and 6.2ng/l for Hs-cTnT. Table 26 shows the difference in proportion of patients with risk factors according to biomarker tertile. KM curves generated by the tertiles of Hs-cTnT (Figure 32) show good discrimination of MACE according to the rank of Hs-cTnT with the clear divergence of the curves, particularly as follow-up progresses. A Mantel-Cox log-rank test reported statistical significance in freedom from MACE ($\chi^2(2)=23.825$, $p<0.001$). Pairwise log-rank comparisons were conducted to determine which groups had different MACE distributions. A Bonferroni correction was made with statistical significance accepted at the $p<0.0167$ level. There was a statistically significant difference in outcome distributions for low vs medium and low vs high levels of Hs-cTnT, but not for medium vs high levels.

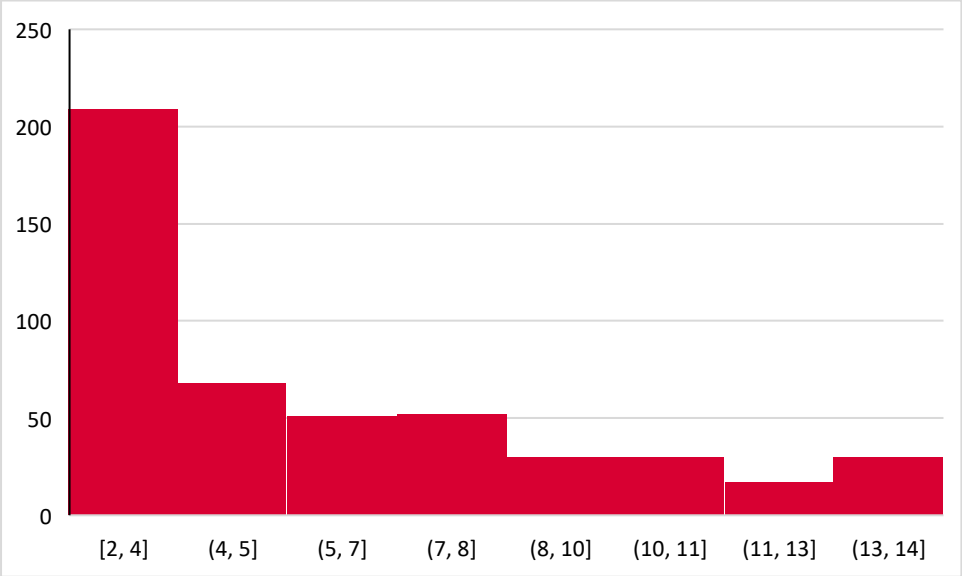
Low Hs-cTnT vs medium Hs-cTnT	$\chi^2(1)=11.954$	$p=0.001$
Low Hs-cTnT vs high Hs-cTnT	$\chi^2(1)=24.557$	$p<0.001$
Medium Hs-cTnT vs high Hs-cTnT	$\chi^2(1)=1.488$	$p=0.223$

Table 26. Patient characteristics according to Hs-cTnT level

	Hs-cTnT		
	Low (116)	Medium (199)	High (172)
Age >75 years	1 (0.9%)	5 (2.5%)	28 (16.2%)
Male	54 (46.6%)	112 (56.3%)	107 (62.2%)
Diabetes	14 (12.1%)	20 (15.1%)	79 (16.2%)
Smoker	39 (33.6%)	77 (38.7%)	36 (20.9%)
Hypertension	44 (37.9%)	83 (41.7%)	105 (61.5%)
Hypercholesterolaemia	47 (40.5%)	86 (43.2%)	106 (61.6%)
ST depression	5 (4.3%)	10 (5.0%)	13 (7.6%)
Previous MI	15 (12.9%)	36 (18.1%)	51 (29.7%)
HEART score high	3 (2.6%)	7 (3.5%)	20 (4.1%)
TIMI score high	1 (0.9%)	1 (0.5%)	2 (1.2%)
GRACE score high	0 (0.0%)	1 (0.5%)	3 (1.7%)
Inpatient MACE	2 (1.7%)	1 (0.5%)	4 (2.3%)
12-month MACE	2 (1.7%)	4 (2.0%)	16 (9.3%)
3-year MACE	2 (1.7%)	9 (4.5%)	25 (14.5%)
Long term MACE	2 (1.7%)	15 (7.5%)	31 (18.0%)

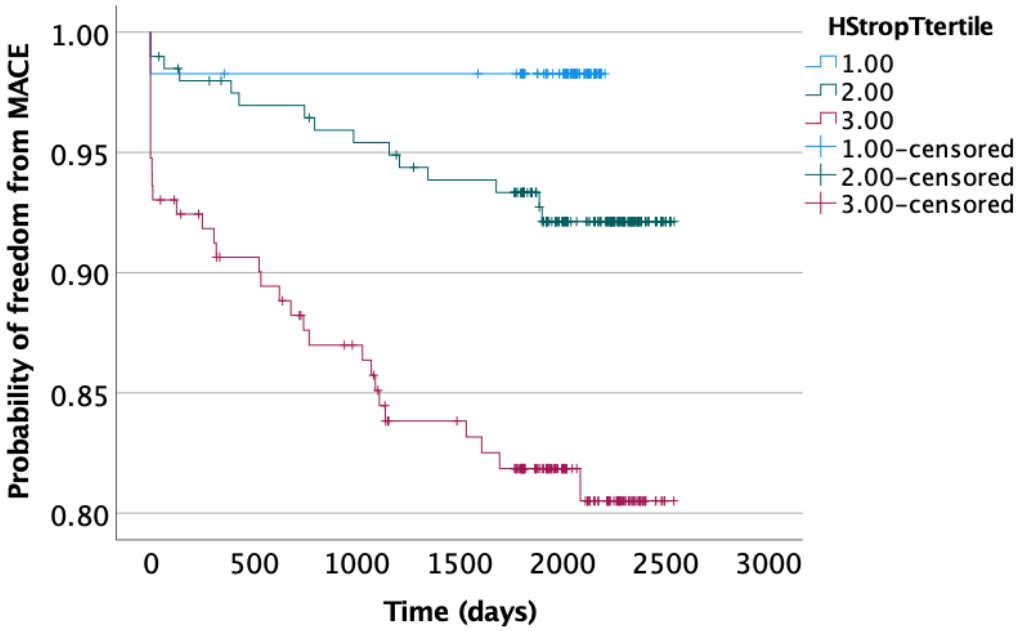
Table showing proportion of baseline characteristics, risk score and MACE according to biomarker level according to tertiles. Total number is displayed in each category along with percentage of group in rounded parenthesis.

Figure 31. Histogram showing distribution of Hs-cTnT level



Histogram showing distribution of biomarker level across the cohort.

Figure 32. KM curves according to Hs-cTnT level



KM curves showing probability of freedom from MACE according to biomarker levels when categorised by tertiles. False origin of y-axis set to near 0.8 to allow better visualisation of divergence of curves.

Relative risk

The relative risk of having a biomarker level in the upper two tertiles or upper tertile compared with the lower tertile are demonstrated in the table below. This shows that patients with a GDF-15 value in the top two tertiles is associated with a relative risk of 3.433, and a value of Hs-cTnT in the top two tertiles is associated with a relative risk of 6.232. HFABP, NTproBNP, Galectin-3, HSCRP and medium to high levels of Hs-cTnI do not have statistically significant relative risk.

Table 27. Relative risk associated with biomarker level and long-term MACE

Biomarker	RR for long-term MACE
GDF-15 level medium or high	**3.433 (1.484-7.945)**
GDF-15 level high	**1.506 (1.100-2.601)**
HFABP level medium or high	1.079 (0.858-1.356)
HFABP level high	1.001 (0.811-1.236)
NTproBNP level medium or high	1.468 (0.861-2.503)
NTproBNP level high	1.229 (0.943-1.602)
Galectin-3 level medium or high	1.251 (0.722-2.167)
Galectin-3 level high	1.079 (0.830-1.402)
HSCRP level medium or high	0.912 (0.600-1.385)
HSCRP level high	1.037 (0.826-1.302)
Hs-cTnT level medium or high	**6.232 (1.590-24.426)**
Hs-cTnT level high	**1.917 (1.301-2.824)**
Hs-cTnI level medium or high	1.364 (0.821-2.266)
Hs-cTnI level high	**1.571 (1.148-2.148)**

Table showing the relative risk associated with levels of biomarker when assessed according to the tertiles.

ROC analysis

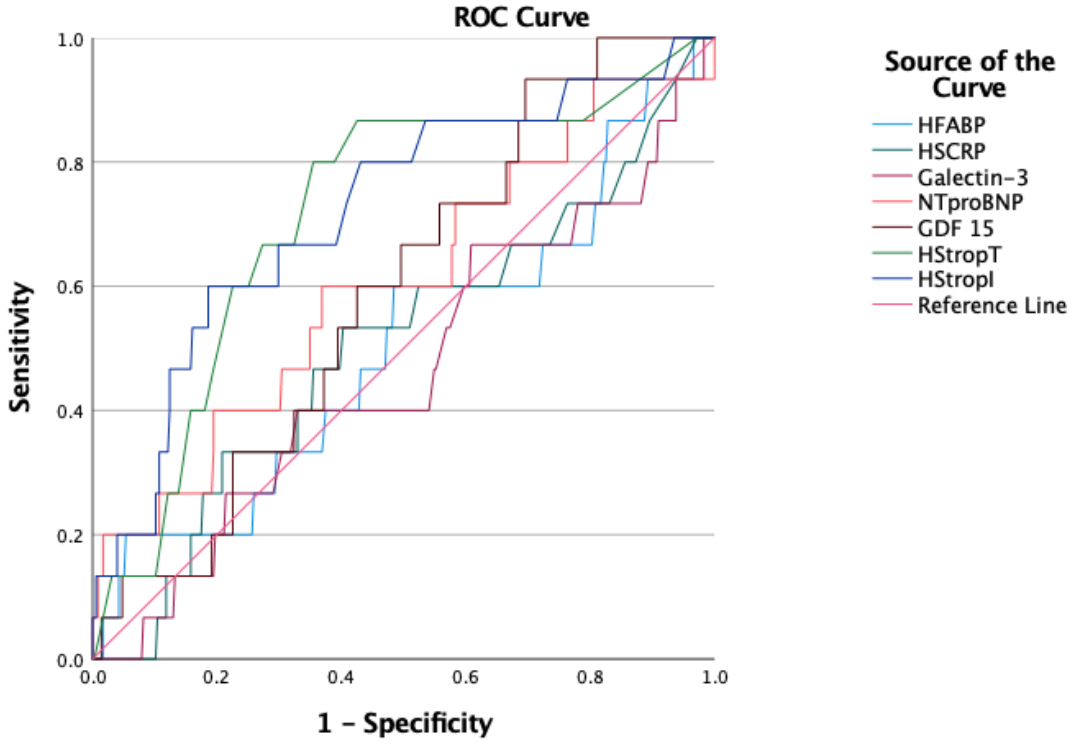
ROC analysis was performed on each of the biomarkers for MACE at 12 months and long term follow up. Table 28 shows the C-statistics for each of the biomarkers at these time points. Using the prespecified cut-off values as specified on page 42 of the Introduction section, Hs-cTnT and Hs-cTnI show fair discrimination of MACE at 12 months, and Hs-cTnT also shows this for long term MACE. Figure 33 and Figure 34 show the ROC curves for MACE at 12-months and long-term.

Table 28. C-statistic for novel biomarkers at different durations of follow up

Biomarker	MACE 12-months	MACE Long term
HFABP	0.504 (0.341-0.665) p=0.965	0.528 (0.426-0.630) p=0.577
GDF-15	0.593 (0.468-0.715 p=0.223	**0.665 (0.583-0.746) p=0.001**
NTproBNP	0.604 (0.445-0.763) p=0.174	**0.644 (0.541-0.746) p=0.004**
HSCRP	0.508 (0.350-0.666) p=0.924	0.482 (0.388-0.576) p=0.719
Galectin-3	0.465 (0.308-0.621) p=0.646	0.538 (0.439-0.638) p=0.444
Hs-cTnI	**0.722 (0.584-0.861) p=0.004**	**0.623 (0.525-0.721) p=0.014**
Hs-cTnT	**0.715 (0.582-0.849) p=0.005**	**0.705 (0.625-0.785) p<0.001**

C-statistic of biomarkers for MACE at 12 months and long term.

Figure 33. ROC curves for MACE at 12 months



ROC curves for biomarkers and MACE at 12 months

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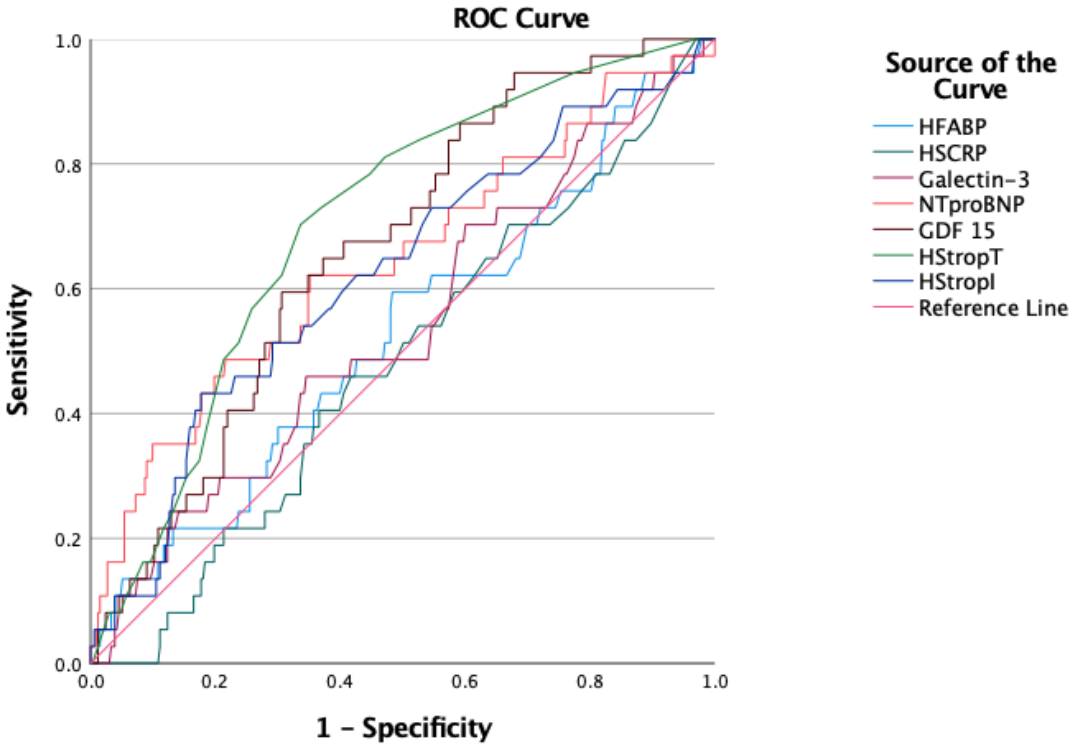
Figure 34. ROC curves for MACE long term.



ROC curves for biomarkers and MACE at long term follow up

Discussion

In this analysis, Galectin-3, HFABP and HSCRP do not appear to provide any prognostic information on their own, whereas NTproBNP, GDF-15, Hs-cTnI and Hs-cTnT do show a potential discrimination of outcome. Hs-cTnT appears to show good discrimination of risk according to tertile of Hs-cTnT level beyond the first year of follow up, but prior to that the low and medium level group suggest similar risk, however Hs-cTnT is likely to be affected by the calibration adjustment and furthermore, precision at these very low levels of biomarker could be inadequate. Hs-cTnI does hold some ability to risk stratify when assessed according to tertiles; high levels are associated with worse prognosis, but low and medium levels convey similar risk. GDF-15 is good at long-term discrimination, but medium and high levels do not discriminate well during the first part of follow up. Low levels of GDF-15 confer a low risk of MACE across the whole study duration. Medium and high levels of Hs-cTnT and GDF-15 have more than a 3-fold greater risk of MACE when compared with low levels of these biomarkers. NTproBNP does seem to show some ability to risk stratify for long-term MACE but does not show ability to predict MACE in the short-term. Hs-cTnT shows fair discrimination for MACE at 12 months and long term, when analysed using ROC curve analysis. Hs-cTnI has fair discrimination for 12-month MACE but is a poor discriminator of long-term MACE. GDF-15 and NTproBNP do show some ability to predict long term MACE in isolation, but this discrimination is still poor according to ROC analysis. This chapter describes only a basic analysis, all biomarkers will still be considered in subsequent analysis, but these initial findings may help contextualise later results. In particular, biomarker levels will

be assessed in their continuous form, as information may be lost when assessing data according to tertile level.

Conclusion

NTproBNP, GDF-15, Hs-cTnI and Hs-cTnT show some ability to predict future MACE. On this initial analysis, Galectin-3, HFABP and HSCRP do not appear to provide any useful prognostic information on their own. Each biomarker will be studied in more detail in future chapters.

CHAPTER 6. BIOMARKERS IN THE RULE OUT OF EARLY MACE

Introduction

Chest pain is one of the most common presenting complaints that lead to hospital attendances. The early identification of ACS facilitates appropriate treatment and investigation, yet most patients who present with chest pain do not have ACS. Patients without elevation in troponin levels are generally considered to be low risk but it is clear from the work in Chapter 3 that this population is not free from risk, with 2.67% experiencing inpatient MACE and 3.29% at 8-weeks. Identifying those patients at truly at low risk of short-term MACE could facilitate expedited discharge, saving NHS resources. As clinical history has repeatedly been shown to be unreliable in the prediction of MI, other methods to help risk stratify are required.(165,166) A biomarker that could assist in the identification in those truly low risk could be an attractive method in early rule out. This chapter provides a preliminary analysis of the role of biomarkers in the rule-out of early MACE, however, due to the low MACE rate at early follow up, the findings would not be powered to provide definite evidence but could be used to provide information to power future larger studies.

Methods

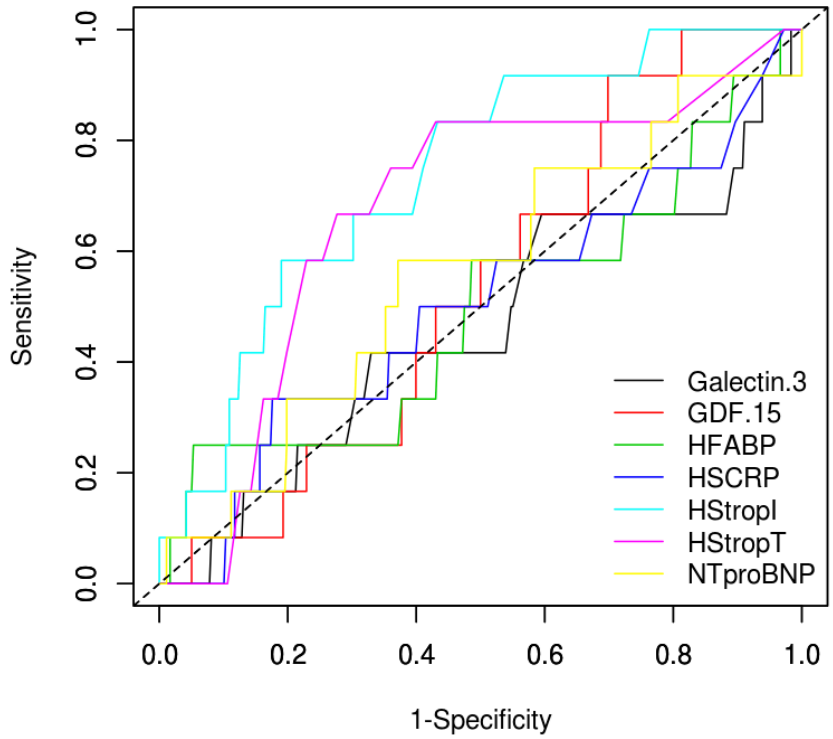
All biomarkers were included in this investigation. Receiver Operator Characteristic (ROC) analysis was performed to produce ROC curves and ROC-derived cut-off values were investigated for a rule-out of MACE at 8 weeks and 6 months, corresponding with an NPV of 99.5%. The acceptable miss-rate of myocardial infarction is an area of much debate. A survey of Emergency Care Physicians had found that a miss rate 0.5% was generally considered to be acceptable. Therefore a NPV of 99.5% was chosen to comply with this miss rate of 0.5%.(167) The proportion of the cohort with a biomarker corresponding to below the value associated with a NPV of 99.5% was calculated along with the sensitivity. Distribution curves and plots comparing those who experienced MACE and those with freedom from MACE were produced. For biomarkers that identified the potential to provide useful information regarding early MACE, algorithms were evaluated to identify rule-out strategies that maximise the number of patients eligible for expedited discharge whilst maintaining a MACE rate of ≤0.5%. This was done by combining biomarker levels and using HEART score, which was previously identified in Chapter 4 as showing value in the rule-out of MACE. MACE was assessed at two time points, 8-weeks and 6-months. Freedom from MACE at 8-weeks was deemed appropriate to facilitate discharge; however, it was acknowledged that proper outpatient investigation, review and treatment might not be completed at this point in an overburdened NHS. MACE at 6-months was also chosen as it was felt most services would be able to complete the appropriate investigations and management over this time frame. ROC analysis was

performed using SPSS version 25 and online software easyROC,(156) which is based on R programming language.

Results

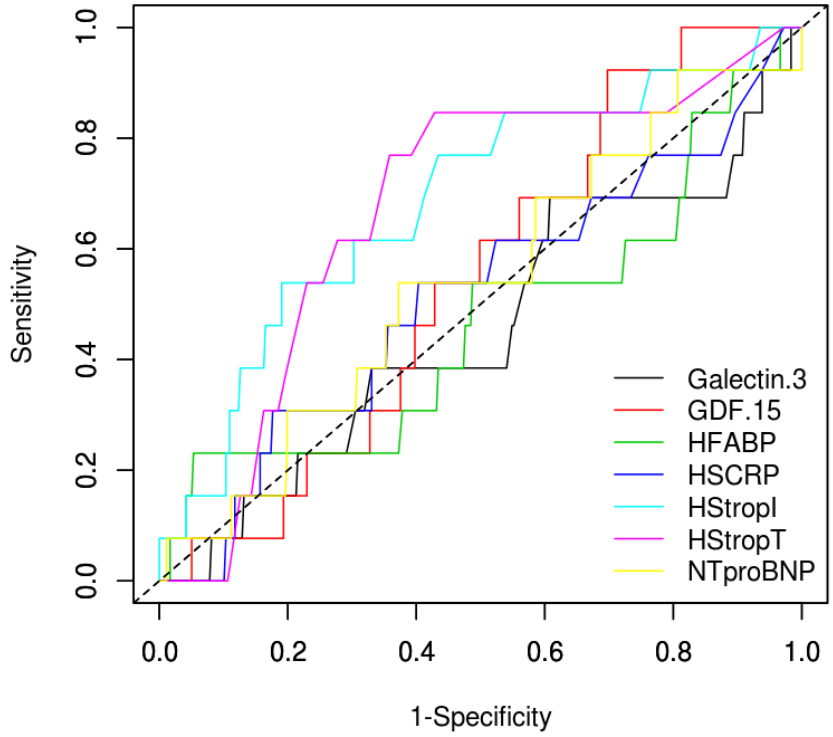
487 patients had values measured for the biomarkers Hs-cTnT, HFABP and GDF-15. Due to the limitation in serum samples remaining for analysis, Hs-cTnI was measured in 479 patients (98.36%), NTproBNP in 471 (96.71%), HSCRP in 445 (91.38%) and Galectin-3 in 386 (79.26%). Of the total population, there were 3.29 and 3.90% MACE events by 8-weeks and 6-months of discharge. These MACE events comprised of UA requiring revascularisation (1.85% at 8 weeks, 2.26% at 6 months) and AMI (1.44% at 8 weeks, 1.85% at 6 months), there were no cardiovascular mortality in this duration of follow up. The ROC curves for each biomarker are demonstrated in Figure 35 and Figure 36, for MACE at 8-weeks and 6-months respectively. These show similar traces for all biomarkers, apart from Hs-cTnI, which on review of the upper right area of the ROC curve has a favourable trace for rule out (as discussed in Chapter 1) at 8 weeks follow up, but is closer to the line of random chance at 6 months. GDF-15 also has a favourable trace for a rule-out test, but this extends to both time points of follow up. The coordinates of the ROC analysis computed were reviewed to establish the lowest value of biomarker that the first MACE occurred and are reported in Table 29. The patient with the lowest level of NTproBNP experienced a MACE at 8-weeks, meaning this biomarker was not assessed any further in this chapter. The biomarker GDF-15 and Hs-cTnI show higher numbers of patients (greater or equal to 100) when an NPV of 99.5% for 8-week MACE, which infer that these may have some utility in the rule out of early MACE. The other non-necrosis biomarkers HFABP, Galectin-3, and HSCRP, have only very low numbers of patients below the values associated with an NPV of 99.5%; therefore, are unlikely to be helpful at ruling out early MACE in this population.

Figure 35. ROC curves of biomarkers and 8-week MACE



ROC curves of each biomarker with outcome at 8 weeks

Figure 36. ROC curves of biomarkers and 6-month MACE



ROC curves of each biomarker with outcome at 6 months

Table 29. Biomarker levels and MACE

Biomarker [cohort size]	Lowest level biomarker 8-week MACE	Lowest level biomarker 6-month MACE
HFABP [487]	1.99ng/ml	1.99ng/ml
GDF-15 [487]	172.106pg/ml	172.106pg/ml
NTproBNP [471]	0.2mg/dl	0.2mg/dl
HSCRP [445]	0.03ng/ml	0.03ng/ml
Galectin-3 [386]	6.8pg/ml	6.8pg/ml
Hs-cTnI [479] total	2.3pg/ml	1.4pg/ml
Hs-cTnT [487] total	3ng/l	3ng/l

Lowest absolute value of biomarker where MACE experienced at 8-weeks and 6-months.

Table 30. ROC optimised cut-off for minimum NPV 99.5%

Biomarker (units) [cohort size]	8-week MACE	Number below this (% of cohort)	6-month MACE	Number below this (% of cohort)
HFABP [487]	1.99ng/ml	16 (3.3%)	1.99ng/ml	16 (3.3%)
GDF-15 [487]	172.106pg/ml	102 (20.9%)	172.368pg/ml	102 (20.9%)
NTproBNP [471]	-	-	-	-
HSCRP [445]	0.03ng/ml	11 (2.5%)	0.03ng/ml	11 (2.5%)
Galectin-3 [386]	6.8pg/ml	6 (1.6%)	6.8pg/ml	6 (1.6%)
Hs-cTnI [479]	3.2pg/ml	229 (47.0%)	1.4pg/ml	36 (7.5%)
Hs-cTnT [487]	3ng/l	10 (2.1%)	3ng/l	10 (2.1%)

ROC optimised cut-off of biomarker levels associated with a NPV of 99.5% for 8-week and 6-month MACE.

ROC curve analysis for Hs-cTnI and GDF-15

Further ROC analysis was performed on the biomarkers Hs-cTnI and GDF-15, in light of the more favourable ROC trace and the larger cohort of patients below the ROC optimised cut-off for a minimum NPV of 99.5%. See Figure 37 and Figure 38.

