High sensitivity Cardiac troponin to rule-out stable coronary artery disease

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the faculty of Biology, Medicine and Health

2023

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Table of Contents

Word count: 34153.

List of tables

List of Figures

List of Abbreviations

ACS	acute coronary syndrome
AEs	adverse events
CVD	cardiovascular disease
cTn	cardiac troponin
cTnI	cardiac troponin I
cTnT	cardiac troponin T
CAD	coronary artery disease
CK-MB	creatine kinase-myocardial band isoenzyme
CV	coefficient of variation
DALYs	disability-adjusted life year
GCP	good clinical practice
hs-cTn	high-sensitivity cardiac troponin
HRA	health research authority
IHD	ischemic heart disease
ICER	incremental cost effectiveness ratio
LVH	left ventricular hypertrophy
LBBB	left bundle branch block
MI	myocardial infraction
MRI	Manchester royal infirmary
NICE	National Institute for Health and Care Excellence
NSTEMI	non-ST elevation myocardial infarction
QALYs	quality-adjusted life years
RACPC	Rapid Access Chest Pain Clinic
STEMI	ST elevation myocardial infarction
RBBB	right bundle branch block
SAEs	serious adverse eventsAbstract
CAD-C model	Coronary Artery Disease Consortium (CAD-C) risk score
QUADAS	Quality Assessment for Diagnostic Accuracy Studies

Abstract

Background:

Stable angina is the most common symptom of stable coronary artery disease (sCAD), typically occurring during physical exertion or emotional stress. At present, diagnosis relies on imaging. However, there has been growing interest in the use of high-sensitivity cardiac troponin (hs-cTn) assays to identify patients with such low probability of sCAD that the diagnosis can be considered 'ruled out'.

Objective:

The overall aim was to explore the role of high-sensitivity cardiac troponin in ruling out sCAD without imaging.

My specific objectives were to: (a) systematically review the literature on this topic; (b) evaluate the diagnostic accuracy of two different hs-cTn assays for stable sCAD; and (c) evaluate the diagnostic accuracy of the hs-cTn assays when used in combination with widely used risk scores for sCAD.

Methods:

To systematically review the literature, two individuals searched two databases and identified relevant papers. Data were extracted and synthesized in a narrative way. A prospective diagnostic test accuracy study was undertaken, including participants attending clinic with suspected stable angina. All patients had blood drawn for hs-cTn and underwent imaging in accordance with routine practice. Samples were tested for two hs-cTn assays (Roche hs-cTnT and Abbott hs-cTnI). Data were collected to calculate several risk scores including Diamond-Forrester and Coronary Artery Disease Consortium (CAD-C).

Results:

The systematic review identified 570 relevant papers evaluating three hs-cTn assays. In summary, hs-cTn assays alone were insufficiently sensitive to rule-out sCAD, although authors had not consistently evaluated the lowest possible cutoff to maximise sensitivity. Early evidence suggests that combining hs-cTn with risk scores could achieve high negative predictive value. The prospective study recruited 306 participants. This identified that hs-cTnT (cutoff 3ng/L) had a sensitivity of 82.6% (95% CI 71.6% – 90.7%) for sCAD; whereas hs-cTnI had a sensitivity of 65.7% (95% 53.1% - 76.9%). Of the risk scores evaluated, Diamond-Forrester had the highest sensitivity, achieving 95.5% (87.3% - 99.1%) when used alone. Adding hs-cTn to Diamond-Forrester achieved a sensitivity of 98.6% (95% CI 92.3% - 100 %) for hs-cTnT and 96.8% (95% CI 88.8 - 100%) for hs-cTnI.

Conclusion:

Neither hs-cTnT (Roche) nor hs-cTnI (Abbott) could rule-out sCAD when used alone. The Diamond-Forrester risk score had higher sensitivity. Combining risk scores and hs-cTn gave slightly higher sensitivity still, but the added value of troponin was small. Future research should focus on alternative biomarkers and improving risk scores in patients with suspected sCAD.

Declaration

I declare that this is my own work, and there is no portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Acknowledgements

Completing my PhD has been a long five-year journey. During this time I had support and guidance from many people who gave me the will and strength to reach the end of this long, hard and wonderful journey. First and