Hs-cTnI

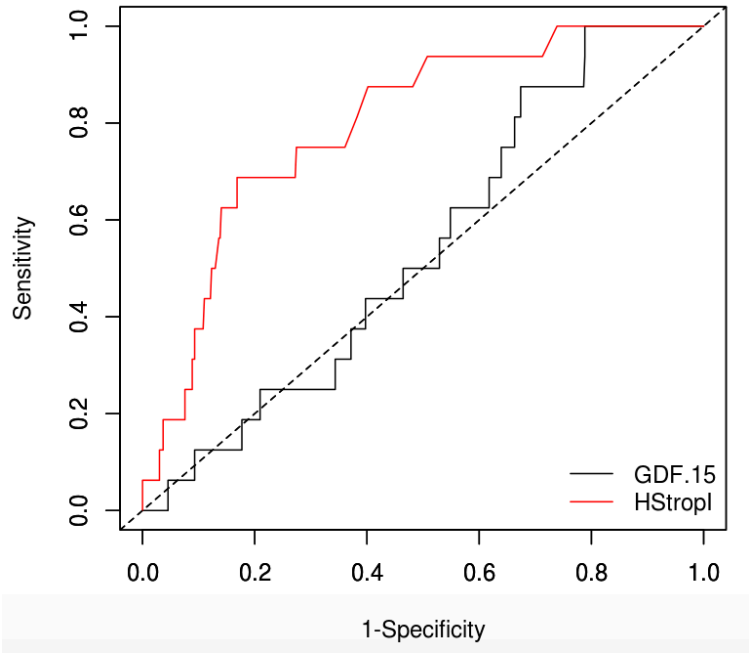
The optimal cut-off for Hs-cTnI based on an NPV for 99.5% for MACE at 8-weeks is 3.2pg/ml (with 47% of the cohort below this value) and 6-months is 1.4pg/ml (with 7.5% below this cohort).These cut-off values give a sensitivity of 93.8 (69.8-99.8) and 100 (82.4-100) for 8-week and 6-month MACE, respectively (See Table 31 and Table 32). Hs-cTnI is the only biomarker that shows a considerable difference in its ability in the rule out of early MACE between 8-weeks and 6-months; suggesting a more powerful ability to rule out very early MACE at 8-weeks, but less so at 6-months, this is reflected in the curves for Hs-cTnI shown in Figure 37 and Figure 38. The distribution graphs are shown in Figure 39 and Figure 40 and demonstrate that those who do not experience early MACE generally have lower Hs-cTnI level.

GDF-15

The optimal cut-off for GDF-15 based on an NPV for 99.5% is 172.106pg/ml for MACE at both 8-weeks and 6-months, with 21% of patients having a GDF-15 below this level. This cut-off gives a sensitivity of 100 (79.4-100) and 1.000 (82.4-100) for 8-week and 6-months MACE, respectively (See Table 33 and Table 34). The distribution graphs are shown in Figure 41; due to the profile and range of GDF-15 it is difficult to fully interpret the findings at the given scale, therefore Figure 43 shows the distribution graphs for GDF-15, specifically for levels below 400pg/ml, to better

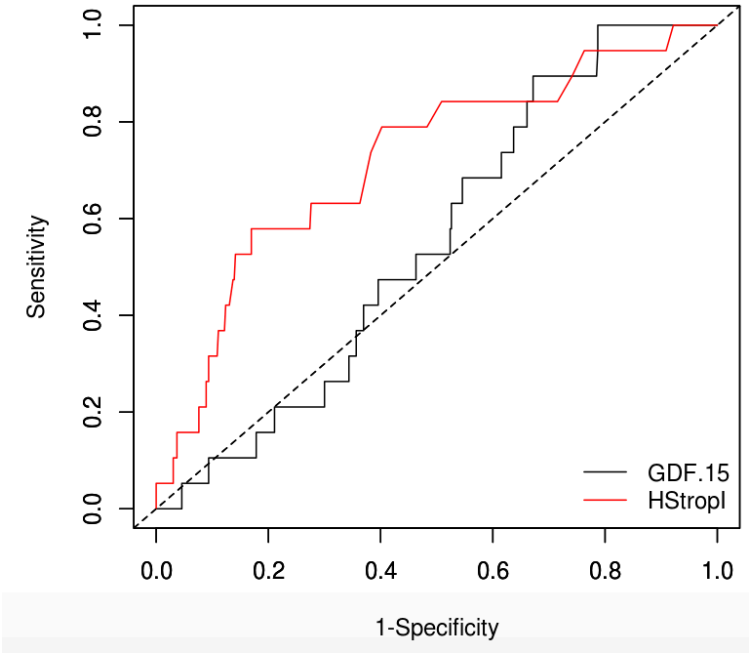
visualise the curves at lower levels, which show the large number of patients that do not experience MACE below the NPV of 99.5%.

Figure 37. ROC curve of GDF-15 and Hs-cTnI for 8-week MACE



ROC curves of biomarkers GDF15 and Hs-cTnI for MACE at 8 weeks.

Figure 38. ROC curve of GDF-15 and Hs-cTnI for 6-month MACE



ROC curves of biomarkers GDF15 and Hs-cTnI for MACE at 6 months.

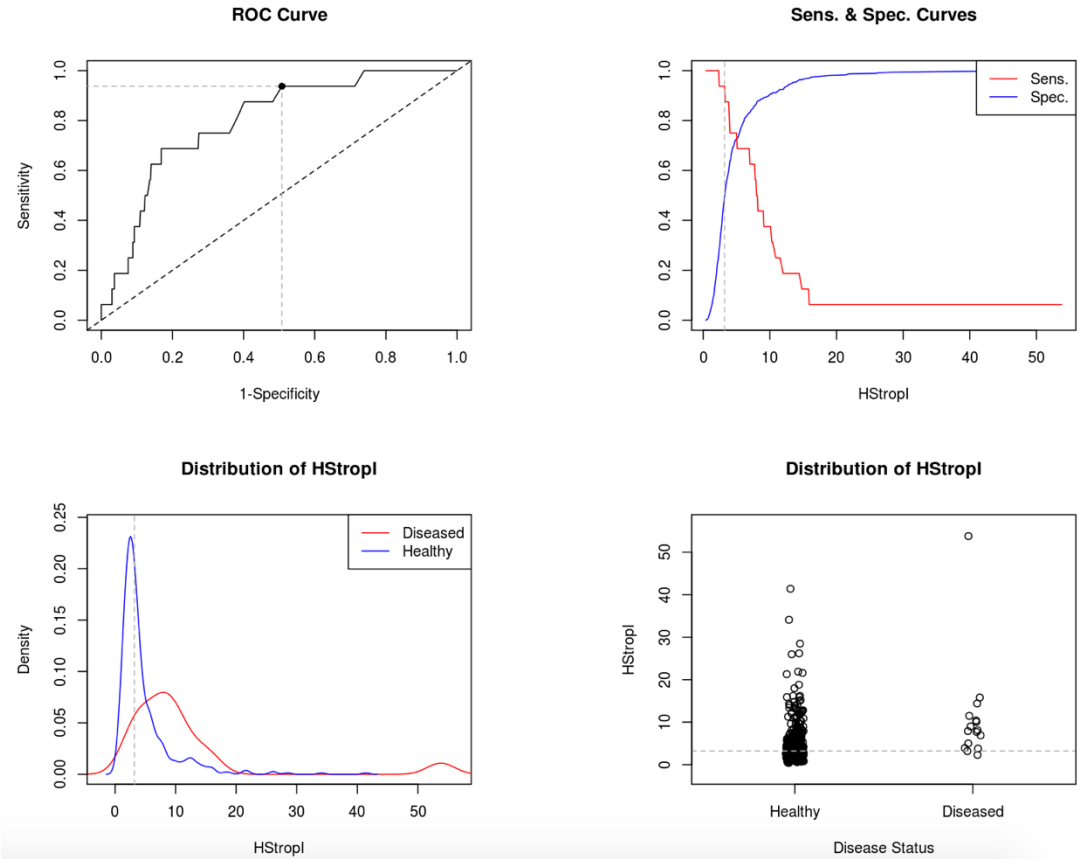
Table 31. Performance measures for Hs-cTnI and MACE at 8-weeks

```
Table 1. Cut-off Results
------------------------------------------------------------
  Optimal cut-off method : MinValueNPV
  Optimal cut-off point  : 3.2
  Optimal criterion      :
------------------------------------------------------------

Table 2. Performance Measures
-------------------------------------------------------------------------------------
                                    Value        Lower Limit     Upper Limit
-------------------------------------------------------------------------------------
Sensitivity                :          0.938          0.698          0.998
Specificity                :          0.492          0.446          0.539
Positive Predictive Value  :          0.060          0.050          0.729
Negative Predictive Value  :          0.996          0.972          0.996
Positive Likelihood Ratio  :          1.847          1.582          2.157
Negative Likelihood Ratio  :          0.127          0.019          0.849
-------------------------------------------------------------------------------------
```

Table showing optimal cut off level and associated sensitivity, specificity, positive and negative predicted value, and positive and negative likelihood ratio of biomarker according to MACE at 8 weeks according to ROC analysis.

Figure 39. Sensitivity, specificity, and distribution of Hs-cTnI



Top left. ROC curve of biomarker showing sensitivity vs 1-specificity. Top right. Plot of sensitivity and specificity. Bottom left. Distribution of biomarker level according to those with MACE and those with freedom from MACE. Bottom right. Distribution of biomarker according to outcome.

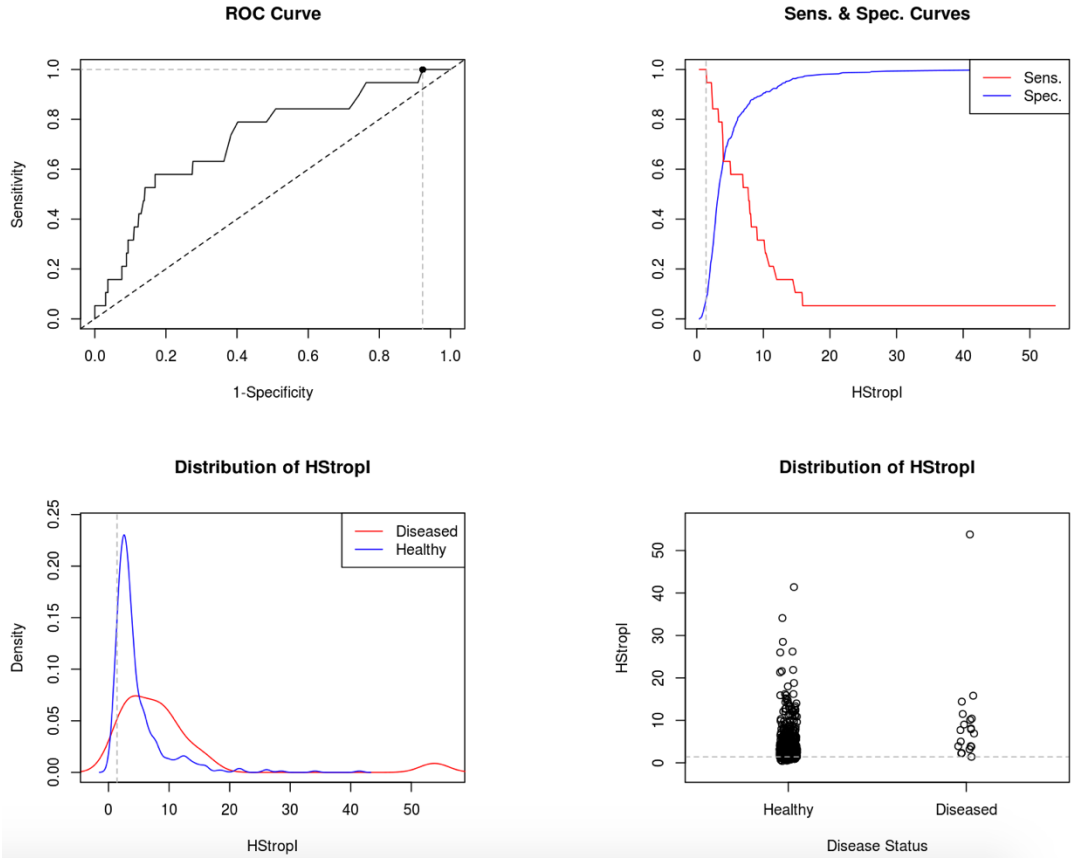
Table 32. Performance measures for Hs-cTnI and MACE at 6 months

```
Table 1. Cut-off Results
------------------------------------------------------------
 Optimal cut-off method : MinValueNPV
 Optimal cut-off point  : 1.4
 Optimal criterion      :
------------------------------------------------------------

Table 2. Performance Measures
-------------------------------------------------------------------------------------
                                  Value        Lower Limit     Upper Limit
-------------------------------------------------------------------------------------
Sensitivity                :      1.000          0.824           NaN
Specificity                :      0.078          0.055           0.107
Positive Predictive Value :       0.043          0.030           NaN
Negative Predictive Value :       1.000          0.898           1.000
Positive Likelihood Ratio :       1.085          1.056           1.114
Negative Likelihood Ratio :       0.000          0.000           NaN
-------------------------------------------------------------------------------------
```

Table showing optimal cut off level and associated sensitivity, specificity, positive and negative predicted value, and positive and negative likelihood ratio of biomarker according to MACE at 6 months according to ROC analysis.

Figure 40. Sensitivity, specificity, and distribution of Hs-cTnI at 6 months



Top left. ROC curve of biomarker showing sensitivity vs 1-specificity. Top right. Plot of sensitivity and specificity. Bottom left. Distribution of biomarker level according to those with MACE and those with freedom from MACE. Bottom right. Distribution of biomarker according to outcome.

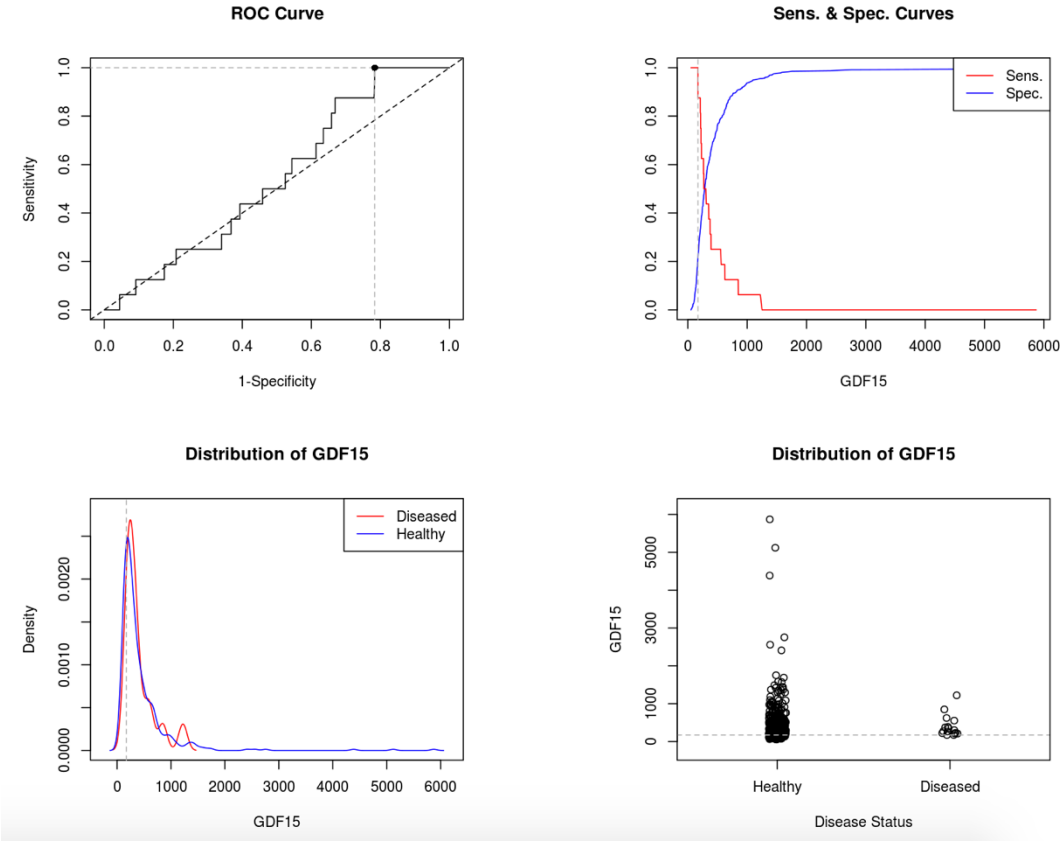
Table 33. Performance measures for GDF-15 and MACE at 8 weeks

```
 Table 1. Cut-off Results
------------------------------------------------------------
  Optimal cut-off method : MinValueNPV
  Optimal cut-off point  : 172.106
  Optimal criterion      :
------------------------------------------------------------

 Table 2. Performance Measures
-------------------------------------------------------------------------------------
                                    Value        Lower Limit     Upper Limit
-------------------------------------------------------------------------------------
 Sensitivity                :          1.000          0.794           NaN
 Specificity                :          0.217          0.180           0.257
 Positive Predictive Value  :          0.042          0.033           NaN
 Negative Predictive Value  :          1.000          0.961           1.000
 Positive Likelihood Ratio  :          1.276          1.217           1.338
 Negative Likelihood Ratio  :          0.000          0.000           NaN
-------------------------------------------------------------------------------------
```

Table showing optimal cut off level and associated sensitivity, specificity, positive and negative predicted value, and positive and negative likelihood ratio of biomarker according to MACE at 6 months according to ROC analysis.

Figure 41. Sensitivity, specificity, and distribution of GDF-15



Top left. ROC curve of biomarker showing sensitivity vs 1-specificity. Top right. Plot of sensitivity and specificity. Bottom left. Distribution of biomarker level according to those with MACE and those with freedom from MACE. Bottom right. Distribution of biomarker according to outcome.

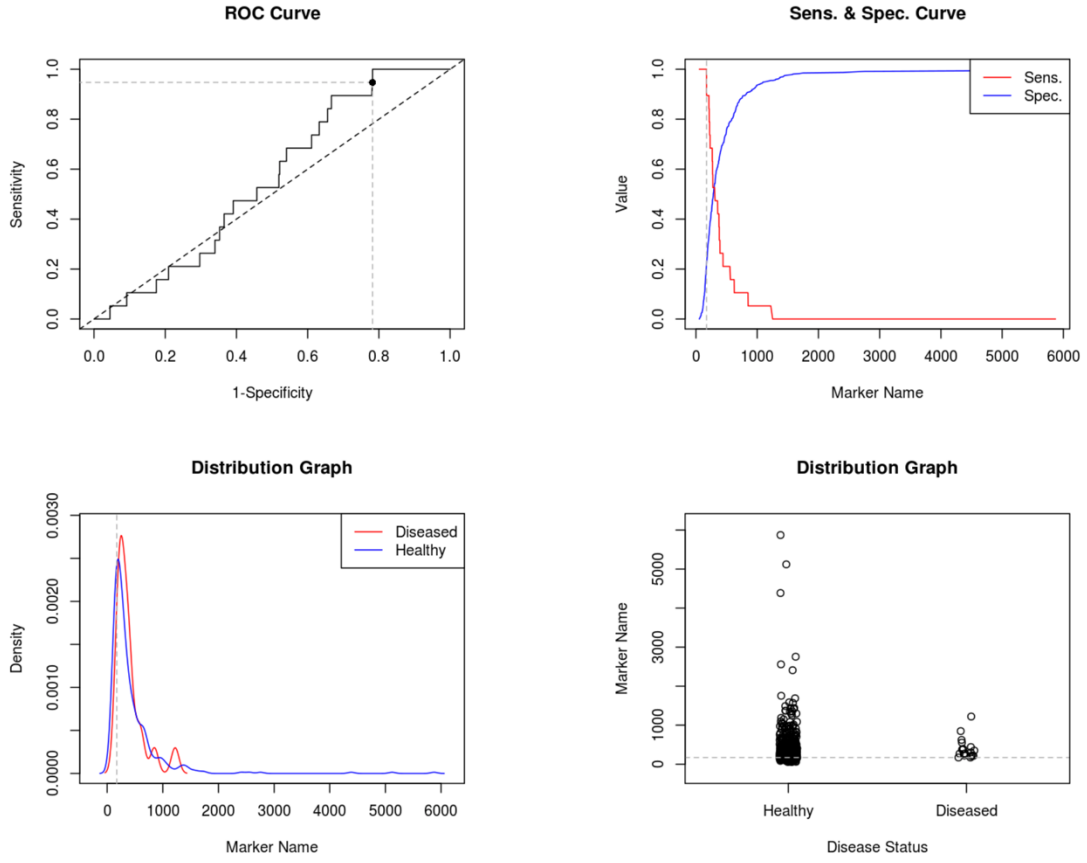
Table 34. Performance measures for GDF-15 and MACE at 6 months

```
Table 1. Cut-off Results
------------------------------------------------------------
 Optimal cut-off method : ValueNPV
 Optimal cut-off point  : 172.368
 Optimal criterion      :
------------------------------------------------------------

Table 2. Performance Measures
------------------------------------------------------------------------------------------
                                    Value        Lower Limit     Upper Limit
------------------------------------------------------------------------------------------
Sensitivity                :          0.947          0.740          0.999
Specificity                :          0.218          0.181          0.258
Positive Predictive Value :           0.047          0.038          0.672
Negative Predictive Value :           0.990          0.942          0.992
Positive Likelihood Ratio :           1.211          1.078          1.361
Negative Likelihood Ratio :           0.241          0.036          1.640
------------------------------------------------------------------------------------------
```

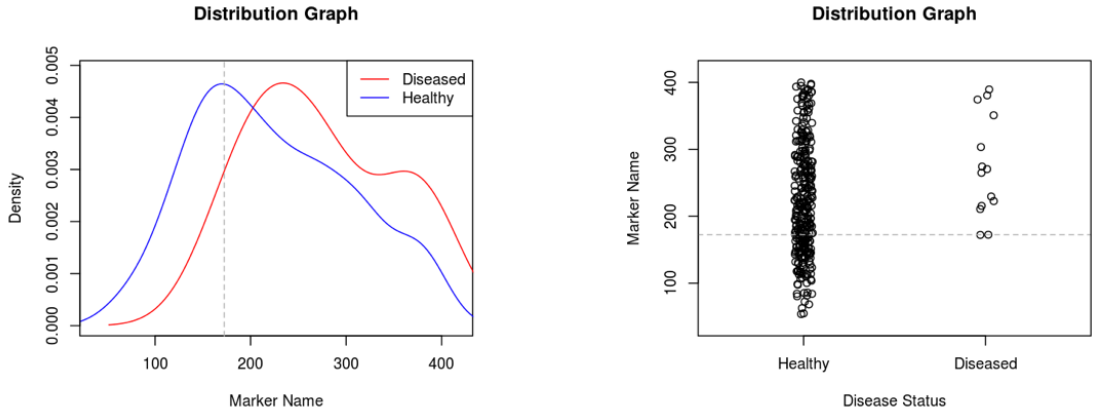
Table showing optimal cut off level and associated sensitivity, specificity, positive and negative predicted value, and positive and negative likelihood ratio of biomarker according to MACE at 6 months according to ROC analysis.

Figure 42. Sensitivity, specificity, and distribution of GDF-15



Top left. ROC curve of biomarker showing sensitivity vs 1-specificity. Top right. Plot of sensitivity and specificity. Bottom left. Distribution of biomarker level according to those with MACE and those with freedom from MACE. Bottom right. Distribution of biomarker according to outcome.

Figure 43. Distribution of GDF-15 for levels below 400pg/ml



Distribution of GDF-15 for those with/without MACE at levels below 400pg/ml to allow better visualisation of distribution between subjects.

Combinations of GDF-15, Hs-cTnI and HEART score to rule out early MACE

Table 35 shows the performance of combining GDF-15 and Hs-cTnI in the early rule out of MACE; for reference, the performance of GDF-15 and Hs-cTnI are also reported. This shows that these cut-offs and combinations do have an appropriate NPV for 8-week MACE of greater than 99.5%. This also shows that the improvement of adding GDF-15<172.106pg/ml to Hs-cTnI <3.2pg/ml increases the number of patients that could be defined as low risk from 47% of the cohort to 56.5%, with acceptable MACE rates at 8 weeks; this change is larger than what could be achieved by combining Hs-cTnI and the HEART score, however, combining Hs-cTnI, GDF-15 and HEART score values does lead to the largest cohort of patients that could be defined as low risk, with 60% of the cohort being eligible for discharge. It is important to note that the acceptable MACE rates do not extend to 6 months in these strategies, therefore early investigation/intervention would be required following discharge.

Table 35. NPV, Sensitivity, Specificity for different discharge strategies

Model	NPV for MACE 8 weeks (95% CI)	Sensitivity for MACE 8 weeks (95% CI)	Discharged N (% of cohort)	MACE 8/52 N (% of discharged)	MACE 6/12 N (% of discharged)
Hs-cTnI <3.2pg/ml	99.6 (97.2-99.6)	93.8 (69.8-99.8)	229 (47.0)	1 (0.44%)	3 (1.31%)
GDF-15 <172.106pg/ml	100 (96.1-100)	100 (0.794-NaN)	102 (20.9%)	0	0
Hs-cTnI or GDF<172.106pg/ml	99.6 (97.7-99.7)	93.8 (69.8-99.8)	275 (56.5%)	1 (0.36%)	3 (1.09%)
Hs-cTnI <3.2pg/ml or HEART <3	99.6 (97.5-99.7)	93.8 (69.8-99.8)	254 (52.2%)	1 (0.39%)	3 (1.18%)
Hs-cTnI<3.2pg/ml or HEART <3 or GDF <172.106pg/ml	99.7 (97.8-99.7)	93.8 (69.8-99.8)	293 (60.2%)	1 (0.34%)	3 (1.02%)

Table showing the NPV and sensitivity with 95% confidence intervals in rounded parenthesis, the number of patients eligible for discharge according to strategy, with proportion as a percentage in rounded parenthesis, and the MACE rates at 8 weeks and 6 months with percentages in rounded parenthesis.

Discussion

The results in this chapter show that using low level cut-off values of GDF-15 and Hs-cTnI might be useful in the early rule out of short-term MACE. GDF-15 appears to improve the number of patients that could be identified as low-risk, in addition to Hs-cTnI. Even if patients are identified as low risk of short-term MACE, care must be made that appropriate outpatient investigation and management continues. GDF-15 is a marker that is a non-specific marker that can be increased for many different reasons, such as having risk factors, any physiological stress; therefore, it is probable that levels are lower in those patients who are less likely to have significant underlying coronary disease or other significant illnesses. It is imperative to note that Hs-cTnT will have been affected by the adjustments made to the raw data because of transformations due to the technical issues with the assays as previously stated. This transformation increased the values of Hs-cTnT for 40% of the study population, which meant that those with very low levels of Hs-cTnT would have had their results amplified. This meant that even when assessing the cut-off at the level of detection, which has previously been known to have a very high sensitivity for MACE, in this population is associated with a miss rate of greater than 1%. HFABP, HSCRP, Galectin-3, NTproBNP do not appear to have a role in identifying patients who are low risk in this analysis.

Conclusion

GDF-15 may be a useful and clinically relevant biomarker in improving the rule out of early MACE in patients who present with suspected ACS and have troponin levels below the 99th percentile. Larger studies assessing GDF-15 in various rule out strategies would be warranted before its use; studies with troponins taken at early time points and in combination with delta change in troponin would be of particular interest. Other important issues to also consider would be the turnaround time of testing and cost effectiveness.

CHAPTER 7. THE INCREMENTAL ADDED VALUE OF BIOMARKERS TO RISK SCORES IN THE PREDICTION OF MACE

Introduction

The vast majority of patients presenting to the ED with suspected ACS do not have elevation of troponin.(161,168) Cardiovascular outcomes in this population, particularly in the longer term, have not been well studied. There are considerable resources deployed to risk stratify these patients, usually in an outpatient setting with cardiac imaging, with uncertain impact on outcomes.(169–172)

This chapter studies the role of serological biomarkers, either alone or in concert with composite risk scores, to effectively risk stratify this population. It is reasonable to assume that improved risk stratification may enable more targeted investigation and intervention, thereby improving outcome.

Methods

All patients were included in the analysis for Hs-cTnT, HFABP and GDF-15. Due to limitations in serum samples, Hs-cTnI was measured in 479 (98.36%), NTproBNP in 471 (96.71%), HSCRP in 445 (91.38%) and Galectin-3 in 386 (79.26%) patients. MACE was defined as a composite endpoint of unstable angina requiring revascularization, type I myocardial infarction and cardiac mortality. For definitions of MACE and method of adjudication see Chapter 2.

Univariable logistic regression was performed to assess risk scores and novel biomarkers with MACE at 3 years following discharge. The risk scores were calculated as per previous chapters, with high sensitivity troponin levels effectively not contributing as these were all below the 99th percentile. The lower levels of high sensitivity troponin levels below the 99th percentile were then added into the analysis. Logistic regression was performed as this method was defined a priori. The assumptions of logistic regression were assessed and met prior to analysis; the Box-Tidwell procedure was used to assess the linear relationship between continuous variables and the logit transformation of the dependent variable. Multicollinearity was assessed by the inspection of correlation coefficients and Variance Inflation Factor (VIF). Biomarkers values were transformed on a natural logarithmic scale prior to logistic regression.

Univariable logistic regression modelling was constructed to calculate odds ratios, 95% confidence intervals and significance level. Variables that were significant predictors of MACE at the 5% level from the univariable logistic regression analysis were used for different multivariate models. Hs-cTnT and Hs-cTnI were not assessed

in the multivariable model due to their moderate correlation. A maximum of 3 variables were used in each multivariable analysis model, to reduce the likelihood of overfitting.(173)

Independent predictors of MACE on multivariable analysis were identified by the backward stepwise (likelihood ratio) elimination method. Variables with a significance value of <0.05 were considered for entry into the model and removed if >0.10. ROC curve analysis with computation of c-statistic was used to assess model discrimination. Logistic regression models were compared using likelihood ratio test, to assess the benefit of incorporating biomarkers that were significant on univariable analysis to risk scores.

Category-free net reclassification index (NRI) was also performed using 3-year MACE to assess how well the addition of biomarkers to risk scores can improve the individual prediction of risk.

Cox Proportional Hazards Regression was performed to assess risk scores and novel biomarkers with MACE over full study follow up. Cox Proportional Hazards Regression was performed due to the lengthy follow up, that was unanticipated during the planning stage. Cox Proportional Hazards Regression takes into consideration censoring from all-cause mortality, which was higher than expected. Univariable Cox regression analysis was performed on variables that fulfil the Cox proportional-hazards (PH) assumptions, giving Hazard Ratios (HR), 95% confidence intervals and statistical significance for long term MACE.

The assumptions of Cox PH regression were assessed and met prior to analysis. Evaluation of the Cox PH assumptions were performed graphically by plotting scaled

Schoenfeld's residuals to assess their independence with transformed time (see Appendix) and confirmed using the p-value for the Schoenfeld Individual Test, with a threshold of 0.05. Continuous covariates were assessed for linearity graphically by plotting the Martingale residuals against a given covariate and using a LOWESS line. Deviance residuals were plotted to evaluate for outliers of continuous variables.

Variables with statistical significance on univariate analysis were used in the multivariable analysis. Due to the moderate correlation between Hs-cTnT and Hs-cTnI, these were not assessed in the same multivariable model. A backward Likelihood Ratio method was used to assess the independent predictors of MACE. A p-value of <0.05 was taken as an entry criterion, and a p-value of > 0.10 was taken as a removal criterion. The results are presented as a hazard ratio with 95% confidence intervals. Each multivariable model was assessed by the adjusted hazards ratio, -2log likelihood and Harrell's C-index.

SPSS version 25 was used for KM analysis, logistic regression and Cox PH regression. NRI was performed in R using the 'NRIcens' package. ROC analysis was performed using SPSS version 25 and online software easyROC(156) (based on R programming language.

Results

i) Biomarkers and MACE at 3 years

There were 36 composite MACE events at 3 years; these included 14 episodes of unstable angina requiring revascularisation and 22 adjudicated type 1 myocardial infarctions. There were no deaths due to cardiovascular causes during this follow-up.

Net Reclassification Index (NRI)

Table 36 shows the Net Reclassification Index (NRI) for the addition of each biomarker to the baseline risk scores; HEART, TIMI and GRACE. As per study design, all high sensitivity troponin T levels were below the 99th percentile, therefore high sensitivity troponin T had no bearing on the calculation of the risk score. However, the addition of high sensitivity troponin T levels below the 99th percentile were added into the model to see if these low levels of high sensitivity troponin contributed. Hs-cTnT is the only biomarker assessed that leads to an improvement in both the prediction of events and non-events, regardless of the risk score assessed. Adding Hs-cTnT to TIMI score led to a 37.63% net improvement in the prediction of a non-event and a 23.35% net improvement in the prediction of an event, with a total NRI of 0.610; an NRI above 0.6 can be considered strong improvement in risk prediction.(145) The addition of Hs-cTnT to the HEART and GRACE score show intermediate strength, as does the addition of Hs-cTnI to each risk score. The non-necrosis biomarker GDF-15 has an intermediate effect with each risk score, and this appears to be in the prediction of non-events.

Whilst the biomarkers HFABP, NTproBNP, HSCRP, Galectin-3 may hold some value in improvement in a single category (either prediction of events or non-events) this is offset by a similar magnitude of decline in the prediction of the other category, leading to a NRI that shows no overall significant effect.

Table 36. NRI of risk scores and with inclusion of biomarkers

Models	Net Reclassification Index (95% CI)	Improvement in the prediction of events (NRI_e)	Improvement in the prediction of non-events (NRI_{ne})
GRACE score + biomarker			
HFABP	0.134 (-0.201,0.418)	44.67%	-31.23%
GDF-15	0.316 (-0.271,0.636)	-14.95%	46.51%
NTproBNP	-0.056 (-0.337, 0.421)	-48.71%	43.10%
HSCRP	0.145 (-0.274,0.360)	57.64%	-43.19%
Galectin3	-0.015 (-0.180,0.479)	6.40%	-7.88%
Hs-cTnT	0.495 (0.149,0.824)	11.48%	38.00%
Hs-cTnI	0.479 (-0.111,0.590)	-11.06%	58.97%
HEART score + biomarker			
HFABP	0.052 (-0.152, 0.388)	39.10%	-33.90%
GDF-15	0.332 (-0.333, 0.673)	-14.68%	47.86%
NTproBNP	0.160 (-0.153, 0.489)	-52.91%	68.94%
HSCRP	-0.018 (-0.421, 0.406)	51.09%	-52.95%
Galectin3	-0.189 (-0.331,0.649)	-1.42%	-17.46%
Hs-cTnT	0.547 (0.180, 0.927)	17.17%	37.58%
Hs-cTnI	0.440 (-0.066, 0.777)	-11.35%	55.34%
TIMI score + biomarker			
HFABP	0.0699 (-0.154, 0.385)	39.12%	-32.12%
GDF-15	0.335 (-0.295, 0.647)	-14.79%	48.30%
NTproBNP	-0.427 (-0.882, 0.729)	-53.75%	11.00%
HSCRP	0.215 (-0.311, 1.047)	51.54%	-30.07%
Galectin3	-0.148 (-0.311, 0.568)	-1.16%	-13.51%
Hs-cTnT	0.610 (0.295, 0.899)	23.35%	37.63%
Hs-cTnI	0.347 (-0.135, 0.740))	-16.96%	51.71%

Table shows individual risk score in combination with individual biomarkers. The NRI shows the total NRI with 95% confidence intervals. NRI_e denotes the net proportion of events assigned as higher risk. NRI_{ne} denotes the net proportion of non-events assigned a lower risk.

Logistic regression

The results from the univariable analysis are displayed in Table 37. Statistically significant variables included TIMI score, HEART score, GRACE score, ln(GDF-15), ln(Hs-cTnT) and ln(Hs-cTnI). For each unit increase in ln(Hs-cTnT) and ln(Hs-cTnI) patients are 4.458 and 2.095 times more likely to experience an event ($p<0.001$), respectively. The only non-necrosis biomarker that was significant on univariable analysis was GDF-15, for each unit increase in ln(GDF-15) patients are 1.804 times more likely to experience MACE ($p=0.007$).

Table 37. Univariate logistic regression

	Total	Events	Odds ratio	95% CI	p value
TIMI	**487**	**36**	**2.727**	**1.738-2.971**	**<0.001**
HEART	**487**	**36**	**2.215**	**1.662-2.950**	**<0.001**
GRACE	**487**	**36**	**1.018**	**1.004-1.031**	**0.009**
Ln(HFABP)	487	36	1.105	0.606-2.016	0.744
Ln(GDF-15)	**487**	**36**	**1.804**	**1.174-2.770**	**0.007**
Ln(Galectin-3)	386	28	1.576	0.443-5.608	0.483
Ln(HSCRP)	445	33	0.969	0.756-1.243	0.805
Ln(NTproBNP)	471	35	1.275	0.969-1.678	0.082
Ln(Hs-cTnT)	**487**	**36**	**4.458**	**2.285-8.699**	**<0.001**
Ln(Hs-cTnI)	**479**	**36**	**2.095**	**1.360-3.228**	**<0.001**

Univariable analysis showing odds ratio of variables with 95% confidence intervals and significance.

Multivariable logistic regression models involving the HEART score

HEART score

For reference, the HEART score in isolation when assessed using logistic regression, was statistically significant, with a $\chi^2(1)$ of 36.933, p<0.001. The HEART score model had an AUC 0.787 (0.721-0.853), p<0.001.

HEART score with the inclusion of biomarkers

Log Model 1A (HEART score and Hs-cTnT)

The variables assessed included HEART score, ln(Hs-cTnT) and ln(GDF15). ln(GDF15) was removed due to lack of statistical significance.

The final variables included in Log Model 1A included HEART score and ln(Hs-cTnT). This model was statistically significant for 3 year MACE [$\chi^2(2)$=46.926, p<0.001]. Table 38 shows the results of the multivariable analysis. For each increase in unit of ln(Hs-cTnT), patients are 2.961 times more likely to experience an adverse outcome when HEART score is kept constant. The AUC for this final model was 0.829 (0.721-0.853), p<0.001 (see Figure 44). When comparing Model 1A over the baseline HEART model, there was a difference between the areas of 0.0419 with a p-value of <0.001 (see Table 42).

Table 38. Model 1A (HEART score/Hs-cTnT). Odds ratios and significance

Variable	Patients	Events	Odds ratio (95% CI)	P value
HEART	487	36	2.027 (1.506-2.727)	<0.001
ln(Hs-cTnT)	487	36	2.961 (1.480-5.924)	0.002

Adjusted odds ratio of variables within the logistic regression model with 95% confidence intervals and significance.

Log Model 1B (HEART score, Hs-cTnI and GDF-15)

The variables for Model 1B include HEART score, ln(Hs-cTnI) and ln(GDF15). All variables remained in the model. This logistic regression model was statistically significant, with a $\chi^2(2)=43.795$, $p<0.001$. Table 39 shows the results of the multivariable analysis.

With each increase in unit of ln(Hs-cTnI), patients are 1.621 times more likely to experience MACE when HEART score and ln(GDF15) are kept constant. For each unit increase in ln(GDF15) there was an increased risk of 1.590.

The AUC for Model 1B was 0.818 (0.760-0.876), $p<0.001$ (see Figure 44). The improvement between the AUC for the HEART score and Model 1B was 0.0314 with a significance level of 0.02 (see Table 42).

Table 39. Model 1B. Odds ratios and significance

Variable	Patients	Events	Odds ratio (95% CI)	P value
HEART score	479	36	2.069 (1.530-2.796)	<0.001
ln(Hs-cTnI)	479	36	1.621 (1.030-2.550)	0.037
Ln(GDF15)	479	36	1.590 (0.947-2.669)	0.080

Adjusted odds ratio of variables within the logistic regression model with 95% confidence intervals and significance.

Multivariable logistic regression models involving the TIMI score

TIMI score

For reference, the TIMI score logistic regression model was statistically significant, with a $\chi^2(1)$ of 41.605, p<0.001. The TIMI score model had an AUC 0.787 (0.709-0.865), p<0.001.

TIMI score with the inclusion of biomarkers

Log model 2A (TIMI score and Hs-cTnT)

The variables used for Model 2A include TIMI score, ln(GDF15) and ln(Hs-cTnT). After backwards selection, both TIMI score and ln(Hs-cTnT) were left in the model. This logistic regression model was statistically significant, with a $\chi^2(2) = 53.425$, $p < 0.001$. Table 40 shows the results of the multivariable analysis. The AUC for this model was 0.825 (0.762-0.888) p<0.001 (see Figure 45).

For each increase in ln(Hs-cTnT) unit, patients are 3.173 times more likely to experience an adverse outcome when TIMI score is kept constant. The improvement in the AUC between the TIMI score and Model 2A was 0.0400, with statistical significance of p<0.001 (see Table 43).

Table 40. Model 2A. Odds ratios and significance

Variable	Patients	Events	Odds ratio (95% CI)	P value
TIMI	471	36	2.162 (1.632-2.865)	<0.001
Ln(Hs-cTnT)	471	36	3.173 (1.582-6.362)	0.001

Adjusted odds ratio of variables within the logistic regression model with 95% confidence intervals and significance.

Log Model 2B (TIMI score and Hs-cTnI)

The variables used for Model 2B include TIMI score, ln(GDF15) and ln(Hs-cTnI). After backwards selection, both TIMI score and ln(Hs-cTnI) were left in the model. This logistic regression model was statistically significant, with a $\chi^2(2)=45.198$, $p<0.001$. Table 41 shows the results of the multivariable analysis. The AUC for this Model 2B was 0.801 (0.732-0.870) $p<0.001$ (see Figure 45). With each increase in unit of TIMI score, patients are 2.272 times more likely to experience an adverse outcome when other variables in the model are kept constant ($p<0.001$). With each increase in ln(Hs-cTnI) unit, patients are 1.573 times more likely to experience an adverse outcome when other variables in the model are kept constant ($p=0.008$). The improvement in the AUC between the TIMI model and Model 2B is 0.0141 with statistical significance of $p=0.028$ (see Table 43).

Table 41. Model 2B. Odds ratios and significance

Variable	Patients	Events	Odds ratio (95% CI)	P value
TIMI	465	36	2.272 (1.648-2.863)	<0.001
Ln(Hs-cTnI)	465	36	1.573 (0.999-2.476)	0.05

Adjusted odds ratio of variables within the logistic regression model with 95% confidence intervals and significance.

Logistic regression models involving the GRACE score

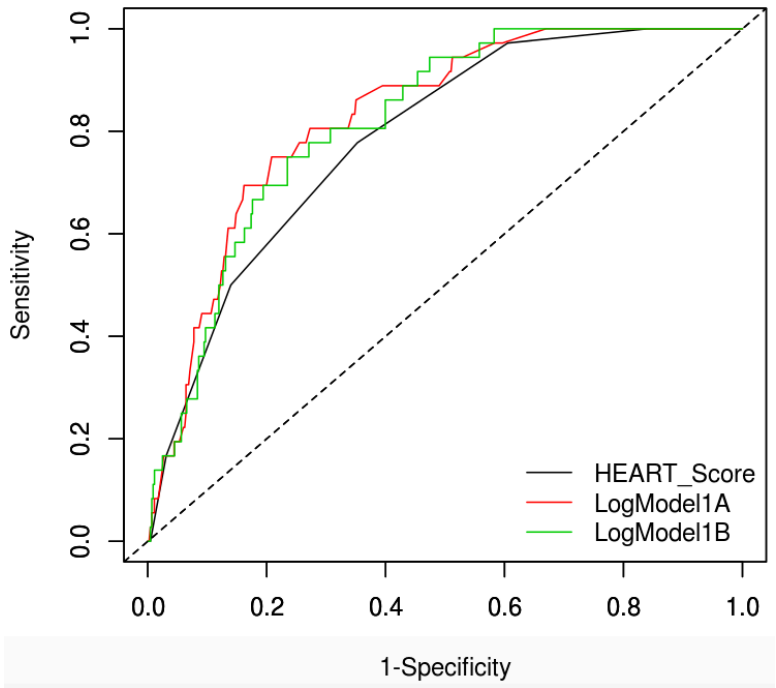
GRACE score

For reference, the GRACE score logistic model was statistically significant, with a $\chi^2(1)$ of 6.502, p=0.034. The GRACE score model had an AUC 0.629 (0.538-0.721), p=0.006.

GRACE score with inclusion of biomarkers

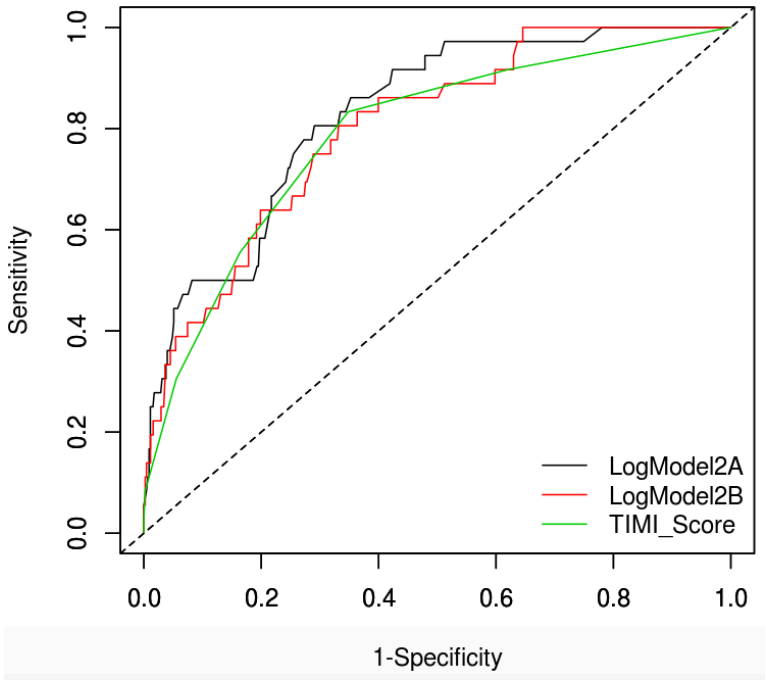
Models were assessed using GRACE score in combination with ln(NTproBNP), ln(Hs-cTnT), but regardless of combination of biomarker assessed, GRACE score was removed due to lack of statistical significance, therefore ROC analysis was not performed on models incorporating the GRACE score.

Figure 44. ROC curve of HEART models



Visual comparison of ROC curves produced by Log Model 1A [ln(Hs-cTnT) and HEART score], Log Model 1B [ln(Hs-cTnI), ln(GDF-15) and HEART score] and HEART Score.

Figure 45. ROC curve of TIMI models



Visual comparison of ROC curves produced by Log Model 2A [ln(Hs-cTnT) and TIMI score], Log Model 2B [ln(Hs-cTnI) and TIMI score] and TIMI Score.

Comparison of logistic regression models

Table 42 shows the performance of the HEART-based logistic regression models with regards to AUC and -2 log likelihood. The HEART based models that include biomarkers outperform HEART score in isolation. The HEART based model with the greatest AUC is Model 1A which incorporates HEART score and Hs-cTnT. Both Model 1A and Model 1B are statistically superior to the HEART score in isolation.

Table 43 shows the performance of the TIMI-based logistic regression models. Again, the TIMI based models that include biomarkers outperform the TIMI score in isolation. The TIMI based model with the greatest AUC is Model 2A, that incorporates TIMI score and Hs-cTnT. Model 2A and Model 2B are statistically superior to the TIMI score in isolation.

Table 42. Comparison of logistic regression HEART models

	HEART score	Model 1A (HEART score, Hs-cTnT)	Model 1B (HEART score, Hs-cTnI, GDF15)
AUC	0.786 (0.719-0.853) p<0.001	0.827 (0.768-0.886) p<0.001	0.818 (0.760-0.876) p<0.001
-2log likelihood	219.880	209.887	211.778
Significance from HEART score	-	<0.001	0.02

Table showing AUC with confidence intervals/p-values and -2log likelihood for HEART based models. Significance from baseline HEART score model calculated by likelihood ratio test.

Table 43. Comparison of logistic regression TIMI models

	TIMI score	Model 2A (TIMI score, Hs-cTnT)	Model 2B (TIMI score, Hs-cTnI)
AUC	0.786 (0.707-0.864) p<0.001	0.825 (0.762-0.888) <0.001	0.801 (0.732-0.870) <0.001
-2log likelihood	215.207	203.926	210.375
Significance from TIMI score	-	<0.001	0.028

Table showing AUC with confidence intervals/p-values and -2log likelihood for TIMI based models. Significance from baseline TIMI score model calculated by likelihood ratio test.

ii) Long term MACE

487 patients were included with a median follow up of 5.8 years. 48 patients experienced a composite end point of MACE; 16 of these consisted of UA requiring revascularisation, 27 patients experienced adjudicated myocardial infarction and 4 had cardiovascular death. 42 patients experienced all-cause mortality during follow up.

Univariable Cox Proportional Hazards Regression

Univariable Cox PH regression was used to analyse individual variables and long-term MACE to produce Hazard Ratios (HR), with confidence intervals. The following variables were significant predictors of a MACE in univariate analysis: ln(NTproBNP), ln(GDF-15), ln(Hs-cTnT), ln(Hs-cTnI), GRACE score, TIMI score and HEART score (See Table 44).

Table 44. Univariable Cox Regression analysis

Variable	Coeff	HR	95% CI	Significance
Ln(HFABP)	0.047	1.048	0.632-1.735	0.856
Ln(GDF-15)	**0.616**	**1.851**	**1.319-2.598**	**<0.001**
Ln(NTproBNP)	**0.341**	**1.407**	**1.116-1.773**	**0.004**
Ln(HSCRP)	-0.037	0.964	0.786-1.183	0.726
Ln(Galectin-3)	0.450	1.568	0.538-4.566	0.407
Ln(Hs-cTnT)	**1.317**	**3.733**	**2.184-6.381**	**<0.001**
Ln(Hs-cTnI)	**0.595**	**1.814**	**1.275-2.580**	**<0.001**
GRACE	**0.016**	**1.016**	**1.004-1.029**	**0.009**
TIMI	**0.503**	**1.654**	**1.371-1.996**	**<0.001**
HEART	**0.590**	**1.804**	**1.436-2.266**	**<0.001**

Univariable analysis of variables showing hazard ratios, 95% confidence intervals and significance.

Multivariable Cox Proportional Hazard Regression models using the HEART score

Univariable Cox proportional regression shows for each unit increase in HEART score, there is a 1.804-fold risk of MACE. HEART score as a continuous variable has a Harrell's c-index of 0.725 (see Table 49).

Cox Model 1A (HEART score, Hs-cTnT and GDF-15)

HEART score, ln(GDF15), ln(NTproBNP) and ln(Hs-cTnT) were assessed in this model in their continuous forms. After backwards selection, ln(NTproBNP) was excluded from the model due to lack of statistical significance. The final Cox Model 1A consisted of HEART score, ln(GDF-15) and ln(Hs-cTnT). The Harrell's c-index for Cox Model 1A is 0.783 (see Table 49 and Figure 46).

The results of the multivariable analysis are shown in Table 45. This shows that for each unit increase in ln(Hs-cTnT), patients are 2.512 times more likely to experience an event when HEART score and ln(GDF15) remain constant. For each unit increase in ln(GDF15), patients are 1.514 times more likely to experience an event when HEART score and ln(Hs-cTnT) are kept constant.

The improvement in the AUC between Cox Model 1A and the baseline HEART score in isolation was 0.0579 with a p-value of <0.001 (see Table 49).

Table 45. Cox Model 1A multivariable analysis

Variable	aHR	95% CI	Significance
HEART score	1.583	1.262-1.985	<0.001
Ln(Hs-cTnT)	2.512	1.445-4.366	<0.001
Ln(GDF-15)	1.514	1.003-2.287	0.048

Adjusted hazard ratio of variables within the Cox Model with 95% confidence intervals and significance.

Cox Model 1B (HEART score, Hs-cTnI and GDF-15)

HEART score, ln(GDF-15), ln(NTproBNP) and ln(Hs-cTnI) were assessed in this model. After backwards selection, again, ln(NTproBNP) was excluded from the model. The final Cox Model 1B consisted of HEART score, ln(GDF-15) and ln(Hs-cTnI). The Harrell's c-index of this model was 0.761 (see Table 50 and Figure 46).

Table 46 shows the results of the multivariable analysis. With each increase in unit of ln(Hs-cTnI), patients are 1.428 times more likely to experience MACE when ln(Hs-cTnI) and HEART score are kept constant. With each increase in unit of ln(GDF-15), patients are 1.706 times more likely to experience an adverse outcome when HEART score and ln(Hs-cTnI) are kept constant.

The improvement in the AUC between Cox Model 1B and the baseline HEART score in isolation was 0.0355 with a p-value of 0.003 (see Table 50).

Table 46. Cox Model 1B multivariable analysis

Variable	aHR	95% CI	Significance
HEART score	1.642	1.315-2.051	<0.001
Ln(Hs-cTnI)	1.428	0.990-2.060	0.057
Ln(GDF-15)	1.706	1.145-2.541	0.009

Adjusted hazard ratio of variables within the Cox Model with 95% confidence intervals and significance.

Multivariable Cox proportional regression models including the TIMI score

Univariable Cox proportional regression of TIMI score has an HR of 1.654. TIMI score as a continuous variable has a Harrell's c-index of 0.733 (see Table 50).

Cox Model 2A (TIMI score and Hs-cTnT)

TIMI score, ln(GDF-15), ln(NTproBNP) and ln(Hs-cTnT) were assessed for inclusion in the model. After backwards selection, ln(NTproBNP) and ln(GDF-15) were excluded from the model. The final Cox Model 2A consisted of TIMI score and ln(Hs-cTnT). Harrell's c-index 0.788 (0.722-0.854) (see Table 50 and Figure 47).

Table 47 shows the results of the multivariable analysis. With each increase in unit of ln(Hs-cTnT), patients are 2.789 times more likely to experience MACE when ln(Hs-cTnT) is kept constant. The improvement in the AUC between Cox Model 2A and the baseline TIMI score is 0.0552 with a p-value <0.001 (see Table 50).

Table 47. Cox Model 2A multivariable analysis

Variable	aHR	95% CI	Significance
TIMI score	1.846	1.490-2.287	P<0.001
Ln(Hs-cTnT)	2.789	1.621-4.799	P=0.001

Adjusted hazard ratio of variables within the Cox Model with 95% confidence intervals and significance.

Cox Model 2B (TIMI score, Hs-cTnI and GDF-15)

TIMI score, ln(GDF-15), ln(NTproBNP) and ln(Hs-cTnI) were assessed for inclusion in the model. After backwards selection, ln(NTproBNP) was excluded. The final Cox Model 2B consisted of TIMI score, ln(Hs-cTnI) and ln(GDF-15). Harrell's c-index was 0.768 (see Table 50 and Figure 47).

Table 48 shows the results of the multivariable analysis. With each increase in unit of ln(Hs-cTnI), patients are 1.373 times more likely to experience MACE when ln(GDF15) and TIMI score are kept constant. With each increase in unit of ln(GDF-15), patients are 1.627 times more likely to experience an adverse outcome when TIMI score and ln(Hs-cTnI) are kept constant. The improvement in the AUC between Cox Model 2B and the TIMI score in isolation is 0.0348 with a p-value <0.001 (see Table 50).

Table 48. Cox Model 2B multivariable analysis

Variable	aHR	95% CI	Significance
TIMI score	1.816	1.466-2.249	<0.001
Ln(Hs-cTnI)	1.373	0.960-1.963	0.083
Ln(GDF-15)	1.627	1.087-2.435	0.018

Adjusted hazard ratio of variables within the Cox Model with 95% confidence intervals and significance.

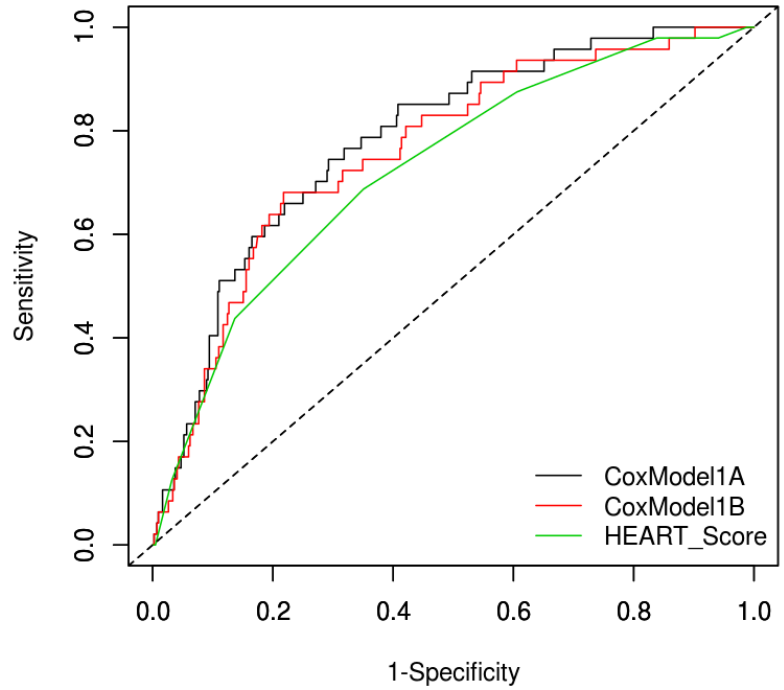
Multivariable Cox regression models including GRACE score

Univariable Cox proportional regression demonstrates that the GRACE score has a hazard ratio of 1.016. GRACE score, when assessed as a continuous variable, has a Harrell's c-index of 0.598.

GRACE score with inclusion of biomarkers

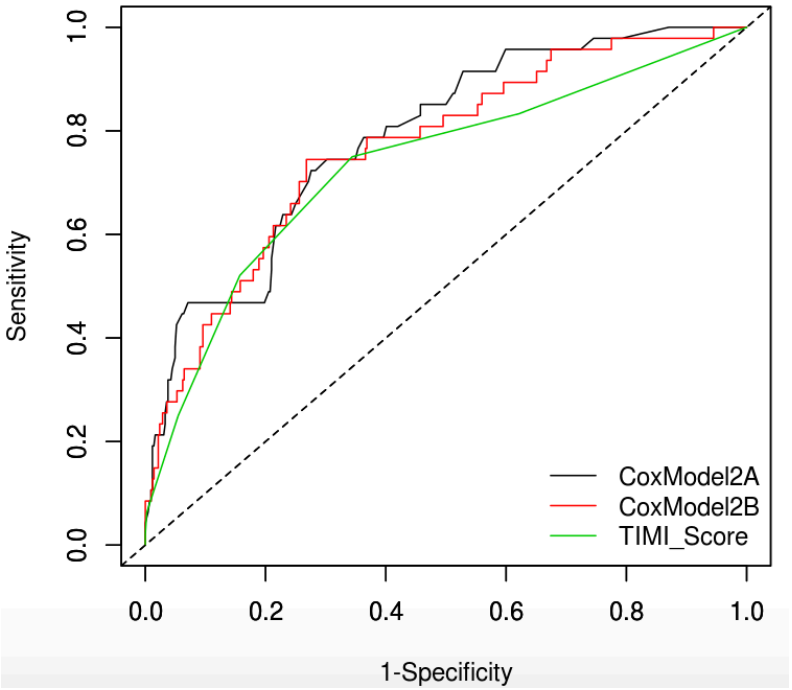
Models were assessed using GRACE score in combination with ln(GDF15), ln(Hs-cTnT), ln(Hs-cTnI) and ln(NTproBNP), but regardless of combination of biomarker assessed, GRACE score was removed by the algorithm, therefore ROC analysis was not performed on models incorporating the GRACE score.

Figure 46. ROC curves comparing HEART Score models



ROC curves produced by Cox Model 1A [ln(Hs-cTnT), ln(GDF-15) and HEART score], Cox Model 1B [ln(Hs-cTnI), ln(GDF-15) and HEART score] and HEART Score.

Figure 47. ROC curves comparing TIMI Score models



ROC curves produced by Cox Model 2A [ln(Hs-cTnT) and TIMI score], Cox Model 2B [ln(Hs-cTnI), ln(GDF-15) and TIMI score] and TIMI Score.

Comparison of Cox PH models

Table 49 shows the performance of the HEART-based Cox PH regression models with regards to AUC and -2 log likelihood. The HEART based models that include biomarkers outperform HEART score in isolation. The HEART based model with the greatest AUC is Cox Model 1A which incorporates HEART score, GDF-15 and Hs-cTnT. Both Cox Model 1A and Model 1B are statistically superior to the HEART score in isolation.

Table 50 shows the performance of the TIMI-based Cox PH regression models. Again, the TIMI based models that include biomarkers outperform the TIMI score in isolation. The TIMI based model with the greatest AUC is Cox Model 2A, that incorporates TIMI score and Hs-cTnT. Cox Model 2A and Cox Model 2B are statistically superior to the TIMI score in isolation.

Table 49. Comparison of HEART-based Cox PH regression models

	HEART score	Cox Model 1A (HEART score, Hs-cTnT, GDF15)	Cox Model 1B (HEART score, Hs-cTnI, GDF15)
AUC	0.725 (0.653-0.798)	0.783 (0.719-0.848)	0.761 (1.690-0.831)
-2log likelihood	554.424	537.856	543.032
Significance from HEART score	-	<0.001	0.003

Table showing AUC with confidence intervals/p-values and -2log likelihood for HEART-based Cox models. Significance from baseline HEART score model calculated by likelihood ratio test.

Table 50. Comparison of TIMI-based Cox PH regression models

	TIMI score	Cox Model 2A (TIMI score, Hs-cTnT)	Cox Model 2B (TIMI score, Hs-cTnI, GDF15)
AUC	0.733 (0.652-0.814)	0.788 (0.722-0.854)	0.768 (0.696-0.840)
-2log likelihood	545.862	531.676	535.472
Significance from TIMI score	-	<0.001	<0.001

Table showing AUC with confidence intervals/p-values and -2log likelihood for TIMI-based Cox models. Significance from baseline TIMI score model calculated by likelihood ratio test.

Discussion

In this analysis, several non-necrosis biomarkers linked to atherosclerosis and coronary disease were studied, to evaluate their ability to improve risk stratification for long term MACE, beyond risk scores, in patients with suspected ACS when MI has been excluded.

The TIMI and HEART score in isolation show fair discrimination in terms of predicting 3-year MACE, both with AUC of 0.786. The GRACE score in isolation has poor discrimination. The GRACE score is likely to perform poorly because of most of the variables included in this score are most likely to be normal in patients who have had MI excluded (such as cardiac arrest at admission, Killip class, troponin level) and the only variables that are likely to be contributary are age, ST depression, and creatinine.

What was very apparent in this analysis was the incremental value of high sensitivity troponins beyond the risk scores. By study design, all index Hs-cTnT values were in the normal range, below the 99th percentile. Whilst some patients still had an elevation in Hs-cTnI, nearly all (98.7%) had Hs-cTnI levels below the 99th percentile. Both the HEART and TIMI score perform well in predicting MACE in isolation, yet the incorporation of absolute levels of Hs-cTnT and Hs-cTnI, even below the 99th percentile, improves the performance of both risk scores and still offers significant prognostic information.

Of the non-necrosis biomarkers assessed, GDF-15 was the only marker that provided independent prognostic information beyond risk scores. GDF-15 was complementary

to the HEART score and Hs-cTnI, when assessed using logistic regression over 3-year follow up. Yet, it was excluded from logistic regression models including the TIMI score and high sensitivity troponin, and, the HEART score in combination with Hs-cTnT.

Similar findings were seen in the longer-term analysis looking at freedom from MACE using Cox PH regression. The TIMI and HEART score had fair discrimination with AUC of 0.725 and 0.733 respectively. The AUC was poor for GRACE score at 0.598. Again, troponins were able to improve the performance of the TIMI and HEART score.

GDF-15 was also informative over longer term freedom from MACE when assessed using Cox regression, showing that it provided incremental information beyond the HEART score and high sensitivity troponin, regardless of the assay. It also provided prognostic information above the TIMI score with Hs-cTnI model, but not with TIMI score and Hs-cTnT.

NTproBNP was a significant predictor of the long-term MACE on univariable Cox regression; however, this was no longer significant on multivariable analysis. HSCRP, Galectin-3, HFABP did not show any prognostic information for long term MACE in this analysis.

Conclusion

HEART and TIMI scores perform well in patients with normal Hs-cTnT levels, yet there is an improvement in the predictive value of these scores for longer term MACE when high sensitivity troponin levels are incorporated. GDF-15 can provide useful prognostic information beyond risk scores and high sensitivity troponins in some multivariable analysis models.

CHAPTER 8. SUMMARY, DISCUSSION AND CONCLUSION

Summary of chapters

The work in Chapter 3 describes the baseline characteristics, management and outcome of the investigative cohort using descriptive statistics. The patients who presented with suspected ACS and had MI excluded had high rates of cardiovascular risk factors and pre-existing cardiovascular disease. 85% of patients had at least one traditional cardiovascular risk factor (not including gender) and nearly a third (28%) of patients had a history of angina. In terms of clinical history, 94% of patients had characteristics of chest pain that would score 1 or 2 in the history component of the HEART score (i.e. moderate or high suspicion). Despite exclusion of MI in this cohort, the high proportion with risk factors for ischaemic heart disease and at least of moderate suspicion of myocardial ischaemia in history challenge the assumption that this is a low-risk population. There were high rates of hospital admission, prolonged hospital stays and resource utilisation. There were large numbers of patients (>40%) who were discharged without a firm diagnosis. Important non-cardiovascular diagnosis included pulmonary embolism in 2 patients, gastric ulceration in 1 patient and malignancy in 1 patient. 3% of patients experienced MACE as an inpatient during their index admission. Over four years, nearly 8.5% had experienced MACE, which was principally type 1 MI (5.95%) or revascularisation due to unstable angina (2.87%). There were no deaths due to cardiovascular causes at 4 years (all-cause mortality was 5.3% at 4 years). It was also worth noting that there was a surprisingly high number of representations with chest pain to the same AED, with 7% of patients representing by 8-weeks and 39% of patients representing by four years. Given the event rates despite a highly sensitive troponin within normal limits, at presentation,

there is a compelling case for effective risk factor stratification and in some, risk factor modification and/or revascularisation to improve outcome

Chapter 4 investigated the utility of HEART, TIMI and GRACE risk score to effectively risk stratify this population. The findings reveal that the GRACE score is a poor discriminator of risk in patients who present with chest pain and have normal Hs-cTnT levels. The TIMI and HEART scores perform well, with AUC being 0.76 and 0.74, respectively, for 8-week MACE. The HEART score has a more favourable profile than the TIMI score of ruling out future MACE, as it has the greatest ability to classify patients at low risk. A HEART score of <3 was present in 15% of patients with no patient experiencing MACE at 6 months. A score of <4 categorised 37% of the cohort, but the 6-month MACE was 0.6%

The work in Chapter 5 analyses the distribution of biomarkers and their ability to risk stratify this population. the analysis reveals that both high sensitivity troponins Hs-cTnT and Hs-cTnI, between limit of detection and 99th percentile are relatively powerful prognostic tools. Moreover these 'outperform' any of the non-necrosis biomarkers. The highest tertile of Hs-cTnT has a much higher event rate than the first and second tertile. Hs-cTnT tertile discriminated particularly well across all tertiles beyond the first 500 days. Surprisingly biomarkers linked directly to ischaemia, such as HFABP and NTproBNP, did not effectively risk stratify the population nor did those indicative of inflammation such as HSCRP. GDF-15 did hold some promise in our population. Low levels of GDF-15 (GDF-15 <172pg/ml) in this cohort, were

associated with a MACE event rate of 0% at 6 months. The second and third tertiles of GDF-15 do not discriminate well during the first 3 years of follow up but do after this time point. This could be reflection of underpowering of the sample size or an association with a 'slow burn' pathology such as atherosclerosis. There was synergy between highly sensitive troponins and GDF-15. Patients with a Hs-cTnT or GDF-15 in their respective second or third tertiles had more than a 3-fold risk of MACE compared with the first tertile. Larger cohorts would be needed to confirm the utility of GDF-15 and whether they are truly additive in a meaningful manner to highly sensitive troponins. Also, larger possibly combined data sets would allow cut-points to be explored.

Chapter 6 analyses trends for short-term MACE association with biomarkers. The study was not powered for short-term MACE and the aim of this chapter was to be hypothesis generating for future studies. The principal finding was that both Hs-cTnI and GDF-15 may have the potential to identify fair proportions of the cohort at low-risk of early MACE. A Hs-cTnI below the ROC optimised cut-off of <3.2pg/ml identified nearly half of the cohort as low-risk of MACE at 8-weeks and GDF-15 identified 21% of the cohort at low risk of early MACE. The additive role of GDF-15 to Hs-cTnI improved the cohort identified suitable for discharge from 47% to 57%.

Chapter 7 investigates longer term (>3 years) MACE and specifically whether the selected non-necrosis biomarkers and highly sensitive troponins below the 99th percentile can provide incremental prognostication beyond conventional HEART,

TIMI and GRACE risk scores. Whilst troponin levels are incorporated into each of these scores already; these are dichotomised at the 99th percentile, therefore troponin levels would not contribute to the risk scores in this study population. We specifically investigated how absolute values of troponin below the 99th percentile could add to composite risk scores in effectively risk stratifying this cohort. In univariable logistic regression at 3-year follow up, values of Hs-cTnT, Hs-cTnI, and GDF-15 were significantly correlated with MACE. On multivariable logistic regression, each risk score was improved by the addition of biomarkers. The addition of high sensitivity troponins significantly improved each of the risk scores in terms of AUC or NRI. GDF-15 was able to provide independent information in a multivariate model incorporating HEART score and Hs-cTnI. The other non-necrosis biomarkers investigated (HFABP, Galectin-3, HSCRP and NTproBNP) do not risk stratify or aid GRACE, TIMI or HEART in multivariable logistic analysis. Net Reclassification Index (NRI) was also analysed in this chapter as an additional but alternative means of assessing the value of adding a specific biomarker to risk stratification. This demonstrated Hs-cTnT, to be the only biomarker that was able to improve the risk scores' prediction of both MACE events and event free survival. Both Hs-cTnI and GDF-15 improved the prediction of event free survival, when added to the risk scores. Chapter 7 also investigated longer-term outcome, a median of 5.8 years, using Cox Proportional Hazards Models. GDF-15, NTproBNP, Hs-cTnT and Hs-cTnI were statistically significant predictors of long-term MACE in univariable analysis. In multivariable models, Hs-cTnT and Hs-cTnI remained statistically significant beyond risk scores. GDF-15 was also significant in multivariable analysis when assessed with the HEART score with either high sensitivity troponin assay and the TIMI score when assessed with Hs-cTnT.

Summary of results

I have determined that 2 of the gold-standard highly sensitive troponin assays provide prognostic value in the 'troponin-negative' suspected ACS population. Furthermore, I have demonstrated, for the first time, that Hs-cTnT and Hs-cTnI outperform many promising non-necrosis biomarkers for both short and long-term MACE and provide complementary prognostic information to composite risk scores. Larger data sets could allow development of cut-points for use in risk stratification for more targeted investigation and risk factor modification and/or revascularisation. The only non-necrosis biomarker that provides complementary information to existing means for risk stratification is GDF-15. GDF-15 was shown to be a promising biomarker primarily due to its ability to provide information beyond what can be collected as part of routine care. Limitations of adding new biomarkers to routine clinical care may include cost, increased turn around-time and there may not be meaningful difference in outcome. These results show that GDF-15 is worthy of further exploration, but the next steps would include assessing the role GDF-15 in accelerated rule out strategies then reviewing cost implications and whether there was an improvement in clinical outcomes.

Discussion

Patients who present with suspected ACS who have had MI excluded are a heterogeneous population that are not free from risk and lead to considerable health care resource utilisation. Understanding this population in greater detail is crucial to plan health care and resource health care services such as cardiac imaging. Accurate risk stratification could save lives and improve the allocation of clinical resources. Identifying patients at medium and high-risk could ascertain those who would benefit from intensive risk factor modification and/or revascularisation thus potentially improving outcome. This body of work is observational and does not demonstrate this strategy would be effective but provides a rationale to test this approach. Although the cohort could be criticised for its relatively small size it did have the advantage of being well-characterised. Knowledge of number and type of symptoms and meticulous documentation of risk factors is testament to its granularity, a feature often lacking in 'big data'.

The primary aim of this thesis was to investigate biomarkers, intimately linked to ischaemia, wall stress, inflammation that are consequences of or triggers for ACS. The hypothesis tested was whether the biomarkers incrementally improved current means of risk-stratification of patients with suspected ACS with MI excluded. This is a diverse but rarely studied subpopulation of suspected ACS. Risk scores can risk stratify patients at no additional cost, therefore, the ability of biomarkers to help beyond standard risk stratification was studied.

Risk scores

The biomarkers were assessed in addition to the TIMI, HEART and GRACE risk scores. The HEART performed best in the ability to 'rule out' future MACE. The HEART and TIMI score had a similar ability to predict future MACE. The GRACE score performed poorly in the rule out and prediction of MACE in this population (and had never been intended for use in those with MI excluded). The HEART and TIMI score do have variables that are more relevant in differentiating this population. Binary logistic regression analysis demonstrated similar AUC for both TIMI and HEART score. However, the use of the HEART score is preferable because of its ability to both identify those at low risk of short-term MACE and those high risk of long-term MACE

High sensitivity troponins

The most striking finding was the incremental value of high sensitivity troponins beyond risk scores, for risk stratification, even when the absolute values was capped by the 99th percentile. This finding was unexpected given the narrow range of absolute values of either highly sensitive troponin assay and the absence of myocyte necrosis implicated by capping at 99th percentile of Hs-cTnT. Whilst some patients still had an elevation in Hs-cTnI, nearly all (98.7%) had Hs-cTnI levels below the 99th percentile. Hs-cTnI performed best in the early rule-out of MACE allowing discharge of 47% of the cohort. Both the HEART and TIMI score perform well in predicting MACE, yet the incorporation of absolute levels of Hs-cTnT and Hs-cTnI, even below the 99th percentile, improves the performance of both risk scores and still offers significant prognostic information.

NRI also demonstrated that the high sensitivity troponins have a role in the reclassification of risk at 3 years. Hs-cTnT was able to lead to an improvement in each risk score's ability to predict both events and event free survival. Hs-cTnI enabled improved prediction of event free survival. High sensitivity troponins also improved upon composite risk score prediction of MACE when analysing outcome over the long term. To the best of our knowledge, this is the first analysis investigating the value of low levels of high sensitivity troponin assays to both TIMI and HEART scores in this cohort of suspected ACS. The mechanisms behind the prognostic role of detectable troponin levels below the 99th percentile were established in this study, yet it is most likely that this is due to the association with the severity of coronary disease and plaque burden. (51–53)

GDF-15

This body of work also provides promising data on GDF 15 for risk stratification in this population and builds on the clinical utility of this biomarker in acute chest pain. This was evident across several different assessment methods and time points of follow up. The preliminary analysis in chapter 6 alluded to GDF-15 levels being able to identify patients who are low risk of early MACE. A discharge strategy using either low levels of GDF-15 or Hs-cTnI could improve the cohort that could be discharged to 57%, when compared with using Hs-cTnI alone that would identify 47%. If these findings were replicated in larger studies, GDF-15 could be additive to low levels of highly sensitive troponins. However much larger, multicentre cohort studies in a range of health care environments are required to study in particular optimal dichotomisation for gdf-15 for rule-out.

The results in Chapter 7 demonstrate that GDF-15 had some ability to provide incremental information beyond standard risk scores in the prediction of MACE over longer term follow up. Logistic and Cox regression modelling using 3-year and 5.8 year follow up, respectively, demonstrated incremental benefit to models containing the HEART score and Hs-cTnI, when adding GDF-15. There was also an improvement in the prediction of some (but not all) models using Hs-cTnT or the TIMI score. Hs-cTnT has been identified as a superior prognostic marker to Hs-cTnI(174,175) which may account for GDF-15 to bolster Hs-cTnI containing models. The TIMI score also shows a heavier weighting to those who may have a higher risk of CAD (due to the variables 'known coronary stenosis' and 'aspirin use in the last 7 days') which are associated with higher GDF-15 levels.(104) To the best of our knowledge, this is the first study to investigate the role of GDF-15 beyond the HEART/TIMI scores in the high sensitivity troponin era. The exact pathophysiology for its prognostic impact in our population is not elucidated. However, as these patients have Hs-cTnT levels below the 99th percentile, it is more likely due to GDF-15's correlation is with atherosclerosis and plaque burden, rather than a relationship with plaque rupture.

NTproBNP

NTproBNP was a significant predictor of the long-term MACE on univariable Cox regression; however, this was no longer significant on multivariable analysis. NTproBNP may not be informative in this study because chronic heart failure was an exclusion criterion for this cohort. Patients with heart failure are more likely to have left ventricular systolic dysfunction, higher natriuretic levels and be at an adverse risk of MACE. However, the results were unexpected as wall stress due to myocardial

ischaemia is likely to trigger natriuretic peptide release and this has been demonstrated previously to be a powerful adverse predictor of outcome in MI.(125) Most of the adverse prognostic impact of natriuretic peptides have been in the MI population.(124,127,176) It is probable that other markers for MACE in this cohort, such as risk score and high sensitivity troponin level, have much greater adverse prognostic impact, in the medium and long term, thus 'drowning out' any influence of natriuretic peptides.

HFABP

HFABP was assessed in a number of ways, but there was no evidence that HFABP predicted short- or long-term MACE. Whilst Viswanathan et al.(93) had demonstrated an impressive prognostic role of HFABP in patients with normal high sensitivity troponin levels, this was not replicated in this study. There are a number of key differences in the study designs that might account for this discrepancy. Viswanathan had assessed troponin levels using Siemens Advia cTnI Ultra assay, whereas we had used Roche High Sensitivity Troponin T to base recruitment on. 5% of the patients included in their study had a history of heart failure. Heart failure was associated with higher HFABP levels and subsequent MACE. We had excluded patients with a history of heart failure. They had evaluated MACE as MI and all-mortality over a median of 18 months follow up and had an event rate of 5.29%. Although we had a lower MACE rate, by our definition, at a comparable time point, we had a similar rate of MI and all-cause mortality at 2 years (29/487 = 5.95%). HFABP has been found to be a predictor of all-cause mortality in community with a 7 year follow up,(91) which may reflect the positive findings of HFABP with their end points. The study had used a cut-off value to define a normal Hs-cTnI as below 50ng/l, but since publication, the cut-off

for 99th percentile for the assay has been reduced to 47.34ng/l, so a small number of patients may have fulfilled criteria for MI by today's standard. The lack of any signal for HFABP is surprising considering ischaemia is likely to have been prevalent in a sizable proportion given the documented symptoms and risk factors in our population. Recent evidence however has 'diminished' ischaemia as a predictor of adverse outcome compared to plaque burden.(177–179)

Galectin-3

Galectin-3 failed to show any meaningful prognostic information in this work. The studies that have shown a potential useful role of Galectin-3 in chest pain, have been in those with confirmed AMI and those who are high-risk. To our knowledge, this is the first study that has looked at the role of Galectin-3 and risk scores in patients with suspected ACS but high sensitivity troponin levels at or below the 99th percentile.

HSCRP

HSCRP did not show any prognostic information. This is similar findings to other studies who have found no role for HSCRP in chest pain patients with MI excluded.(118–120) Although inflammation is central to atherosclerosis and its progression(180) there are competing and more reliable barometers of the degree of inflammation, such as GDF-15. It is also possible competing non-cardiovascular diagnoses for chest pain and an endpoint of cardiovascular death, but not all cause mortality, 'diminished' the impact of HSCRP.

Strengths of the study

There are a number of strengths to this study. This was a prospective design of a fair population size using several statistical analysis methods. the population was well-

characterised with detailed documentation of both nature and quality of symptoms and likelihood of myocardial ischaemia. The follow-up length was also sufficient to document the temporal risk of this important subgroup of suspected ACS. Very few similar studies have documented follow-up to 5 years and beyond thus enabling a more complete profile of the outcome of these patients.

Although studied historically there are few contemporary analyses of cohorts of this size in the highly sensitive troponin era. Very few studies have analysed the much greater proportion of patients (compared to confirmed ACS or troponin positive suspected ACS). There is no pathway or consistent plan for the cohort of MI excluded chest pain presentations to accident and emergency, as opposed to confirmed MI. Whilst early discharge is appropriate targeting and/or type of investigation for underlying IHD remain variable.

This study provides novel evidence beyond risk scores. High sensitivity troponins, even in the narrow range studied could allow for targeting of investigation and intensive risk factor modification in an effort to forestall downstream MACE. The implications of this study and its analysis are clear. Targeting of investigations such as coronary CT scans to those identified as potentially high risk is useful. The burden of testing and follow-up for this largest subpopulation of suspected ACS is onerous for any healthcare system. Identification of high plaque burden or ischaemia would allow intensive treatment and risk factor modification. However, the validity of this approach need testing in large scale prospective multicentre studies and ideally for the latter strategy a randomised controlled trial. Such as study may well randomise between testing for all approach for instance with coronary CTs (with treatment and RF modification for those at risk) versus targeted investigations based on composite

risk scores allied to absolute values of high sensitivity troponins and risk factor modification and treatment for those identified. An assessment of outcome and cost-effectiveness could thus be derived.

There was assessment of multiple relevant non-necrosis biomarkers in isolation and the incremental role that these biomarkers provide beyond standard risk stratification methods, including high sensitivity troponin levels and risk scores. Two high sensitivity troponin assays that are widely available and are considered gold standard were assessed.

The MACE in this analysis was defined as cardiovascular death, non-fatal myocardial infarction and unstable angina requiring revascularisation. Cardiovascular death and non-fatal myocardial infarction are hard endpoints. Unstable angina requiring revascularisation is an important endpoint, but the clinical diagnosis is open to interpretation; however, the clinicians and operators did not have access to the novel biomarker results. The diagnosis of type 1 myocardial infarction was adjudicated by independent 2 physician assessment with blinding of index presentation troponins and biomarkers. Such an approach is considered gold standard.

Study limitations

As with all studies, there are some limitations. Hs-cTnT was analysed in real-time in keeping with clinical need, but the other biomarkers were analysed once recruitment was complete, after being stored at -70 degrees centigrade. Each of the biomarkers has been demonstrated to be stable after freeze/thaw cycles, but there may be some variation relative to the same sample taken on admission.(181–185)

A very small percentage of NTproBNP results could have been affected by calibrators. It was unclear which samples may have been affected but was approximately 1%; this is unlikely to have had a meaningful impact on the analysis. Whilst adjustments were made for the downward shift experienced by batches of the Hs-cTnT assays, some truly low values will have been erroneously amplified.

Several follow up methods were used to identify patients who had experienced MACE. Still, it is possible some patients experienced MACE outside of our geographical area that we did not identify. A small number of silent events or events occurring outside of the Merseyside region would not be expected to produce any bias to the results. Other large studies in peer-reviewed journals have used similar follow-up methods.(186)

Finally, there was additional data we had hoped to collect and analyse in this study, including exercise echocardiography, changes in biomarkers before and after exercise, and heart rate variability, but these were reluctantly forfeited to improve recruitment rates.

Future work

Despite the limitations of this work, it does highlight areas for future research. Whilst GDF-15 may improve the identification of low-risk patients; it is unclear whether this would lead to any improvement in outcome or resource utilisation. Assessing the role of novel biomarkers, particularly GDF-15, taken at earlier time points in the admission and whether these can assist or expand rapid rule-out strategies is important. Rapid testing using point of care may improve the overall length of stay and early identification of those at risk. Optimal ranges of GDF-15 would need to be established. GDF-15 may also be helpful in those with low-level elevation in troponin where MI

is excluded. The investigation of the performance of biomarkers according to gender would also be welcomed.

Conclusion

The HEART score should be used in preference over the TIMI or GRACE score for the risk stratification of patients who present with suspected ACS and have MI excluded. Absolute high sensitivity troponin levels should be incorporated to the conventional HEART score in this large subpopulation of suspected ACS to aid risk stratification. GDF-15 could help expand early safe discharge. However, studies into the clinical and cost effectiveness of this strategy are required before implementation to clinical practice.

REFERENCES

1. No Title. Br Hear Found Cardiovasc Dis Stat UK Factsheet 2015.
2. Scarborough P, Wickramasinghe K, Bhatnagar P, Rayner M. Trends in coronary heart disease , 1961-2011. Hear Dis. 2011;1961–2011.
3. Public Health England. Action plan for cardiovascular disease prevention , 2017 to 2018. Public Heal Engl. 2017;
4. WHO. Fact Sheet: cardiovascular disease. Cardiovasc Dis. 2017;
5. Wilkins E, Wilson L, Wickramasinghe K, Bhatnagar P. European Cardiovascular Disease Statistics 2017. European Heart Network. 2017.
6. WHO. Global action plan for the prevention and control of noncommunicable diseases 2013-2020. World Heal Organ. 2013;
7. Kapur N. The NHS Long Term Plan. SUSHRUTA J Heal Policy Opin. 2020;
8. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction (2018). Eur Heart J. 2019;40(3):237–69.
9. Ricci B, Cenko E, Varotti E, Emilio Puddu P, Manfrini O. Atypical Chest Pain in ACS: A Trap Especially for Women. Curr Pharm Des. 2016;
10. Goodacre SW, Locker T, Morris F, Campbell S. How useful are clinical features in the diagnosis of acute, undifferentiated chest pain? Acad Emerg Med. 2002;
11. Canto JG, Fincher C, Kiefe CI, Allison JJ, Li Q, Funkhouser E, et al. Atypical Presentations Among Medicare Beneficiaries With Unstable Angina Pectoris. Am J Cardiol. 2002;90:248–53.
12. Brieger D, Eagle KA, Goodman SG, Steg PG, Budaj A, White K, et al. Acute coronary syndromes without chest pain, an underdiagnosed and undertreated high-risk group: Insights from the global registry of acute coronary events. Chest. 2004;
13. Goodacre S, Cross E, Arnold J, Angelini K, Capewell S, Nicholl J. The health care burden of acute chest pain. Heart [Internet]. 2005 Feb [cited 2013 Sep 19];91(2):229–30. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1768669&tool=pmcentrez&rendertype=abstract
14. Murphy NF, MacIntyre K, Capewell S, Stewart S, Pell J, Chalmers J, et al. Hospital discharge rates for suspected acute coronary syndromes between 1990 and 2000: population based analysis. BMJ [Internet]. 2004 Jun 12 [cited 2013 Sep 15];328(7453):1413–4. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=421781&tool=pmcentrez&rendertype=abstract
15. MacIntyre K, Murphy NF, Chalmers J, Capewell S, Frame S, Finlayson a, et al. Hospital burden of suspected acute coronary syndromes: recent trends. Heart [Internet]. 2006 May [cited 2013 Sep 15];92(5):691–2. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1860941&tool=pmcentrez&rendertype=abstract
16. (GISSI) GI per lo S della S nell'Infarto M. Effectiveness of intravenous thrombolytic treatment in acute myocardial infarction. Lancet. 1986;1:397–402.
17. Deparment of Health. Cardiovascular Disease Outcomes Strategy Improving

outcomes for people with or at risk of cardiovascular disease. 2013;
18. Robinson JG, Wallace R, Limacher M, Sato A, Cochrane B, Wassertheil-Smoller S, et al. Elderly women diagnosed with nonspecific chest pain may be at increased cardiovascular risk. J Women's Heal. 2006;
19. Sekhri N, Feder GS, Junghans C, Hemingway H, Timmis a D. How effective are rapid access chest pain clinics? Prognosis of incident angina and non-cardiac chest pain in 8762 consecutive patients. Heart [Internet]. 2007 Apr [cited 2013 Sep 15];93(4):458–63. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1861500&tool=pmcentrez&rendertype=abstract
20. Wilhelmsen L, Rosengren A, Hagman M, Lappas G. "Nonspecific" chest pain associated with high long-term mortality: Results from the primary prevention study in Goteborg, Sweden. Clin Cardiol. 1998;
21. Ruddox V, Mathisen M, Otterstad JE. Prevalence and prognosis of non-specific chest pain among patients hospitalized for suspected acute coronary syndrome - a systematic literature search. BMC Med. 2012;
22. Ravn-Fischer A, Karlsson T, Santos M, Bergman B, Johanson P, Herlitz J. Chain of care in chest pain - Differences between three hospitals in an urban area. Int J Cardiol [Internet]. 2013;166(2):440–7. Available from: http://dx.doi.org/10.1016/j.ijcard.2011.10.139
23. Libby P. Inflammation and Atherosclerosis. Circulation [Internet]. 2002 Mar 5 [cited 2013 Aug 6];105(9):1135–43. Available from: http://circ.ahajournals.org/cgi/doi/10.1161/hc0902.104353
24. Falk E. Pathogenesis of Atherosclerosis. Vol. 47, Journal of the American College of Cardiology. 2006.
25. DeGraba TJ. Immunogenetic susceptibility of atherosclerotic stroke: Implications on current and future treatment of vascular inflammation. Stroke. 2004;35(11 SUPPL. 1):2712–9.
26. White SJ, Newby AC, Johnson TW. Endothelial erosion of plaques as a substrate for coronary thrombosis. Thromb Haemost. 2016;115(3):509–19.
27. Moreno PR, Falk E, Palacios IF, Newell JB, Fuster V, Fallon JT. Macrophage infiltration in acute coronary syndromes. Implications for plaque rupture. Circulation. 1994;90(2):775–8.
28. Kolodgie FD, Burke AP, Farb A, Weber DK, Kutys R, Wight TN, et al. Differential accumulation of proteoglycans and hyaluronan in culprit lesions: Insights into plaque erosion. Arterioscler Thromb Vasc Biol. 2002;22(10):1642–8.
29. Arbustini E, Dal Bello B, Morbini P, Burke a P, Bocciarelli M, Specchia G, et al. Plaque erosion is a major substrate for coronary thrombosis in acute myocardial infarction. Heart [Internet]. 1999;82(3):269–72. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1729173&tool=pmcentrez&rendertype=abstract
30. Burke, AP. Farb, A. Malcom, GT. Liang, Y. Smialek, J. Virmani R. Effect of risk factors on the mechanism of acute thrombosis and sudden coronary death in women. Circulation. 1998;97(21):2110.
31. Glaser R, Selzer F, Faxon DP, Laskey WK, Cohen HA, Slater J, et al. Clinical progression of incidental, asymptomatic lesions discovered during culprit vessel coronary intervention. Circulation. 2005;111(2):143–9.
32. Burke a P, Farb a, Malcom GT, Liang YH, Smialek J, Virmani R. Coronary risk factors and plaque morphology in men with coronary disease who died

suddenly. N Engl J Med. 1997;336(18):1276–82.
33. OpenStax, CC BY 4.0 <https://creativecommons.org/licenses/by/4.0> via WC. Thick and thin filaments [Internet]. Available from: https://commons.wikimedia.org/wiki/File:1003_Thick_and_Thin_Filaments.jpg
34. OpenStax, CC BY 4.0 <https://creativecommons.org/licenses/by/4.0> via WC. Skeletal muscle contraction [Internet]. [cited 2021 Jun 14]. Available from: https://commons.wikimedia.org/wiki/File:1008_Skeletal_Muscle_Contraction.jpg
35. Katus HA, Remppis A, Scheffold T, Diederich KW, Kuebler W. Intracellular compartmentation of cardiac troponin T and its release kinetics in patients with reperfused and nonreperfused myocardial infarction. AmJCardiol. 1991 Jun 15;67(0002-9149 (Print)):1360–7.
36. Adams III JE, Schechtman KB, Landt Y, Ladenson JH, Jaffe AS. Comparable detection of acute myocardial infarction by creatine kinase MB isoenzyme and cardiac troponin I. ClinChem. 1994 Jul;40(0009-9147 (Print)):1291–5.
37. Wu AH, Feng YJ. Biochemical differences between cTnT and cTnI and their significance for diagnosis of acute coronary syndromes. EurHeart J. 1998 Nov;19 Suppl N(0195-668X (Print)):N25–9.
38. Heuser J. AMI bloodtests engl [Internet]. [cited 2021 Jun 14]. Available from: https://commons.wikimedia.org/wiki/File:AMI_bloodtests_engl.png
39. Mair J, Thome-Kromer B, Wagner I, Lechleitner P, Dienstl F, Puschendorf B, et al. Concentration time courses of troponin and myosin subunits after acute myocardial infarction. CoronArtery Dis. 1994 Oct;5(0954-6928 (Print)):865–72.
40. Solecki K, Dupuy AM, Kuster N, Leclercq F, Gervasoni R, Macia JC, et al. Kinetics of high-sensitivity cardiac troponin T or troponin I compared to creatine kinase in patients with revascularized acute myocardial infarction. Clin Chem Lab Med. 2015;53(5):707–14.
41. Mair J, Wagnera I, Jakob G, Lechleitnerb P, Dienstlb F, Puschendorf B, et al. Different time courses of cardiac contractile proteins after acute myocardial infarction *. 1994;231:47–60.
42. Seino Y, Tomita Y, Hoshino K, Setsuta K, Takano T, Hayakawa H. Pathophysiological analysis of serum troponin T release kinetics in evolving ischemic myocardial injury. JpnCircJ. 1996 May;60(0047-1828 (Print)):265–76.
43. Michielsen ECHJ, Diris JHC, Kleijnen VWVC, Wodzig WKWH, Dieijen-visser MP Van. Investigation of release and degradation of cardiac troponin T in patients with acute myocardial infarction. 2007;40:851–5.
44. Younger J, Plein S, Barth J, Ridgway J. Troponin-I concentration 72 h after myocardial infarction correlates with infarct size and presence of microvascular obstruction. Heart [Internet]. 2007 [cited 2013 Oct 4];93(12):1547–51. Available from: http://heart.bmj.com/content/93/12/1547.short
45. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, et al. Early Diagnosis of Myocardial Infarction with Sensitive Cardiac Troponin Assays. N Engl J Med. 2009;
46. Ebell MH, White LL, Weismantel D. A systematic review of troponin T and I values as a prognostic tool for patients with chest pain. J Fam Pr [Internet]. 2000;49(8):746–53. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&

dopt=Citation&list_uids=10947143
47. Lindahl B, Venge P, James S. The new high-sensitivity cardiac troponin T assay improves risk assessment in acute coronary syndromes. Am Heart J. 2010;160(2):224–9.
48. May EAB, Bonaca MP, Jarolim P, Antman EM, Braunwald E, Giugliano RP, et al. Prognostic performance of a high-sensitivity cardiac troponin i assay in patients with Non-ST-Elevation acute coronary syndrome. Clin Chem. 2014;60(1):158–64.
49. Sanchis J, Bodí V, Llácer A, Núñez J, Consuegra L, Bosch MJ, et al. Risk stratification of patients with acute chest pain and normal troponin concentrations. Heart [Internet]. 2005;91(8):1013–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1769052&tool=pmcentrez&rendertype=abstract
50. Beatty AL, Ku IA, Christenson RH, DeFilippi CR, Schiller NB, Whooley MA. High-sensitivity cardiac troponin t levels and secondary events in outpatients with coronary heart disease from the heart and soul study. JAMA Intern Med. 2013;
51. Oemrawsingh RM, Cheng JM, García-García HM, Kardys I, van Schaik RHN, Regar E, et al. High-sensitivity Troponin T in relation to coronary plaque characteristics in patients with stable coronary artery disease; results of the ATHEROREMO-IVUS study. Atherosclerosis. 2016;
52. Januzzi JL, Suchindran S, Coles A, Ferencik M, Patel MR, Hoffmann U, et al. High-Sensitivity Troponin I and Coronary Computed Tomography in Symptomatic Outpatients With Suspected CAD: Insights From the PROMISE Trial. JACC Cardiovasc Imaging. 2019;
53. Bouzas-Mosquera A, Peteiro J, Broullón FJ, Constanso IP, Rodríguez-Garrido JL, Martínez D, et al. Troponin levels within the normal range and probability of inducible myocardial ischemia and coronary events in patients with acute chest pain. Eur J Intern Med. 2016;
54. Roos A, Bandstein N, Lundbäck M, Hammarsten O, Ljung R, Holzmann MJ. Stable High-Sensitivity Cardiac Troponin T Levels and Outcomes in Patients With Chest Pain. J Am Coll Cardiol. 2017;
55. Body R, Carlton E, Sperrin M, Lewis PS, Burrows G, Carley S, et al. Troponin-only Manchester Acute Coronary Syndromes (T-MACS) decision aid: Single biomarker re-derivation and external validation in three cohorts. Emerg Med J. 2017;
56. Collet J-P, Thiele H, Barbato E, Barthélémy O, Bauersachs J, Bhatt DL, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J. 2020;
57. Shah ASV, Anand A, Strachan FE, Ferry A V., Lee KK, Chapman AR, et al. High-sensitivity troponin in the evaluation of patients with suspected acute coronary syndrome: a stepped-wedge, cluster-randomised controlled trial. Lancet. 2018;392:919–28.
58. NICE. High-sensitivity troponin tests for the early rule out of NSTEMI. 2020;(August 2020):42.
59. Than M, Herbert M, Flaws D, Cullen L, Hess E, Hollander JE, et al. What is an acceptable risk of major adverse cardiac event in chest pain patients soon after discharge from the Emergency Department? A clinical survey. Int J Cardiol. 2013;
60. Wildi K, Boeddinghaus J, Nestelberger T, Twerenbold R, Badertscher P,

Wussler D, et al. Comparison of fourteen rule-out strategies for acute myocardial infarction. Int J Cardiol. 2019;
61. Pickering JW, Than MP, Cullen L, Aldous S, Ter Avest E, Body R, et al. Rapid rule-out of acute myocardial infarction with a single high-sensitivity cardiac troponin t measurement below the limit of detection: A collaborative meta-analysis. Ann Intern Med. 2017;166(10):715–24.
62. Gray AJ, Roobottom C, Smith JE, Goodacre S, Oatey K, O'Brien R, et al. Early computed tomography coronary angiography in patients with suspected acute coronary syndrome: Randomised controlled trial. BMJ. 2021;374:1–12.
63. Poldervaart JM, Langedijk M, Backus BE, Dekker IMC, Six AJ, Doevendans PA, et al. Comparison of the GRACE, HEART and TIMI score to predict major adverse cardiac events in chest pain patients at the emergency department. Int J Cardiol. 2017;
64. Six AJ, Backus BE, Kelder JC. Chest pain in the emergency room: Value of the HEART score. Netherlands Hear J. 2008;
65. Antman EM, Cohen M, Bernink PJ, McCabe CH, Horacek T, Papuchis G, et al. The TIMI risk score for unstable angina/non-ST elevation MI: A method for prognostication and therapeutic decision making. JAMA. 2000 Aug 16;284(0098-7484 (Print)):835–42.
66. Fox K a a, Dabbous OH, Goldberg RJ, Pieper KS, Eagle K a, Van de Werf F, et al. Prediction of risk of death and myocardial infarction in the six months after presentation with acute coronary syndrome: prospective multinational observational study (GRACE). BMJ [Internet]. 2006 Nov 25 [cited 2013 Sep 17];333(7578):1091. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1661748&tool=pmcentrez&rendertype=abstract
67. Scirica BM, Cannon CP, Antman EM, Murphy SA, Morrow DA, Sabatine MS, et al. Validation of the Thrombolysis In Myocardial Infarction (TIMI) risk score for unstable angina pectoris and non-ST-elevation myocardial infarction in the TIMI III registry. Am J Cardiol. 2002;
68. Chase M, Robey JL, Zogby KE, Sease KL, Shofer FS, Hollander JE. Prospective Validation of the Thrombolysis in Myocardial Infarction Risk Score in the Emergency Department Chest Pain Population. Ann Emerg Med. 2006;
69. Pollack C V., Sites FD, Shofer FS, Sease KL, Hollander JE. Application of the TIMI risk score for unstable angina and non-ST elevation acute coronary syndrome to an unselected emergency department chest pain population. Acad Emerg Med. 2006;
70. Tang EW, Wong CK, Herbison P. Global Registry of Acute Coronary Events (GRACE) hospital discharge risk score accurately predicts long-term mortality post acute coronary syndrome. Am Heart J. 2007;
71. Huang W, FitzGerald G, Goldberg RJ, Gore J, McManus RH, Awad H, et al. Performance of the GRACE Risk Score 2.0 Simplified Algorithm for Predicting 1-Year Death After Hospitalization for an Acute Coronary Syndrome in a Contemporary Multiracial Cohort. Am J Cardiol. 2016;
72. Backus BE, Six AJ, Kelder JC, Bosschaert MAR, Mast EG, Mosterd A, et al. A prospective validation of the HEART score for chest pain patients at the emergency department. Int J Cardiol. 2013;
73. Backus BE, Six AJ, Kelder JC, Mast TP, Van Den Akker F, Mast EG, et al. Chest pain in the emergency room: A multicenter validation of the heart score.

Crit Pathw Cardiol. 2010;
74. Fernando SM, Tran A, Cheng W, Rochwerg B, Taljaard M, Thiruganasambandamoorthy V, et al. Prognostic Accuracy of the HEART Score for Prediction of Major Adverse Cardiac Events in Patients Presenting With Chest Pain: A Systematic Review and Meta-analysis. Academic Emergency Medicine. 2019.
75. Wamala H, Aggarwal L, Bernard A, Scott IA. Comparison of nine coronary risk scores in evaluating patients presenting to hospital with undifferentiated chest pain. Int J Gen Med. 2018;
76. Liu N, Ng JCJ, Ting CE, Sakamoto JT, Ho AFW, Koh ZX, et al. Clinical scores for risk stratification of chest pain patients in the emergency department: an updated systematic review. J Emerg Crit Care Med. 2018;
77. Long B, Koyfman A. Best Clinical Practice: Current Controversies in the Evaluation of Low-Risk Chest Pain with Risk Stratification Aids. Part 2. Journal of Emergency Medicine. 2017.
78. Carlton EW, Khattab A, Greaves K. Identifying patients suitable for discharge after a single-presentation high-sensitivity troponin result: A comparison of five established risk scores and two high-sensitivity assays. Ann Emerg Med. 2015;66(6):635-645e1.
79. Reaney PDW, Elliott HI, Noman A, Cooper JG. Risk stratifying chest pain patients in the emergency department using HEART, GRACE and TIMI scores, with a single contemporary troponin result, to predict major adverse cardiac events. Emergency Medicine Journal. 2018;
80. Poldervaart JM, Langedijk M, Backus BE, Dekker IMC, Six AJ, Doevendans PA, et al. Comparison of the GRACE, HEART and TIMI score to predict major adverse cardiac events in chest pain patients at the emergency department. Int J Cardiol. 2017;
81. Chen Y, Tao Y, Zhang L, Xu W, Zhou X. Diagnostic and prognostic value of biomarkers in acute myocardial infarction. Postgrad Med J. 2019;95(1122):210–6.
82. Morrow DA, Cannon CP, Jesse RL, Newby LK, Ravkilde J, Storrow AB, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Clinical characteristics and utilization of biochemical markers in acute coronary syndromes. Clinical Chemistry. 2007.
83. Glatz JF, Van BM, Paulussen RJ, Veerkamp JH, van der Vusse GJ, Reneman RS. Release of fatty acid-binding protein from isolated rat heart subjected to ischemia and reperfusion or to the calcium paradox. BiochimBiophysActa. 1988 Jul 1;961(0006-3002 (Print)):148–52.
84. Storch J, Thumser AE. The fatty acid transport function of fatty acid-binding proteins. BiochimBiophysActa. 2000 Jun 26;1486(0006-3002 (Print)):28–44.
85. Veerkamp JH, Peeters R a, Maatman RG. Structural and functional features of different types of cytoplasmic fatty acid-binding proteins. Biochim Biophys Acta [Internet]. 1991 Jan 4;1081(1):1–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1991151
86. Pelsers MM, Hermens WT, Glatz JF. Fatty acid-binding proteins as plasma markers of tissue injury. ClinChimActa. 2005 Feb;352(0009-8981 (Print)):15–35.
87. Kleine a H, Glatz JF, Van Nieuwenhoven F a, Van der Vusse GJ. Release of heart fatty acid-binding protein into plasma after acute myocardial infarction in man. Mol Cell Biochem [Internet]. 1992 Oct 21;116(1–2):155–62. Available

from: http://www.ncbi.nlm.nih.gov/pubmed/1480144
88. Ming M, Meng X. [Immunohistochemical observation of heart-type fatty acid binding-protein in early myocardial ischemia]. Fa Yi Xue Za Zhi [Internet]. 2004 Jan [cited 2013 Oct 4];20(1):15–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15150874
89. Meng X, Ming M, Wang E. Heart fatty acid binding protein as a marker for postmortem detection of early myocardial damage. Forensic Sci Int [Internet]. 2006 Jun 27 [cited 2013 Oct 4];160(1):11–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16182485
90. Kilcullen N, Viswanathan K, Das R, Morrell C, Farrin A, Barth JH, et al. Heart-type fatty acid-binding protein predicts long-term mortality after acute coronary syndrome and identifies high-risk patients across the range of troponin values. J Am Coll Cardiol [Internet]. 2007 Nov 20 [cited 2013 Sep 17];50(21):2061–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18021874
91. Otaki Y, Watanabe T, Takahashi H, Hirayama A, Narumi T, Kadowaki S, et al. Association of heart-type fatty acid-binding protein with cardiovascular risk factors and all-cause mortality in the general population: The Takahata study. PLoS One. 2014;
92. Body R, Carley S, McDowell G, Pemberton P, Burrows G, Cook G, et al. The Manchester Acute Coronary Syndromes (MACS) decision rule for suspected cardiac chest pain: Derivation and external validation. Heart. 2014;
93. Viswanathan K, Kilcullen N, Morrell C, Thistlethwaite SJ, Sivananthan MU, Hassan TB, et al. Heart-type fatty acid-binding protein predicts long-term mortality and re-infarction in consecutive patients with suspected acute coronary syndrome who are troponin-negative. J Am Coll Cardiol [Internet]. 2010 Jun 8 [cited 2013 Sep 17];55(23):2590–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20513600
94. Jones JD, Chew PG, Dobson R, Wootton A, Ashrafi R, Khand A. The Prognostic Value of Heart Type Fatty Acid Binding Protein in Patients with Suspected Acute Coronary Syndrome: A Systematic Review. Curr Cardiol Rev. 2017;
95. Claassens DMF, Coers DO, Bergmeijer TO, Chan Pin Yin DRPP, Ten Berg JM. Is there a place for heart-type fatty acid binding protein in the era of high-sensitive cardiac troponin T for the diagnosis of acute myocardial infarction? A systematic review. Emerg Care J [Internet]. 2019;15(2):51–6. Available from: https://www.pagepressjournals.org/index.php/ecj/article/view/8049
96. Van Hise CB, Greenslade JH, Parsonage W, Than M, Young J, Cullen L. External validation of heart-type fatty acid binding protein, high-sensitivity cardiac troponin, and electrocardiography as rule-out for acute myocardial infarction. Clin Biochem. 2018;
97. Kempf T, Eden M, Strelau J, Naguib M, Willenbockel C, Tongers J, et al. The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. Circ Res [Internet]. 2006 Feb 17 [cited 2013 Sep 6];98(3):351–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16397141
98. Xu J, Kimball TR, Lorenz JN, Brown D a, Bauskin AR, Klevitsky R, et al. GDF15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation. Circ Res [Internet]. 2006 Feb 17 [cited 2013 Sep 5];98(3):342–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16397142

99. Fluschnik N, Ojeda F, Zeller T, Jørgensen T, Kuulasmaa K, Becher PM, et al. Predictive value of long-term changes of growth differentiation factor-15 over a 27-year-period for heart failure and death due to coronary heart disease. PLoS One. 2018;
100. Lindholm D, James SK, Gabrysch K, Storey RF, Himmelmann A, Cannon CP, et al. Association of Multiple Biomarkers with Risk of All-Cause and Cause-Specific Mortality after Acute Coronary Syndromes: A Secondary Analysis of the PLATO Biomarker Study. JAMA Cardiol. 2018;
101. Wollert KC, Kempf T, Peter T, Olofsson S, James S, Johnston N, et al. Prognostic value of growth-differentiation factor-15 in patients with non-ST-elevation acute coronary syndrome. Circulation [Internet]. 2007 Feb 27 [cited 2013 Aug 22];115(8):962–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17283261
102. Eggers KM, Kempf T, Allhoff T, Lindahl B, Wallentin L, Wollert KC. Growth-differentiation factor-15 for early risk stratification in patients with acute chest pain. Eur Heart J [Internet]. 2008 Oct [cited 2013 Sep 15];29(19):2327–35. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2556729&tool=pmcentrez&rendertype=abstract
103. Zhang S, Dai D, Wang X, Zhu H, Jin H, Zhao R, et al. Growth differentiation factor-15 predicts the prognoses of patients with acute coronary syndrome: A meta-analysis. BMC Cardiovasc Disord. 2016;
104. Tzikas S, Palapies L, Bakogiannis C, Zeller T, Sinning C, Baldus S, et al. GDF-15 predicts cardiovascular events in acute chest pain patients. PLoS One. 2017;
105. Widera C, Pencina MJ, Meisner A, Kempf T, Bethmann K, Marquardt I, et al. Adjustment of the GRACE score by growth differentiation factor 15 enables a more accurate appreciation of risk in non-ST-elevation acute coronary syndrome. Eur Heart J. 2012;33(9):1095–104.
106. Hagström E, Held C, Stewart RAH, Aylward PE, Budaj A, Cannon CP, et al. Growth differentiation factor 15 predicts all-cause morbidity and mortality in stable coronary heart disease. Clin Chem. 2017;
107. Schaub N, Reichlin T, Twerenbold R, Reiter M, Steuer S, Bassetti S, et al. Growth differentiation factor-15 in the early diagnosis and risk stratification of patients with acute chest pain. Clin Chem. 2012;
108. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. JClinInvest. 2003 Jun;111(0021-9738 (Print)):1805–12.
109. Rattazzi M, Puato M, Faggin E, Bertipaglia B, Zambon A, Pauletto P. C-reactive protein and interleukin-6 in vascular disease: culprits or passive bystanders? J Hypertens [Internet]. 2003 Oct [cited 2013 Oct 4];21(10):1787–803. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14508181
110. James SK, Armstrong P, Barnathan E, Califf R, Topol EJ, Venge P, et al. Troponin and C-Reactive Protein Have Different Relations to Subsequent Mortality and Myocardial Infarction After Acute Coronary Syndrome. 2003;41(6).
111. Brunetti ND, Troccoli R, Correale M, Pellegrino PL, Di Biase M. C-reactive protein in patients with acute coronary syndrome: correlation with diagnosis, myocardial damage, ejection fraction and angiographic findings. Int J Cardiol [Internet]. 2006 May 10 [cited 2013 Sep 15];109(2):248–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16055214
112. Kavsak PA, MacRae AR, Newman AM, Lustig V, Palomaki GE, Ko DT, et al.

Elevated C-reactive protein in acute coronary syndrome presentation is an independent predictor of long-term mortality and heart failure. ClinBiochem. 2007 Mar;40(0009-9120 (Print)):326–9.
113. Scirica BM, Morrow DA, Cannon CP, Lemos JA De, Murphy S, Sabatine MS, et al. Clinical Application of C-Reactive Protein Across the Spectrum of Acute Coronary Syndromes. 2007;1807:1800–7.
114. Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. N Engl J Med [Internet]. 2000 Oct 19 [cited 2013 Oct 4];343(16):1139–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11036119
115. He LP, Tang XY, Ling WH, Chen WQ, Chen YM. Early C-reactive protein in the prediction of long-term outcomes after acute coronary syndromes: a meta-analysis of longitudinal studies. Heart. 2010 Mar;96(1468-201X (Electronic)):339–46.
116. Correia LCL, Lima JC, Rocha MS, D'Oliveira A, Péricles Esteves J. Does high-sensitivity C-reactive protein add prognostic value to the TIMI-Risk Score in individuals with non-ST elevation acute coronary syndromes? Clin Chim Acta. 2007;
117. Correia LCL, Vasconcelos I, Garcia G, Kalil F, Ferreira F, Silva A, et al. Does C-reactive Protein Add Prognostic Value to GRACE Score in Acute Coronary Syndromes? Arq Bras Cardiol. 2014;
118. Ionescu CN, Chrissoheris MP, Slim HB, Slade M, Oen-Hsiao J, Donohue TJ, et al. Prospective chest pain evaluation in the emergency department with use of high-sensitivity C-reactive protein (Proceed-CRP study). Connecticut Medicine. 2014.
119. Banu S, Tanveer S, Manjunath CN. Comparative study of high sensitivity troponin T and heart-type fatty acid-binding protein in STEMI patients. Saudi J Biol Sci. 2015;
120. Diercks DB, Kirk JD, Naser S, Turnipseed S, Amsterdam EA. Value of high-sensitivity C-reactive protein in low risk chest pain observation unit patients. Int J Emerg Med. 2011;
121. Morita E, Yasue H, Yoshimura M, Ogawa H. Increased Plasma Levels of Brain Natriuretic Peptide in Patients With Acute Myocardial Infarction. 1993;
122. Arakawa N, Nakamura M, Aoki H, Hiramori K. Relationship between plasma level of brain natriuretic peptide and myocardial infarct size. Cardiol. 1994;
123. De Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. Lancet. 2003.
124. Jernberg T, Stridsberg M, Venge P, Lindahl B. N-terminal pro brain natriuretic peptide on admission for early risk stratification of patients with chest pain and no ST-segment elevation. J Am Coll Cardiol. 2002;
125. Wolsk E, Claggett B, Pfeffer MA, Diaz R, Dickstein K, Gerstein HC, et al. Role of B-type natriuretic peptide and N-terminal prohormone BNP as predictors of cardiovascular morbidity and mortality in patients with a recent coronary event and type 2 diabetes mellitus. J Am Heart Assoc. 2017;
126. De Lemos JA, Morrow DA, Bentley JH. The prognostic value of B-type natriuretic peptide in patients with acute coronary syndromes. ACC Curr J Rev. 2002;
127. Morrow DA, De Lemos JA, Sabatine MS, Murphy SA, Demopoulos LA,

DiBattiste PM, et al. Evaluation of B-type natriuretic peptide for risk assessment in unstable angina/non-ST-elevation myocardial infarction: B-type natriuretic peptide and prognosis in TACTICS-TIMI 18. J Am Coll Cardiol. 2003;
128. Meune C, Drexler B, Haaf P, Reichlin T, Reiter M, Meissner J, et al. The GRACE score's performance in predicting in-hospital and 1-year outcome in the era of high-sensitivity cardiac troponin assays and B-type natriuretic peptide. Heart [Internet]. 2011;97(18):1479–83. Available from: http://heart.bmj.com/cgi/doi/10.1136/hrt.2010.220988%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/21444339
129. Tesak M, Kala P, Jarkovsky J, Poloczek M, Bocek O, Jerabek P, et al. The value of novel invasive hemodynamic parameters added to the TIMI risk score for short-term prognosis assessment in patients with ST segment elevation myocardial infarction. Int J Cardiol. 2016;
130. Ochieng J, Fridman R, Nangia-Makker P, Kleiner DE, Liotta LA, Stetler-Stevenson WG, et al. Galectin-3 Is a Novel Substrate for Human Matrix Metalloproteinases-2 and -9. Biochemistry. 1994;
131. Bivona G, Bellia C, Lo Sasso B, Agnello L, Scazzone C, Novo G, et al. Short-term Changes in Gal 3 Circulating Levels After Acute Myocardial Infarction. Arch Med Res. 2016;
132. Atay E, Guzel M, Amanvermez R, Demir MT, Erenler AK, Ozgen E, et al. Role of Gal-3 and H-FABP in the early diagnosis of acute coronary syndrome. Bratislava Med J. 2019;
133. Liu FT, Patterson RJ, Wang JL. Intracellular functions of galectins. Biochimica et Biophysica Acta - General Subjects. 2002.
134. Dumic J, Dabelic S, Flögel M. Galectin-3: An open-ended story. Biochimica et Biophysica Acta - General Subjects. 2006.
135. de Boer RA, van Veldhuisen DJ, Gansevoort RT, Muller Kobold AC, van Gilst WH, Hillege HL, et al. The fibrosis marker galectin-3 and outcome in the general population. J Intern Med. 2012;
136. Asleh R, Enriquez-Sarano M, Jaffe AS, Manemann SM, Weston SA, Jiang R, et al. Galectin-3 Levels and Outcomes After Myocardial Infarction: A Population-Based Study. J Am Coll Cardiol. 2019;
137. Winter MP, Wiesbauer F, Alimohammadi A, Blessberger H, Pavo N, Schillinger M, et al. Soluble galectin-3 is associated with premature myocardial infarction. Eur J Clin Invest. 2016;
138. Gagno G, Padoan L, Stenner E, Beleù A, Ziberna F, Hiche C, et al. Galectin 3 and Galectin 3 Binding Protein Improve the Risk Stratification after Myocardial Infarction. J Clin Med. 2019;8(5):570.
139. Kittanakom S, Hill S, Worster A, Kavsak P. The utility of galectin-3 and high-sensitivity cardiac troponin assays to predict short-term serious outcomes in patients presenting early after chest-pain onset. Clin Biochem. 2014;
140. Tian L, Chen K, Han Z. Correlation between Galectin-3 and Adverse Outcomes in Myocardial Infarction Patients: A Meta-Analysis. Cardiology Research and Practice. 2020.
141. Safari S, Baratloo A, Elfil M, Negida A. Evidence Based Emergency Medicine; Part 5 Receiver Operating Curve and Area under the Curve. Emerg (Tehran, Iran). 2016;
142. Park SH, Goo JM, Jo CH. Receiver operating characteristic (ROC) curve: Practical review for radiologists. Korean Journal of Radiology. 2004.

143. Pencina MJ, D'Agostino RB, D'Agostino RB, Vasan RS. Evaluating the added predictive ability of a new marker: From area under the ROC curve to reclassification and beyond. Stat Med. 2008;
144. Pencina MJ, D'Agostino RB, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. Stat Med. 2011;
145. Pencina MJ, D'Agostino RB, Pencina KM, Janssens ACJW, Greenland P. Interpreting incremental value of markers added to risk prediction models. Am J Epidemiol. 2012;
146. Leening MJG, Vedder MM, Witteman JCM, Pencina MJ, Steyerberg EW. Net Reclassification Improvement: Computation, Interpretation, and Controversies. Ann Intern Med. 2014;
147. DCLG. The English Indices of Deprivation 2015. Neighb Stat Release. 2015;
148. Group LCC. One Liverpool : 2018 - 2021. The Long and Winding Road to a Healthier Life. 2018.
149. Information Services Division Scotland. Scottish Heart Disease Statistics Year ending 31 March 2017. 2018;(February):18. Available from: https://www.statisticsauthority.gov.uk/national-statistician/types-of-official-statistics/%0Ahttp://www.isdscotland.org/Health-Topics/Heart-Disease/Publications/2017-02-21/2017-02-21-Heart-Disease-Report.pdf%0Ahttps://www.isdscotland.org/Health-Topics/Hea
150. Leslie SJ, Rysdale J, Lee AJ, Eteiba H, Starkey IR, Pell J, et al. Unemployment and deprivation are associated with a poorer outcome following percutaneous coronary angioplasty. Int J Cardiol. 2007;122(2):168–9.
151. Santema BT, Chan MMY, Tromp J, Dokter M, van der Wal HH, Emmens JE, et al. The influence of atrial fibrillation on the levels of NT-proBNP versus GDF-15 in patients with heart failure. Clin Res Cardiol. 2020;
152. Otaki Y, Arimoto T, Takahashi H, Kadowaki S, Ishigaki D, Narumi T, et al. Prognostic value of myocardial damage markers in patients with chronic heart failure with atrial fibrillation. Intern Med. 2014;
153. Gong M, Cheung A, Wang QS, Li G, Goudis CA, Bazoukis G, et al. Galectin-3 and risk of atrial fibrillation: A systematic review and meta-analysis. J Clin Lab Anal. 2020;
154. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. J Chronic Dis. 1987;
155. Da Molin S, Cappellini F, Falbo R, Signorini S, Brambilla P. Verification of an immunoturbidimetric assay for heart-type fatty acid-binding protein (H-FABP) on a clinical chemistry platform and establishment of the upper reference limit. Clin Biochem. 2014;
156. Goksuluk D, Korkmaz S, Zararsiz G, Karaagaoglu AE. EasyROC: An interactive web-tool for roc curve analysis using r language environment. R J. 2016;
157. Sanchis J, García-Blas S, Mainar L, Mollar A, Abellán L, Ventura S, et al. High-sensitivity versus conventional troponin for management and prognosis assessment of patients with acute chest pain. Heart [Internet]. 2014;1591–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24947318
158. Cullen L, Greenslade JH, Carlton EW, Than M, Pickering JW, Ho A, et al. Sex-specific versus overall cut points for a high sensitivity troponin I assay in predicting 1-year outcomes in emergency patients presenting with chest pain.

Heart. 2016;
159. Backus BE, Six a J, Kelder JH, Gibler WB, Moll FL, Doevendans P a. Risk scores for patients with chest pain: evaluation in the emergency department. Curr Cardiol Rev [Internet]. 2011 Feb;7(1):2–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3131711&tool=pmcentrez&rendertype=abstract
160. Bularga A, Lee KK, Stewart S, Ferry A V., Chapman AR, Marshall L, et al. High-Sensitivity Troponin and the Application of Risk Stratification Thresholds in Patients with Suspected Acute Coronary Syndrome. Circulation. 2019;
161. Chew PG, Frost F, Mullen L, Fisher M, Zadeh H, Grainger R, et al. A direct comparison of decision rules for early discharge of suspected acute coronary syndromes in the era of high sensitivity troponin. Eur Hear J Acute Cardiovasc Care. 2019;
162. Vorlat A, Claeys MJ, De Raedt H, Gevaert S, Vandekerckhove Y, Dubois P, et al. TIMI risk score underestimates prognosis in unstable angina/non-ST segment elevation myocardial infarction. Acute Card Care. 2008;
163. Fox KA. GRACE Risk Table - Center for Outcomes Research [Internet]. Available from: https://www.outcomes-umassmed.org/GRACE/default.aspx
164. Giannitsis E, Mueller-Hennessen M, Zeller T, Schuebler A, Aurich M, Biener M, et al. Gender-specific reference values for high-sensitivity cardiac troponin T and I in well-phenotyped healthy individuals and validity of high-sensitivity assay designation. Clin Biochem. 2020;
165. Body R, Carley S, Wibberley C, McDowell G, Ferguson J, Mackway-Jones K. The value of symptoms and signs in the emergent diagnosis of acute coronary syndromes. Resuscitation. 2010;
166. Bruyninckx R, Aertgeerts B, Bruyninckx P, Buntinx F. Signs and symptoms in diagnosing acute myocardial infarction and acute coronary syndrome: A diagnostic meta-analysis. British Journal of General Practice. 2008.
167. Than M, Herbert M, Flaws D, Cullen L, Hess E, Hollander JE, et al. What is an acceptable risk of major adverse cardiac event in chest pain patients soon after discharge from the Emergency Department? A clinical survey. International Journal of Cardiology. 2013.
168. Shah ASV, Anand A, Strachan FE, Ferry A V., Lee KK, Chapman AR, et al. High-sensitivity troponin in the evaluation of patients with suspected acute coronary syndrome: a stepped-wedge, cluster-randomised controlled trial. Lancet. 2018;392(10151):919–28.
169. Roifman I, Sivaswamy A, Chu A, Austin PC, Ko DT, Douglas PS, et al. Clinical effectiveness of cardiac noninvasive diagnostic testing in outpatients evaluated for stable coronary artery disease. J Am Heart Assoc. 2020;
170. Mudrick DW, Cowper PA, Shah BR, Patel MR, Jensen NC, Peterson ED, et al. Downstream procedures and outcomes after stress testing for chest pain without known coronary artery disease in the United States. Am Heart J. 2012;
171. Tahhan AS, Sandesara P, Hayek SS, Hammadah M, Alkhoder A, Kelli HM, et al. High-sensitivity troponin I levels and coronary artery disease severity, progression, and long-term outcomes. J Am Heart Assoc. 2018;
172. Ford I, Shah ASV, Zhang R, McAllister DA, Strachan FE, Caslake M, et al. High-Sensitivity Cardiac Troponin, Statin Therapy, and Risk of Coronary Heart Disease. J Am Coll Cardiol. 2016;
173. Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in

logistic and cox regression. Am J Epidemiol. 2007;
174. Árnadóttir Á, Vestergaard KR, Pallisgaard J, Sölétormos G, Steffensen R, Goetze JP, et al. High-sensitivity cardiac troponin T is superior to troponin I in the prediction of mortality in patients without acute coronary syndrome. Int J Cardiol. 2018;
175. Tveit SH, Myhre PL, Hoff NJS, Le TM, Seljeflot I, Røysland R, et al. Superiority of high sensitivity cardiac troponin T vs. I for long-term prognostic value in patients with chest pain; data from the Akershus cardiac Examination (ACE) 3 study. Clin Biochem. 2020;
176. Cannon CP, McCabe CH, Wilcox RG, Langer a., Caspi a., Berink P, et al. Oral Glycoprotein IIb/IIIa Inhibition With Orbofiban in Patients With Unstable Coronary Syndromes (OPUS-TIMI 16) Trial. Circulation [Internet]. 2000 Jul 11 [cited 2014 Mar 11];102(2):149–56. Available from: http://circ.ahajournals.org/cgi/doi/10.1161/01.CIR.102.2.149
177. Maron DJ, Hochman JS, Reynolds HR, Bangalore S, O'Brien SM, Boden WE, et al. Initial Invasive or Conservative Strategy for Stable Coronary Disease. N Engl J Med. 2020;
178. Mortensen MB, Dzaye O, Steffensen FH, Bøtker HE, Jensen JM, Rønnow Sand NP, et al. Impact of Plaque Burden Versus Stenosis on Ischemic Events in Patients With Coronary Atherosclerosis. J Am Coll Cardiol. 2020;
179. Villines TC, Rodriguez Lozano P. Transitioning From Stenosis to Plaque Burden in the Cardiac CT Era: The Changing Risk Paradigm. Journal of the American College of Cardiology. 2020.
180. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med. 2017;
181. Kempf T, Horn-Wichmann R, Brabant G, Peter T, Allhoff T, Klein G, et al. Circulating concentrations of growth-differentiation factor 15 in apparently healthy elderly individuals and patients with chronic heart failure as assessed by a new immunoradiometric sandwich assay. Clin Chem. 2007;
182. Collinson PO, Barnes SC, Gaze DC, Galasko G, Lahiri A, Senior R. Analytical performance of the N terminal pro B type natriuretic peptide (NT-proBNP) assay on the Elecsys™ 1010 and 2010 analysers. European Journal of Heart Failure. 2004.
183. Hartweg J, Gunter M, Perera R, Farmer A, Cull C, Schalkwijk C, et al. Stability of soluble adhesion molecules, selectins, and C-reactive protein at various temperatures: Implications for epidemiological and large-scale clinical studies. Clin Chem. 2007;
184. Kavsak PA, Ainsworth C, Arnold DM, Scott T, Clark L, Ivica J, et al. The potential role of a turbidimetric heart-type fatty acid-binding protein assay to aid in the interpretation of persistently elevated, non-changing, cardiac troponin I concentrations. Clin Biochem. 2018;
185. Egger M, Dieplinger B, Mueller T. One-year in vitro stability of cardiac troponins and galectin-3 in different sample types. Clin Chim Acta. 2018;
186. Viswanathan K, Kilcullen N, Morrell C, Thistlethwaite SJ, Sivananthan MU, Hassan TB, et al. Heart-type fatty acid-binding protein predicts long-term mortality and re-infarction in consecutive patients with suspected acute coronary syndrome who are troponin-negative. JAmCollCardiol. 2010 Jun 8;55(1558-3597 (Electronic)):2590–8.

Appendix A

Appendix for Chapter 2.

Original PIS Version 5.

Patient Information Sheet

Biomarkers in Acute Chest Pain Study

We would like to invite you to take part in our research study. However, before you decide, we need you to understand the reason for the research and what it involves. **A member of our Research Team will go through the information sheet with you and answer any questions you may raise.** This will take about 20 minutes. You may talk to others about the study if you so wish. Part 1 tells you the purpose of the study and what to expect if you take part. Part 2 gives you more detailed information about the conduct of the study. Please ask, if there is anything you do not understand.

Background

Every year, approximately 700,000 people with chest pain attend Accident and Emergency Departments in the UK. A proportion of these patients are admitted to hospital. Chest pain can be the result of a sudden blockage of a coronary artery, which may be easily identified by the findings on an ECG or by a blood test measuring of Troponin (part of heart muscle released into the blood when there has been damage to the heart muscle). However, these tests can sometimes be normal despite the presence of significant narrowing of the coronary arteries. There is no easy way to identify patients with chest pain who are not having a heart attack but who may still be at considerable risk. Further tests take time, resources and specialist interpretation to determine an individual patient's risk. New biomarkers (chemicals released by the body into the bloodstream associated with either an interrupted blood supply to the heart muscle or a 'severe stress' to the body) are continuously being identified, but further research is required to find more accurate biomarkers which may help identify those at risk of a heart attack or death in the near future. Furthermore, there is a strong link between diabetes and heart disease, but less is known about how a pre-diabetic state could be responsible for triggering angina or heart pain in some of these patients.

What is the purpose of the study?

We propose to study a number of biomarkers. This may grant an insight into an individual's risk of death or heart attack in the near future. We aim to compare the performance of these biomarkers to standard investigations and Exercise Echocardiography (a specialist test to examine the heart under stress). There could be a potential to improve care by allowing safe, early discharge to patients identified to be a low risk thereby reassuring patients and freeing up hospital beds. We also want to look for the presence of the pre-diabetic state in people who present with chest pain, which can be achieved by a blood test.

Why have I been invited?

You have been invited to take part in this study as you have been admitted to hospital with chest pain but neither your ECG, nor your blood tests, indicate that you have had a heart attack. We want to assess whether a number of biomarkers will allow us to determine, more precisely, the risk of individuals like yourself suffering a heart attack in the future. We intend to recruit 500 patients to take part in the project and 25 healthy volunteers.

Do I have to take part?

It is up to you to decide whether to join this study or not. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw from this study at any time, without giving a reason. This would not affect your medical care or legal rights.

What will happen to me if I take part?

Normal investigations will not be withheld as part of this study. However, we will be performing a number of additional tests (see below), some of which can be done on admission. Others tests will require a return visit to hospital. We aim to keep these visits to a minimum. At most, they would require two further visits for investigations which should occur in the 2 months following discharge. We may also invite you back for a clinic appointment with the researcher in a few months to see how you are doing. This would take part at University Hospital Aintree and last for twenty minutes at most.

With your permission, information will be gathered by the research team from your medical notes (medical history, medications, investigation results) and we will also ask you a number of questions related to the risk of heart disease and complications of heart disease.

The only people who will have access to your medical notes as part of this study are a few members of the research team who are qualified doctors or nurses working in the NHS, and the regulatory bodies overseeing the research.

Blood tests: As part of this study, an additional 4 blood samples (at most 40mls of blood) will be required; one during admission and one fasting sample (one of these samples can be taken the same time as blood taken for clinical reasons), and the other two, before and after exercise (these will be taken from a cannula – see below). These are to look at biomarkers and for the pre-diabetic state. These blood samples will be stored in the biochemistry laboratory at University Hospital Aintree. Most of these tests will be performed at University Hospital Aintree laboratory, but, with your permission, we may have to send some of these tests which will have your personal details removed, to an outside laboratory for analysis.

Transthoracic echocardiogram: This is an ultrasound scan used to look at the heart. This is a safe procedure which takes about 30 minutes to perform. This is most likely to be performed during your initial admission, but may be performed in the cardio-respiratory department of the hospital, as an outpatient, at a later date.

Cannulation: Before the exercise echocardiogram begins, a cannula will be inserted (a plastic sheath inserted into one of the veins in your arm using a needle). This will allow us to take blood samples before and after exercise using a needle only once.

Exercise Echocardiogram: If you are able to exercise then an ultrasound scan of your heart will be undertaken whilst you are pedalling a bicycle or immediately after a

treadmill test. This is a simple ultrasound scan to assess how your heart responds to exercise and rest; this should take less than 30 minutes. In order to be able to get the desired heart rate, if you are taking medications that may slow down your heart rate, then these may need to be stopped 48 hours before the test. If you are unable to exercise at all then you would not undergo this aspect of the research. If we cannot clearly see the pictures of your heart using ultrasound, then echo contrast will be injected to visualise your heart more clearly.
Blood pressure readings: Blood pressure will be measured on the arm and ankle using a standard blood pressure cuff, this will be performed during your initial admission or when you return for one of the additional tests.
10 minute ECG recording: This will be performed by attaching a number of sticky pads across your chest and monitoring your heart rate whilst you are resting.
Follow up: We would like to contact everyone included in this study in 6 months, 1 year and then at yearly intervals over 5 years. We will contact you by your preferred method of communication (post, telephone, email). We would also like to look at your hospital records and may contact your GP. We would also appreciate if you would contact us if you have any heart problems after you have seen your GP or have attended hospital.

What will I have to do?

If, after reading this leaflet, you wish to take part, one of our research team will speak with you to make sure that you understand what is involved. You will then be asked to sign a consent form.

We will try to carry out as many investigations whilst you are still an inpatient, but some of these tests require special equipment which is not always available – therefore, we will arrange a mutually convenient time for you to return to the hospital.

With regards to follow up, we will ask you if you would prefer to be contacted by post, telephone or email (you may change this at any time). We will be contacting you to see how you are doing, and if you have developed any new health problems or started any new medication.

What are the possible disadvantages or risks of taking part?

Inconvenience of having further investigations (blood tests, echocardiogram, exercise echocardiogram, 10 minute ECG recordings).

Inconvenience of further visits to the hospital for these investigations (maximum 2 visits).

Inconvenience of being contacted by the research team.

The possible disadvantages and risks of the additional investigations are:

Blood tests: There may be discomfort, bleeding and bruising as a result of having additional blood tests. Blood tests will only be taken by a member of staff qualified to do so.

10 minute ECG: Occasionally the sticky electrodes can cause skin irritation.

Echocardiography: Ultrasound is a safe procedure. Sticky electrodes are also used during this procedure which may cause skin irritation. You will be asked to lie towards your left-hand side during this scan, which may be uncomfortable.

Exercise Echocardiography: Exercise tests are generally regarded as safe. There is a rare risk of heart attack, abnormal heart rhythm or collapse. This investigation will take place on the main hospital site, you will be monitored throughout and a doctor who is trained in dealing with these complications will be present.

Cannulation: a cannula is inserted using a needle similar to those used in taking standard blood tests. This may cause pain, bleeding or bruising. This is usually slightly more uncomfortable than a blood test but once the cannula is in place should not cause you any real discomfort. This will be placed by a member of staff trained to do so. This cannula will be removed once the test is complete (less than 2 hours) unless you become unwell during the test and require this cannula for medical reasons. An additional risk of cannulation is infection. A previous study of hospital patients reported the risk of serious infection of 2/10,000. The risk should be less as part of this study as the cannula will only be in place for a short time.

Stopping medication prior to exercise: if you are taking medications such as a betablockers or calcium channel antagonist (the research team will tell you if you are taking these) then these may need to be stopped 48 hours before your exercise echocardiogram in order for the desired heart rate to be achieved. The research team will determine if they think it is safe for you to temporarily withhold these medications, and only then will this be suggested. As a result of stopping these medications for 48 hours your blood pressure may rise slightly during this time or you may develop palpitations.

Echo Contrast: In some people it may not be possible to get clear enough pictures of the heart using ultrasound alone. If this is the case it would be extremely difficult to fully assess the heart muscles response to exercise. This problem can be overcome by using a small injection of contrast/dye. The product we would be using in this research project is called Sonovue®. Sonovue® is frequently used to assist in stress echocardiography at University Hospital Aintree. Sonovue® is generally regarded as a safe product and is licensed for this use. In a study of 4440 patients, the side effects tended to be mild and short-lived, and include: Headache (2.3%), nausea (1%), chest pain (0.7%), chest discomfort (0.7%), injection-site pain (0.5%) and feeling hot (0.5%). There is a small possibility of an allergy-like or anaphylactic type reaction which may occur in approximately 1 in 10,000 patients. During administration of this product a doctor will be present who is trained to deal with these problems should they arise.

What are the side effects of any treatment received when taking part?

See above for side effect profile of Sonovue® which may be used in this study.

What are the possible benefits of taking part?

We cannot promise that the study will help you but the information we gather may help improve the management of people who present with chest pain and identify those who are at an increased risk of suffering from a heart attack or conversely those that can be safely discharged from hospital earlier.

As a result of the additional investigations, we may discover that you have an underlying heart problem or have a narrowing in the coronary arteries, which may require further evaluation or treatment. Any abnormal results will be discussed with you and the results of these investigations will be made available to the doctor responsible for you during your initial admission.

What happens when the research study stops?

If there is an important finding that has an implication in the way you should be managed, we may invite you to attend for further discussions/tests if we think you are at increased risk of having a heart problem.

Once the initial investigations are collected we would like to follow up as stated above.

What if there is a problem?

Any complaints about the way you have been treated during the study or any possible harm you may suffer will be addressed. Detailed information regarding this option is given in Part 2.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practices and all information about you will be handled in confidence. The details are included in Part 2. If the information in Part 1 has interested you and you are considering participating, please read the additional information in Part 2 before making a decision.

Part 2
What if relevant new information becomes available?

On receiving new information, your doctor may consider it to be in your best interests to withdraw from further investigations in the study. For instance, if your Echocardiogram or routine Exercise Tolerance Test suggests there is a reason why it may be unsafe to put you through the exercise echocardiogram. If anything does arise, your research doctor will explain the reason and inform your medical team and GP.

What will happen if I don't want to carry on with the study?

If you wish to withdraw from the study, you can contact the Research Team to notify them whether you want to withdraw from the study entirely, or just from part of it.
We will remove you from our follow-up database if you do not want to be contacted again.
We will destroy all blood samples collected and stored as part of the research.
We would be unable to destroy the results of investigations already performed as part of this study which may assist standard medical care. (Echocardiogram, Exercise Echocardiogram).
We would be unable to destroy any information from your general medical notes.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions and can be contacted via The Aintree Cardiac Centre on 0151 529 2343. If you remain unhappy and wish to complain formally, you can do this via the NHS Complaints Procedure. Details can be obtained from the Research Team or through the Patient Advice and Liaison Services on 0151 529 2400.

This study is covered by The NHS Indemnity Scheme. In the event that something does go wrong and you are harmed during the research, and, this is due to someone's negligence, then you may have grounds for a legal action for compensation against University Hospital Aintree NHS Trust but you may have to pay your own legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

With your consent we would normally inform your general practitioner that you are participating in the study.

The results of your Exercise Echocardiogram will be made available to the team who looked after you during your admission with chest pain and reports of these investigations will be placed in your medical notes.

All patient information collected in this study will be stored on password protected computers or in locked rooms at University Hospital Aintree. When the results of the study are reported, individuals who have taken part will not be identified in any way.

All data will remain confidential and no personal details will be made available to any third parties or transferred outside of the hospital. Details about you will be stored on computer during the research project but your data will only be looked at by members of the research team. Blood will be stored in a secure storage facility at the University Hospital Aintree NHS Trust. If some blood tests need to be analysed in an alternative lab, these samples will be anonomysed beforehand. The data will only be used for research purposes in this project. No other use of the data will be undertaken without seeking your prior consent.

Involvement of the General Practitioner/Family doctor (GP)

With your consent, your GP will be notified of your participation in the study. If any investigations show results which may affect the way you should be treated medically, your GP will be made aware of these changes.

What will happen to any samples I give?

Blood samples will be frozen and stored on-site in the biochemistry laboratory in University Hospital Aintree.

All blood samples will be handled as any other blood samples are at Aintree Hospital. All samples will be kept securely and only accessed by research and laboratory staff.

Some blood tests may need to be sent to different laboratories, but these samples would have your personal details removed.

I understand that by giving my blood sample I will be donating that sample as a gift for use in future research.

Once the study is complete all blood samples will be destroyed.

Will any genetic tests be done?

No

What will happen to the results of the research study?

You will not be personally identified in any report or publication. The results of the study will be disseminated via the hospital intranet scheme, electronic and paper medical journals as well as presentations in various major medical societies throughout the world.

Who is organising and funding the research?

The study is being funded largely by in-house funds from Aintree University Hospital NHS Trust. There will also be a contribution from industry and possibly various research groups. The research group performing this research project has no conflict of interests. Your research nurse/doctor will not receive any additional money for including you in this study.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by North West 8 Research Ethics Committee – Greater Manchester East.

Detailed summary

The purpose of this research project is to investigate a number of cardiac biomarkers in patients presenting with chest pain which is suspicious of being heart related but initial tests have excluded a heart attack. Participants who give their consent will be asked a number of questions about their general medical history, have a physical examination and additional blood tests looking for a number of biomarkers and the pre-diabetic state. At most, 4 samples of blood may need to be taken (at most 10mls of blood for each sample), some of these tests can be taken at the same time as blood tests taken for clinical reasons. Most samples will be analysed in the biochemistry laboratory at University Hospital Aintree, however, some tests may need to be carried out in an independent laboratory. If this is the case then samples will be anonymised. Participants will also undergo an Exercise Echocardiogram and a 10 minute ECG recording which may require a return visit to the hospital. The aim of this study is to help determine those individuals who are at low risk and can be safely discharged. We intend to recruit 500 patients and carry out follow up by preferred method of contact (post, telephone or email) at 6 months, 12 months, then yearly for 5 years. Your participation is entirely voluntary and you would be able to withdraw from follow up at any time. During this study, it is possible that we may identify a heart abnormality or narrowing of the coronary arteries. If this is the case, the research doctor will discuss this with you and arrange the appropriate course of action.

Further information and contact details

Further information regarding this project can be obtained from The Research Team, who can be contacted by calling the Aintree Cardiac Centre on 0151 529 2343.

Dr Julia Jones, Clinical Research Fellow, principally involved in the day to day running of the study.

Dr Aleem Khand, Consultant Interventional Cardiologist, Chief Investigator of the study.

Sister Doireann McGowan, Chief Research Nurse.

Further information about research in general can be found at the National Institute for Health Research, at the following web address: www.nihr.ac.uk/research

If you develop any concerns about the study, you can contact any member of the research team above.

Updated PIS version 6 for patients who could exercise.

Patient Information Sheet

Biomarkers in Acute Chest Pain Study

We would like to invite you to take part in our research study. However, before you decide, we need you to understand the reason for the study and what it involves. Please take time to read the following information and discuss it with others if you wish. Please ask the research team if there is anything that is not clear or if you would like more information.

Background

Every year, thousands of people with chest pain attend Accident and Emergency Departments in the UK. Chest pain can be the result of a sudden blockage of a coronary artery, which may be easily identified by the ECG or blood tests. However, these tests can sometimes be normal despite the presence of significant narrowing of the coronary arteries. There is no easy way to identify patients with chest pain who are not having a heart attack but who may still be at considerable risk. New biomarkers (natural chemicals released by the body into the bloodstream) are continuously being identified, but further research is required to find more accurate biomarkers which may help identify those at higher risk.

What is the purpose of the study?

To study a number of biomarkers to see if these can predict an individual's risk of future heart problems. This could improve care by allowing early discharge for low risk patients.

Why have I been invited?

You have been invited to take part in this study as you have come to hospital with chest pain but your ECG and blood test indicate that you have not had a heart attack.

Do I have to take part?

It is up to you to decide whether to join this study. If you decide to take part, we will ask you to sign a consent form. You are free to withdraw from this study at any time, without giving a reason. This would not affect your medical care or legal rights.

What will happen to me if I take part?

We will be performing a number of additional tests (see below), some of which can be done on admission. Others tests will require a return visit to hospital but we aim to keep these visits to a minimum. At most, they would require two further visits for investigations which should occur in the 2 months following discharge.

Information will be gathered by the research team from your medical notes and we will also ask you a number of questions related to the risk of heart disease and complications of heart disease.

The only people who will have access to your medical notes as part of this study are a few members of the research team who are qualified doctors or nurses working in the NHS, and the regulatory bodies overseeing the research.

Blood tests: 4 samples (30mls of blood) will be needed, 2 can probably be taken the same time as clinical blood tests. The 2 before and after exercise blood samples will

be taken through a cannula. Samples will be stored at Aintree Hospital and will be anonymised if sent outside of the hospital.
Transthoracic echocardiogram: This is an ultrasound scan used to look at the heart. This is a safe procedure which takes about 30 minutes to perform.
Cannulation: Before the exercise echocardiogram begins, a cannula will be inserted (a plastic sheath inserted into one of the veins in your arm using a needle). This will allow us to take blood samples before and after exercise using a needle only once.
Exercise Echocardiogram: This is an ultrasound scan of your heart whilst you are pedalling a bicycle. This is a simple test to see how your heart responds to exercise; this should take less than 30 minutes. If we cannot clearly see the pictures of your heart using ultrasound, then echo contrast will be injected to visualise your heart more clearly.
Blood pressure readings: Blood pressure will be measured on the arm and ankle using a standard blood pressure cuff.
ECG recordings: This will be performed by attaching a number of sticky pads across your chest and monitoring your heart rate whilst you are resting.
Follow up: We would like to contact you at 6 months, 1 year and then at yearly intervals over 5 years. This can be done by your preferred method (post, telephone, email). We would also look at your hospital records and may contact your GP.

What will I have to do?

If you wish to take part, our research team will speak with you to make sure that you understand what is involved. You will then be asked to sign a consent form. We will try to carry out as many investigations whilst you are still an inpatient, but some of these tests require special equipment which is not always available – therefore, we will either not perform these tests, or arrange a time for you to return. For follow up we will contact you to see how you are doing, and if you have developed any heart condition.

What are the possible disadvantages or risks of taking part?

Inconvenience of having further investigations (blood tests, echocardiogram, exercise echocardiogram, ECG recordings) and being contacted by the research team

The possible disadvantages and risks of the additional investigations are:
Blood tests: There may be discomfort, bleeding and bruising as a result of blood tests. Blood will only be taken by a member of staff qualified to do so.
Echocardiography: Ultrasound is a safe procedure. You will be asked to lie towards your left-hand side during this scan, which may be uncomfortable.
Exercise Echocardiography: Exercise tests are generally regarded as safe. There is a rare risk of heart attack, abnormal heart rhythm or collapse. You will be monitored throughout and a team trained in dealing with complications will be present.
Cannulation: There may cause slight pain, bleeding or bruising. This cannula will be removed once the test is complete unless you become unwell and require this for medical reasons. There is a very small risk of infection with cannulas. In hospital patients the risk of serious infection is 2/10,000 but the risk should be less in this study as the cannula will only be in place for a very short time.
Stopping medication prior to exercise: if you are taking medications which affect your heart rate then these may need to be stopped 48 hours before your exercise echocardiogram. This will only be done if thought safe to do so. As a result of stopping these medications for 48 hours your blood pressure may rise slightly during this time or you may develop palpitations.
Echo Contrast: In some people it may not be possible to get clear enough pictures of the heart using ultrasound alone, clearer pictures can be obtained by using a small injection of contrast/dye. The product we would use is called Sonovue®. Sonovue® is frequently used in stress echocardiography at Aintree Hospital. In a study of 4440 patients, side effects were mild and short-lived, and included: Headache (2.3%), nausea (1%), chest pain (0.7%), chest

discomfort (0.7%), injection-site pain (0.5%) and feeling hot (0.5%). There is a small possibility of an allergy-like or anaphylactic type reaction which may occur in approximately 1 in 10,000 patients. During administration of this product a doctor will be present who is trained to deal with these problems should they arise.

What are the side effects of any treatment received when taking part?
See above for side effect profile of Sonovue® which may be used in this study.

What are the possible benefits of taking part?
We cannot promise that the study will help you but the information we gather may help people who present with chest pain in the future.
As a result of the additional investigations, we may discover that you have an underlying heart problem, which may require further evaluation or treatment. Any abnormal results will be discussed with you and the doctor responsible for you during your admission.

What happens when the research study stops?
Once the initial investigations are collected we would like to follow up as stated above.

What if there is a problem?
Complaints about how you have been treated during the study, or possible harm you may suffer, will be addressed. Information regarding this option is given regarding this later on.

Will my taking part in the study be kept confidential?
Yes. We will follow ethical and legal practices and all information about you will be handled in confidence. Details included later in the form.

What if relevant new information becomes available?
If anything relevant does arise, your research doctor will inform you.

What will happen if I don't want to carry on with the study?
If you wish to withdraw from the study, you can contact the Research Team to notify them whether you want to withdraw from the study entirely, or just from part of it.
Removal from our follow-up database if you do not want to be contacted again.
We can destroy blood samples collected and stored as part of the research.
We would be unable to destroy the results of investigations already performed as part of this study which may assist standard medical care.

What if there is a problem?
If you have concerns about the study, you should speak with the researchers. If you remain unhappy or wish to complain formally, this can be done via the NHS Complaints Procedure. Details can be obtained from the research team or through PALS on 0151 529 2400.

This study is covered by The NHS Indemnity Scheme. In the event that something does go wrong and you are harmed during the research, and, this is due to someone's negligence, then you may have grounds for a legal action for compensation against University Hospital Aintree NHS Trust but you may have to pay your own legal costs. The normal NHS complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?
With your consent we would inform your GP that you are participating in the study.
The results of your Exercise Echocardiogram will be made available to the team who looked after you during your admission with chest pain and reports of these investigations will be placed in your medical notes.

All patient information collected in this study will be stored on password protected computers or in locked rooms at Aintree Hospital. When the results of the study are reported, individuals who have taken part will not be identified in any way.

All data will remain confidential and no personal details will be made available to any third parties or transferred outside of the hospital. Details about you will be stored on computer during the research project but your data will only be looked at by members of the research team. Blood will be stored in a secure storage facility at the Aintree Hospital and any samples sent to external laboratories will be anonymised. Data will only be used for research purposes in this project. No other use of the data will be undertaken without seeking prior consent.

What will happen to any samples I give?

Blood samples will be frozen and stored on-site in the biochemistry laboratory in University Hospital Aintree. All blood samples will be handled as any other blood samples are at Aintree Hospital. All samples will be kept securely and only accessed by research and laboratory staff. Some blood tests may need to be sent to different laboratories, but these samples would have your personal details removed.

The blood samples you donate would be treated as a gift for use in future research.

Once the study is complete all blood samples will be destroyed.

What will happen to the results of the research study?

You will not be personally identified in any report or publication. The results of the study will be disseminated via the hospital intranet, medical meetings and medical journals.

Who is organising and funding the research?

The study is being funded largely by in-house funds from Aintree University Hospital.

Who has reviewed the study?

This study has been reviewed by North West 8 Research Ethics Committee.

Detailed summary

The purpose of this research project is to investigate a number of cardiac biomarkers in patients presenting with chest pain which is suspicious of being heart related but initial tests have excluded a heart attack. Participants who give their consent will be asked a number of questions about their general medical history, have a physical examination and additional blood tests looking for a number of biomarkers and the pre-diabetic state. At most, 2 samples of blood may need to be taken (at most 10mls of blood for each sample). The aim of this study is to help determine those individuals who are at low risk and can be safely discharged. We intend to recruit 500 patients and carry out follow up by preferred method of contact (post, telephone or email) at 6 months, 12 months, then yearly for 5 years. Your participation is entirely voluntary and you would be able to withdraw from follow up at any time. During this study, it is possible that we may identify a heart abnormality. If this is the case, the research doctor will discuss this with you and arrange the appropriate course of action.

Further information and contact details

Further information regarding this project can be obtained from The Research Team, who can be contacted by calling the Aintree Cardiac Centre on 0151 529 2343.

Dr Julia Jones, Clinical Research Fellow, principally involved in the day to day running of the study.

Dr Aleem Khand, Consultant Interventional Cardiologist, Chief Investigator of the study.

Sister Doireann McGowan, Chief Research Nurse.

Further information about research in general can be found at the National Institute for Health Research, at the following web address: www.nihr.ac.uk/research

If you develop any concerns about the study, you can contact any member of the research team above.

Updated PIS version 6 for patients who could not exercise.

Patient Information Sheet

Biomarkers in Acute Chest Pain Study

We would like to invite you to take part in our research study. However, before you decide, we need you to understand the reason for the research and what it involves. **A member of our Research Team will go through the information sheet with you and answer any questions you may raise.** This will take about 20 minutes. You may talk to others about the study if you so wish. Part 1 tells you the purpose of the study and what to expect if you take part. Part 2 gives you more detailed information about the conduct of the study. Please ask, if there is anything you do not understand.

Background

Every year, approximately 700,000 people with chest pain attend Accident and Emergency Departments in the UK. A proportion of these patients are admitted to hospital. Chest pain can be the result of a sudden blockage of a coronary artery, which may be easily identified by the findings on an ECG or by a blood test measuring of Troponin (part of heart muscle released into the blood when there has been damage to the heart muscle). However, these tests can sometimes be normal despite the presence of significant narrowing of the coronary arteries. There is no easy way to identify patients with chest pain who are not having a heart attack but who may still be at considerable risk. Further tests take time, resources and specialist interpretation to determine an individual patient's risk. New biomarkers (chemicals released by the body into the bloodstream associated with either an interrupted blood supply to the heart muscle or a 'severe stress' to the body) are continuously being identified, but further research is required to find more accurate biomarkers which may help identify those at risk of a heart attack or death in the near future. Furthermore, there is a strong link between diabetes and heart disease, but less is known about how a pre-diabetic state could be responsible for triggering angina or heart pain in some of these patients.

What is the purpose of the study?

We propose to study a number of biomarkers. This may grant an insight into an individual's risk of death or heart attack in the near future. We aim to compare the performance of these biomarkers to standard investigations. There could be a potential to improve care by allowing safe, early discharge to patients identified to be a low risk thereby reassuring patients and freeing up hospital beds. We also want to look for the presence of the pre-diabetic state in people who present with chest pain, which can be achieved by a blood test.

Why have I been invited?

You have been invited to take part in this study as you have been admitted to hospital with chest pain but neither your ECG, nor your blood tests, indicate that you have had a heart attack. We want to assess whether a number of biomarkers will allow us to determine, more precisely, the risk of individuals like yourself suffering a heart attack in the future. We intend to recruit 500 patients to take part in the project and 25 healthy volunteers.

Do I have to take part?

It is up to you to decide whether to join this study or not. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw from this study at any time, without giving a reason. This would not affect your medical care or legal rights.

What will happen to me if I take part?

Normal investigations will not be withheld as part of this study. However, we will be performing a number of additional tests (see below), some of which can be done on admission.
With your permission, information will be gathered by the research team from your medical notes (medical history, medications, investigation results) and we will also ask you a number of questions related to the risk of heart disease and complications of heart disease.
The only people who will have access to your medical notes as part of this study are a few members of the research team who are qualified doctors or nurses working in the NHS, and the regulatory bodies overseeing the research.
Blood tests: As part of this study, an additional 2 blood samples (at most 40mls of blood) will be required; one during admission and one fasting sample (these samples will most likely be taken the same time as blood taken for clinical reasons). These are to look at biomarkers and for the pre-diabetic state. These blood samples will be stored in the biochemistry laboratory at University Hospital Aintree. Most of these tests will be performed at University Hospital Aintree laboratory, but, with your permission, we may have to send some of these tests which will have your personal details removed, to an outside laboratory for analysis.
Transthoracic echocardiogram: This is an ultrasound scan used to look at the heart. This is a safe procedure which takes about 30 minutes to perform. This is most likely to be performed during your initial admission, but may be performed in the cardio-respiratory department of the hospital, as an outpatient, at a later date.
Follow up: We would like to contact everyone included in this study in 6 months, 1 year and then at yearly intervals over 5 years. We will contact you by your preferred method of communication (post, telephone, email). We would also like to look at your hospital records and may contact your GP. We would also appreciate if you would contact us if you have any heart problems after you have seen your GP or have attended hospital.

What will I have to do?

If, after reading this leaflet, you wish to take part, one of our research team will speak with you to make sure that you understand what is involved. You will then be asked to sign a consent form.

We will try to carry out as many investigations whilst you are still an inpatient, but some of these tests require special equipment which is not always available – therefore, we will either not perform these tests, or arrange a mutually convenient time for you to return to the hospital.

With regards to follow up, we will ask you if you would prefer to be contacted by post, telephone or email (you may change this at any time). We will be contacting you to see how you are doing, and if you have developed any new health problems or started any new medication.

What are the possible disadvantages or risks of taking part?

Inconvenience of having further investigations (echocardiogram, blood tests).

Inconvenience of being contacted by the research team.

The possible disadvantages and risks of the additional investigations are:

Blood tests: There may be discomfort, bleeding and bruising as a result of having additional blood tests. Blood tests will only be taken by a member of staff qualified to do so.
Echocardiography: Ultrasound is a safe procedure. Sticky electrodes are also used during this procedure which may cause skin irritation. You will be asked to lie towards your left-hand side during this scan, which may be uncomfortable.

What are the side effects of any treatment received when taking part?

No treatment will be given as part of this research project.

What are the possible benefits of taking part?

We cannot promise that the study will help you but the information we gather may help improve the management of people who present with chest pain and identify those who are at an increased risk of suffering from a heart attack or conversely those that can be safely discharged from hospital earlier.

As a result of the additional investigations, we may discover that you have an underlying heart problem or have a narrowing in the coronary arteries, which may require further evaluation or treatment. Any abnormal results will be discussed with you and the results of these investigations will be made available to the doctor responsible for you during your initial admission.

What happens when the research study stops?

If there is an important finding that has an implication in the way you should be managed, we may invite you to attend for further discussions/tests if we think you are at increased risk of having a heart problem.

Once the initial investigations are collected we would like to follow up as stated above.

What if there is a problem?

Any complaints about the way you have been treated during the study or any possible harm you may suffer will be addressed. Detailed information regarding this option is given in Part 2.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practices and all information about you will be handled in confidence. The details are included in Part 2. If the information in Part 1 has interested you and you are considering participating, please read the additional information in Part 2 before making a decision.

Part 2

What if relevant new information becomes available?

On receiving new information, your doctor may consider it to be in your best interests to withdraw from further investigations in the study. If anything does arise, your research doctor will explain the reason and inform your medical team and GP.

What will happen if I don't want to carry on with the study?

If you wish to withdraw from the study, you can contact the Research Team to notify them whether you want to withdraw from the study entirely, or just from part of it.
We will remove you from our follow-up database if you do not want to be contacted again.
We will destroy all blood samples collected and stored as part of the research.
We would be unable to destroy the results of investigations already performed as part of this study which may assist standard medical care.
We would be unable to destroy any information from your general medical notes.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions and can be contacted via The Aintree Cardiac Centre on 0151 529 2343. If you remain unhappy and wish to complain formally, you can do this via the NHS Complaints Procedure. Details can be obtained from the Research Team or through the Patient Advice and Liaison Services on 0151 529 2400.

This study is covered by The NHS Indemnity Scheme. In the event that something does go wrong and you are harmed during the research, and, this is due to someone's negligence, then you may have grounds for a legal action for compensation against

University Hospital Aintree NHS Trust but you may have to pay your own legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

With your consent we would normally inform your general practitioner that you are participating in the study.

All patient information collected in this study will be stored on password protected computers or in locked rooms at University Hospital Aintree. When the results of the study are reported, individuals who have taken part will not be identified in any way.

All data will remain confidential and no personal details will be made available to any third parties or transferred outside of the hospital. Details about you will be stored on computer during the research project but your data will only be looked at by members of the research team. Blood will be stored in a secure storage facility at the University Hospital Aintree NHS Trust. If some blood tests need to be analysed in an alternative lab, these samples will be anonomysed beforehand. The data will only be used for research purposes in this project. No other use of the data will be undertaken without seeking your prior consent.

Involvement of the General Practitioner/Family doctor (GP)

With your consent, your GP will be notified of your participation in the study. If any investigations show results which may affect the way you should be treated medically, your GP will be made aware of these changes.

What will happen to any samples I give?

Blood samples will be frozen and stored on-site in the biochemistry laboratory in University Hospital Aintree.

All blood samples will be handled as any other blood samples are at Aintree Hospital. All samples will be kept securely and only accessed by research and laboratory staff.

Some blood tests may need to be sent to different laboratories, but these samples would have your personal details removed.

I understand that by giving my blood sample I will be donating that sample as a gift for use in future research.

Once the study is complete all blood samples will be destroyed.

Will any genetic tests be done?

No

What will happen to the results of the research study?

You will not be personally identified in any report or publication. The results of the study will be disseminated via the hospital intranet scheme, electronic and paper medical journals as well as presentations in various major medical societies throughout the world.

Who is organising and funding the research?

The study is being funded largely by in-house funds from Aintree University Hospital NHS Trust. There will also be a contribution from industry and possibly various research groups. The research group performing this research project has no conflict of interests. Your research nurse/doctor will not receive any additional money for including you in this study.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by North West 8 Research Ethics Committee – Greater Manchester East.

Detailed summary

The purpose of this research project is to investigate a number of cardiac biomarkers in patients presenting with chest pain which is suspicious of being heart related but initial tests have excluded a heart attack. Participants who give their consent will be asked a number of questions about their general medical history, have a physical examination and additional blood tests looking for a number of biomarkers and the pre-diabetic state. At most, 2 samples of blood may need to be taken (at most 10mls of blood for each sample), some of these tests can be taken at the same time as blood tests taken for clinical reasons. Most samples will be analysed in the biochemistry laboratory at University Hospital Aintree, however, some tests may need to be carried out in an independent laboratory. If this is the case then samples will be anonymised. The aim of this study is to help determine those individuals who are at low risk and can be safely discharged. We intend to recruit 500 patients and carry out follow up by preferred method of contact (post, telephone or email) at 6 months, 12 months, then yearly for 5 years. Your participation is entirely voluntary and you would be able to withdraw from follow up at any time. During this study, it is possible that we may identify a heart abnormality. If this is the case, the research doctor will discuss this with you and arrange the appropriate course of action.

Further information and contact details

Further information regarding this project can be obtained from The Research Team, who can be contacted by calling the Aintree Cardiac Centre on 0151 529 2343.

Dr Julia Jones, Clinical Research Fellow, principally involved in the day to day running of the study.

Dr Aleem Khand, Consultant Interventional Cardiologist, Chief Investigator of the study.

Sister Doireann McGowan, Chief Research Nurse.

Further information about research in general can be found at the National Institute for Health Research, at the following web address: www.nihr.ac.uk/research

If you develop any concerns about the study, you can contact any member of the research team above.

Appendix for Chapter 3.

Investigations requested

279 (57.3%) patients did not have any investigations beyond a standard 12 lead ECG, routine haematology and biochemistry, echocardiography and a chest x ray. 178 (36.6%) patients went on to have an investigation for a coronary cause.

Troponin levels

The mean index troponin level was 5.28µg/l with a median of 4 µg/l (IQR 3 – 7 µg/l). Pain to index troponin was between 6 and 12 hours; the mean duration was 10.3 hours, with a median of 12 hours. 277 (56.9%) patients had a second troponin level measured. The median 2nd troponin value was 4 (IQR 2-8). The time interval between first and second Hs-cTnT was 6.3 hours (IQR 5.1 – 9.6 hours) and the median delta change in first and second troponin was 11µg/l (IQR 0-33). 12 of the patients (4.3%) the second sample of Hs-cTnT was elevated (>99th percentile) 12 (2.5%) patients had a troponin elevation at some time during admission and of these, 6 patients had a troponin >50% of baseline troponin. r patients (%) were adjudicated to have suffered an MI. (>20% in all patients).

ECG

The majority of patients (70.8%) had an ECG that was within normal limits. Most patients were in sinus rhythm, with a small proportion with new onset atrial fibrillation. T wave inversion occurred in 98 (20.1%) patients, which was deep inversion 2mm or greater in 8 (1.6%) patients. 28 (5.7%) patients had ST depression. 20 patients had a QRS duration of over 120ms. 41 (8.4%) patients had abnormal Q waves.

	Total 487	12-month MACE 22	Long-term MACE 48
ECG			
Atrial fib	18 (3.6%)	1 (5.5%)	2 (11.1%)
T wave inversion (any)	98 (20.1%)	7 (7.1%)	13 (13.3%)
T wave >2mm	8 (1.6%)	1 (12.5%)	2 (25%)
ST depression	28 (5.7%)	4 (18.0%)	8 (16.2%)
BBB	20 (4.1%)	1 (5%)	4 (20%)
Q waves	41 (8.4%)	2 (4.9%)	8 (19.5%)

Exercise tolerance tests

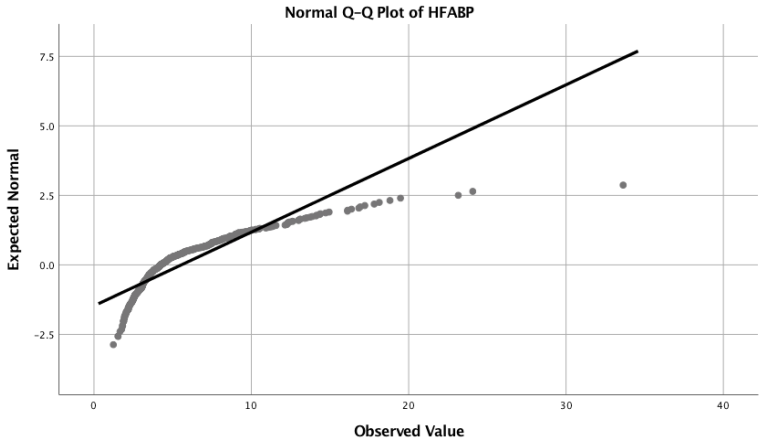
Exercise tolerance tests were requested as a first line investigation in 63 (12.9%) patients, 39 (8.0%) of these as an inpatient investigation and 24 (4.9%) as an outpatient 1 (0.2%) further exercise tolerance test was performed as a second line investigation as an inpatient. There were 3 (0.6%) patients in which an outpatient ETT was intended to occur, but this did not happen. For those who exercised, the intensity varied between 3.3 and 15.3 METS. 15 (3.1%) patients went on to have further investigation; 6 (1.2%) had an inpatient coronary angiogram, 2 (0.4%) had outpatient coronary angiograms, 5 (1.0%) outpatient stress echocardiogram, 1 (0.2%) outpatient CT and 1 (0.2%) OGD. Figure 10 illustrates these investigations and results. PCI was undertaken in 2 (0.4%) patients as a consequence of ETT and subsequent investigations.

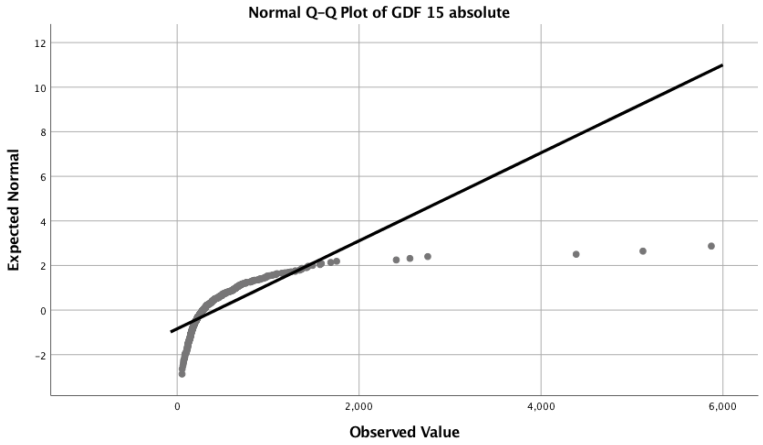
Non-cardiovascular investigations

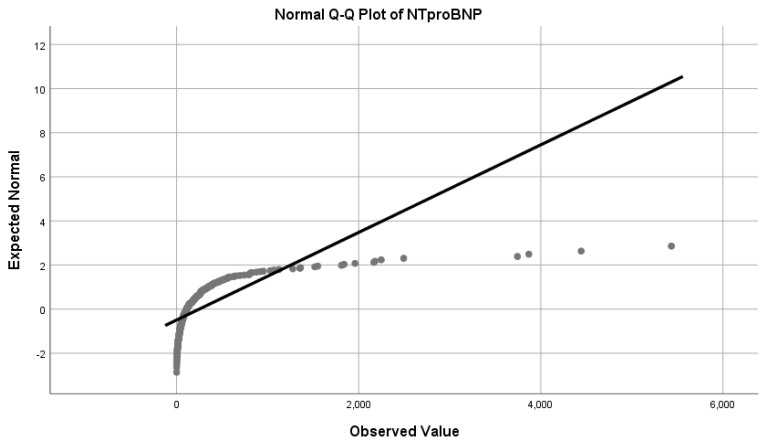
There were 12 OGD requested, 2 of these as an inpatient. 2 inpatient gastritis and gave. There were 10 outpatient OGDs, these demonstrated gastritis in 5 patients, 2 cases of hiatus hernia, 1 ulcer, 1 DNA and 1 was normal. 1 patient who had an outpatient OGD (which was reported as gastritis) went on to suffer MACE at 127 days. There were 8 patients who went on to have a CTPA or VQ scan, 2 of these shown PE.

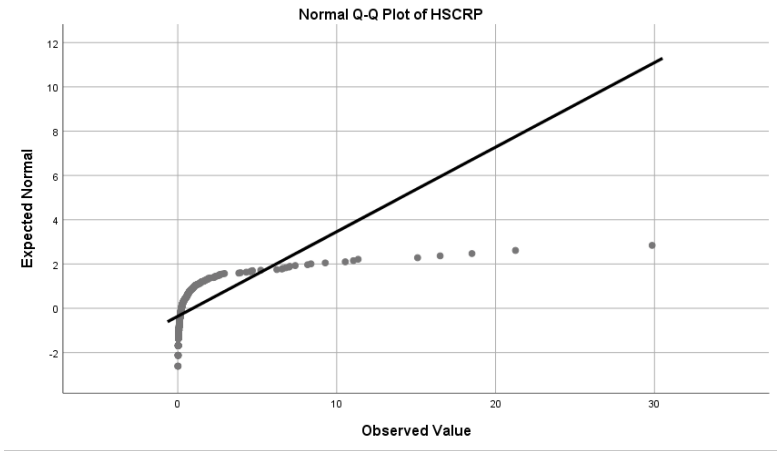
Type of investigation	First line Investigation	Second line Investigation	Third line Investigation	Overall
Nil	279 (57.3%)	453 (93.0%)	485 (99.6%)	-
Inpatient ETT	39 (8.0%)	1 (0.2%)	0	40 (8.2%)
Outpatient ETT	24 (4.9%)	0	0	24 (4.9%)
Inpatient angio	33 (6.8%)	10 (2.0%)	0	43 (8.8%)
Outpatient angio	11 (2.2%)	6 (1.2%)	0	17 (3.5%)
Inpatient SE	4 (0.8%)	0	0	4 (0.8%)
Outpatient SE	46 (9.4%)	12 (2.5%)	2 (0.4%)	59 (12.1%)
Inpt CT	2 (0.2%)	0	0	2 (0.4%)
OP CT	10 (2.1%)	2 (0.4%)	0	12 (2.5%)
CTPA / VQ	9 (1.8%)	1 (0.2%)	0	10 (2.0%)
MIBI	5 (1.0%)	1 (0.2%)	2 (0.4%)	8 (1.6%)
OGD/pH studies	10 (2.1%)	1 (0.2%)	1 (0.2%)	12 (2.5%)
Cath LHCH	4 (0.8%)	0	0	4 (0.8%)
PW study intracoronary	0	2 (0.4%)	0	2 (0.4%)
Other imaging	4 (0.8%)	1 (0.2%)	0	5 (1.0%)
Previous relevant investigation	14 (2.9%)	-	-	14 (2.9%)

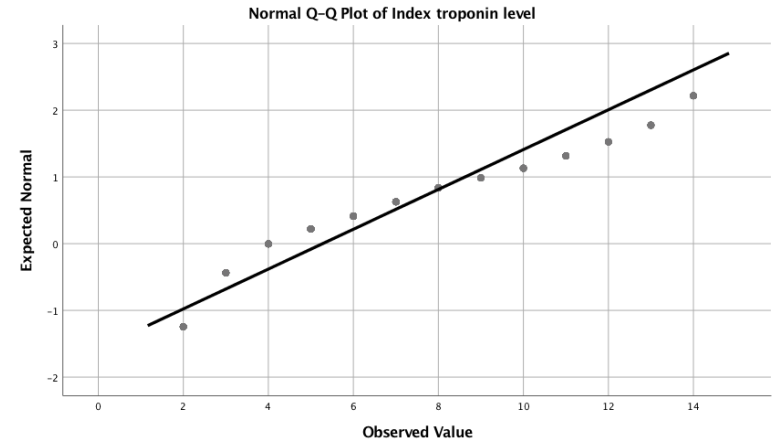
Appendix for Chapter 5.





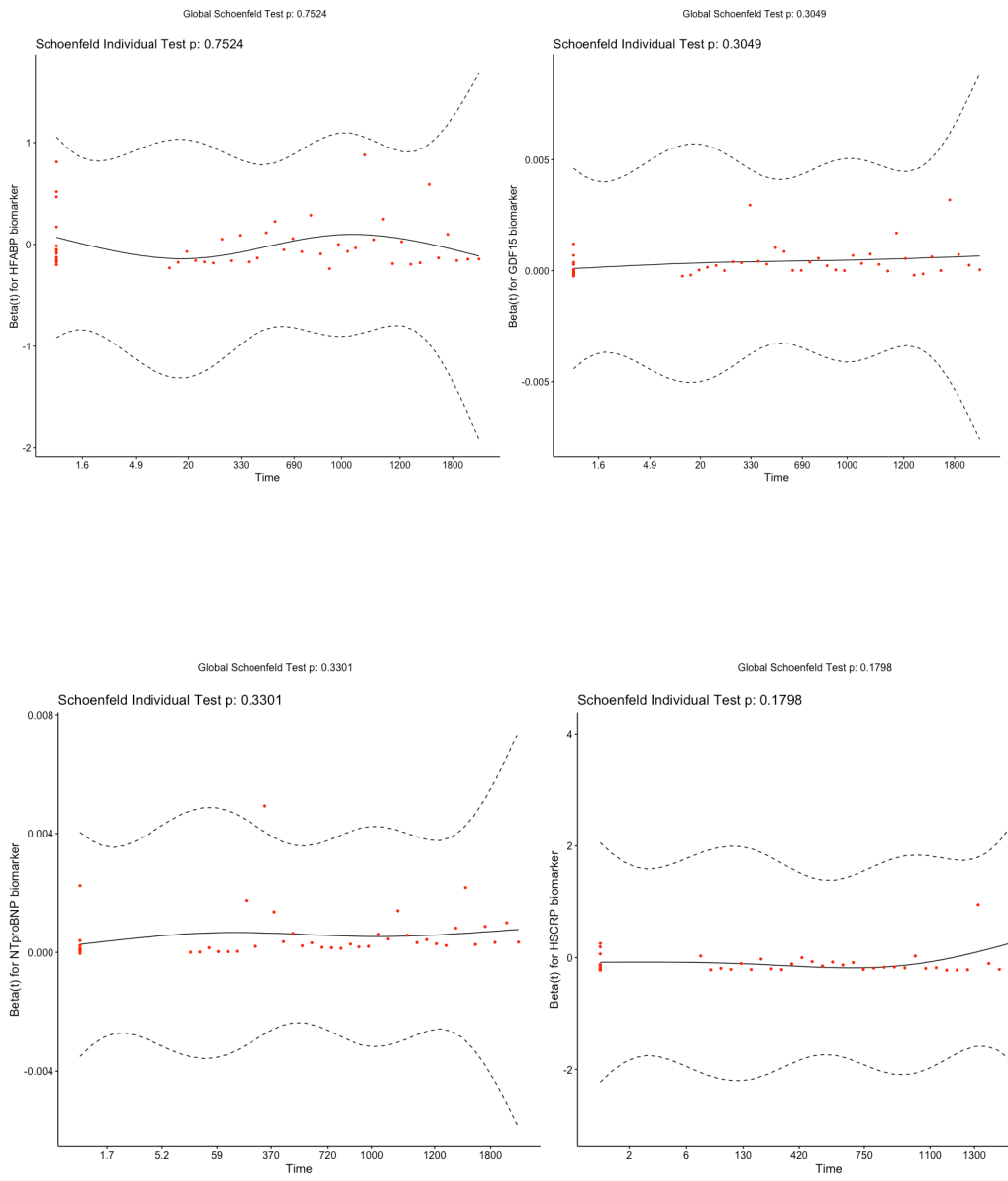


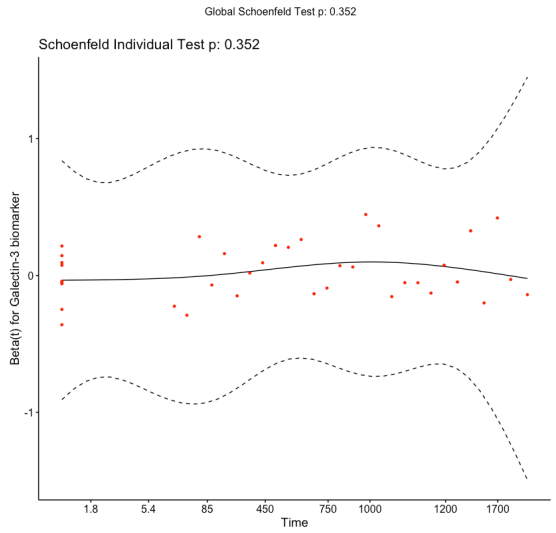

Normal Q-Q Plot of HSCRP
Expected Normal
Observed Value
12
10
8
6
4
2
0
-2
0
10
20
30

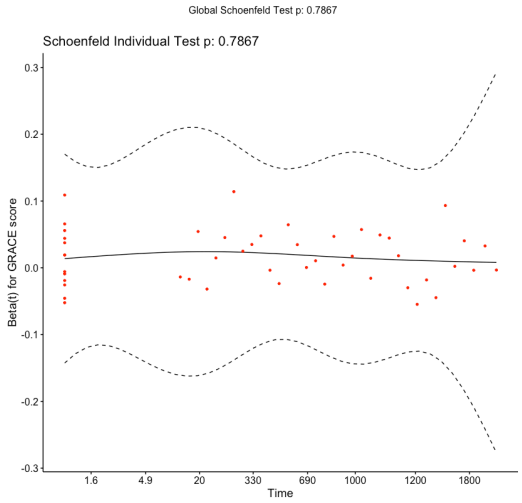

Normal Q-Q Plot of Index troponin level
Expected Normal
Observed Value
3
2
1
0
-1
-2
0
2
4
6
8
10
12
14

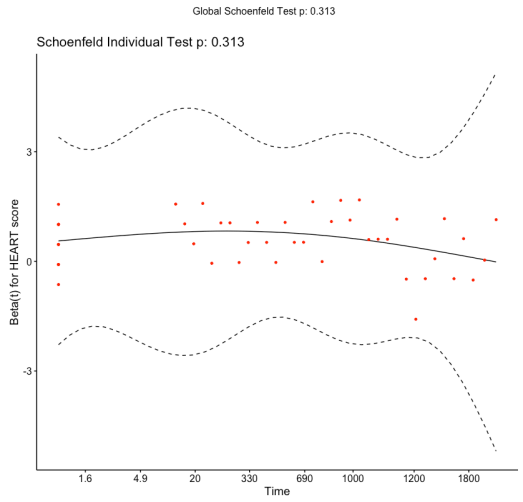
Appendix for Chapter 8.

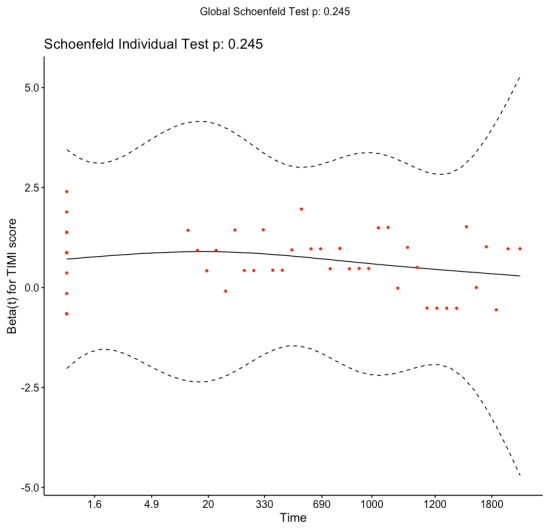
Schoenfeld residuals



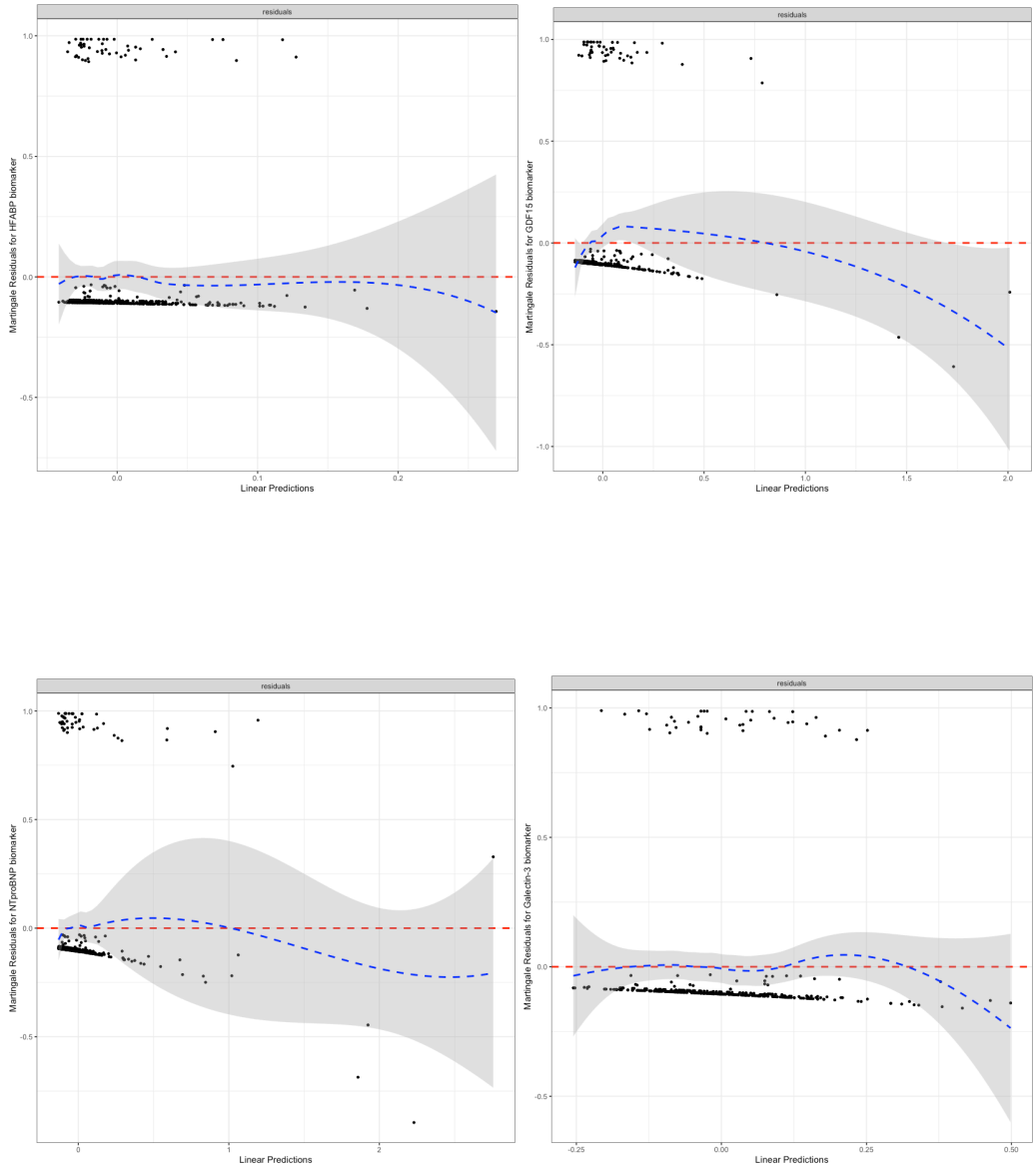

Global Schoenfeld Test p: 0.352
Schoenfeld Individual Test p: 0.352
Beta(t) for Galectin-3 biomarker
1
0
-1
1.8
5.4
85
450
750
1000
1200
1700
Time

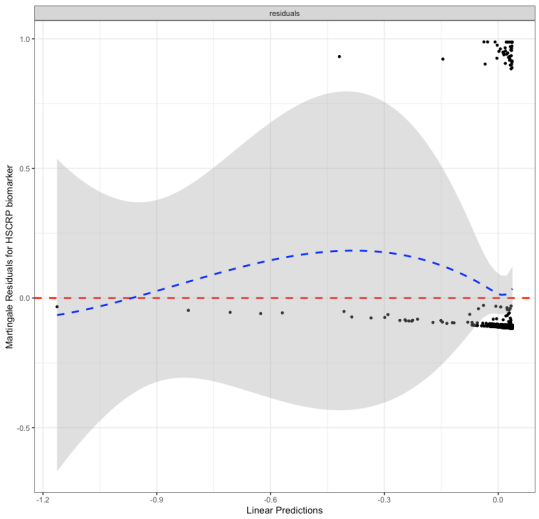

Global Schoenfeld Test p: 0.7867
Schoenfeld Individual Test p: 0.7867
Beta(t) for GRACE score
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
1.6
4.9
20
330
690
1000
1200
1800
Time

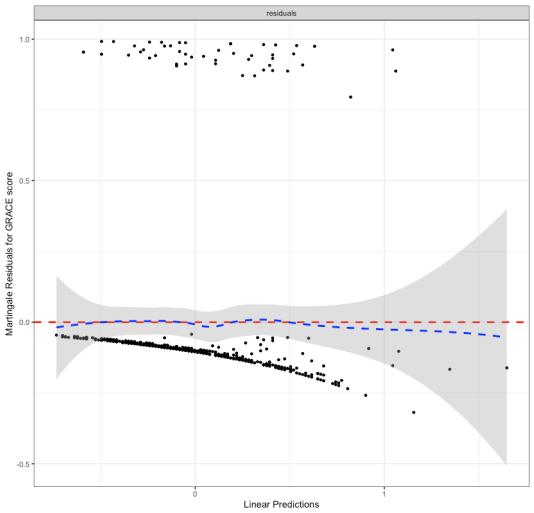

Global Schoenfeld Test p: 0.313
Schoenfeld Individual Test p: 0.313
Beta(t) for HEART score
3
0
-3
1.6
4.9
20
330
690
1000
1200
1800
Time

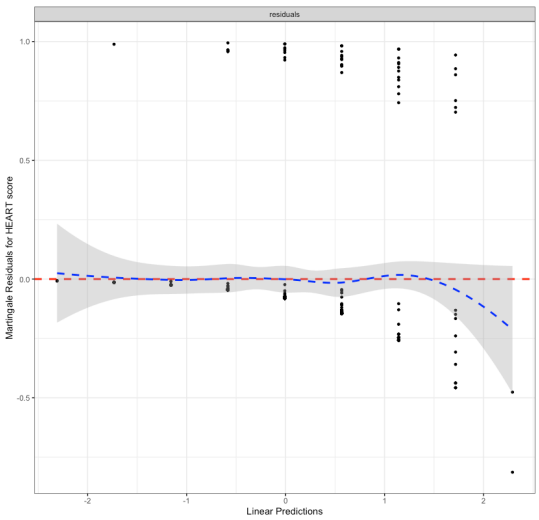

Global Schoenfeld Test p: 0.245
Schoenfeld Individual Test p: 0.245
Beta(t) for TIMI score
5.0
2.5
0.0
-2.5
-5.0
1.6
4.9
20
330
690
1000
1200
1800
Time

Martingale residuals




residuals
Martingale Residuals for HSCRP biomarker
1.0
0.5
0.0
-0.5
-1.2
-0.9
-0.6
-0.3
0.0
Linear Predictions


residuals
Martingale Residuals for GRACE score
1.0
0.5
0.0
-0.5
0
1
Linear Predictions


residuals
Martingale Residuals for HEART score
1.0
0.5
0.0
-0.5
-2
-1
0
1
2
Linear Predictions

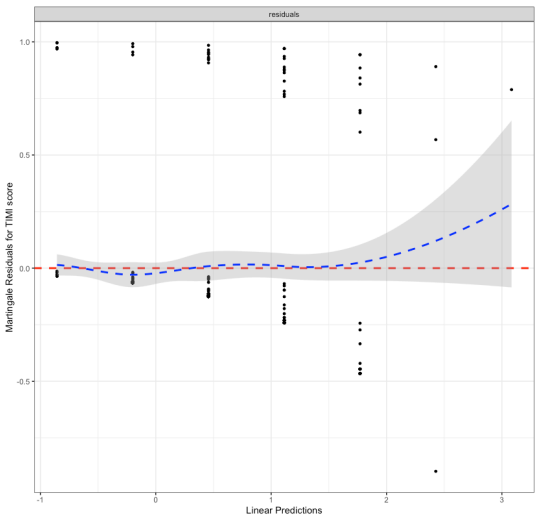

residuals
Martingale Residuals for TIMI score
1.0
0.5
0.0
-0.5
-1
0
1
2
3
Linear Predictions

Table. Correlation of biomarkers.

		Hs-cTnT	Hs-cTnI	HFABP	GDF15	NTproBNP	HSCRP	Galectin-3	Creatinine	Age
Hs-cTnT	Correlation Coefficient	1	0.447	0.220	0.114	0.283	0.037	0.104	0.102	0.386
	Sig. (2-tailed)	.	<0.001	<0.001	0.012	<0.001	0.433	0.042	0.024	<0.001
	N	487	479	487	487	471	445	386	485	487
Hs-cTnI	Correlation Coefficient	Moderate	1	0.231	0.142	0.354	0.026	0.093	0.219	0.314
	Sig. (2-tailed)		.	<0.001	0.002	<0.001	0.591	0.07	0<0.001	<0.001
	N		479	479	479	465	443	384	477	479
HFABP	Correlation Coefficient	Weak	Weak	1	0.147	0.128	0.078	0.110	0.203	0.199
	Sig. (2-tailed)			.	0.001	0.006	0.1	0.03	<0.001	<0.001
	N			487	487	471	445	386	485	487
GDF15	Correlation Coefficient	Very weak	Very weak	Very weak	1	0.240	0.125	0.165	0.218	0.332
	Sig. (2-tailed)				.	<0.001	0.008	0.001	<0.001	<0.001
	N				487	471	445	386	485	487
NTproBNP	Correlation Coefficient	Weak	Weak	Very weak	Weak	1	0.027	0.285	-0.013	0.458
	Sig. (2-tailed)					.	0.571	<0.001	0.786	<0.001
	N					471	434	377	469	471
HSCRP	Correlation Coefficient	Very weak	Very weak	Very weak	Very weak	Very weak	1	0.163	0.038	-0.024
	Sig. (2-tailed)						.	0.001	0.428	0.611
	N						445	380	443	445
Galectin3	Correlation Coefficient	Very weak	Very weak	Very weak	Very weak	Weak	Very weak	1	0.005	0.302
	Sig. (2-tailed)							.	0.927	<0.001
	N							386	384	386
Creatinine	Correlation Coefficient	Very weak	Weak	Weak	Weak	Very weak	Very weak	Very weak	1	0.039
	Sig. (2-tailed)								.	0.393
	N								485	485
Age	Correlation Coefficient	Weak	Weak	Very weak	Weak	Moderate	Very weak	Weak	Very weak	1
	Sig. (2-tailed)									.
	N									487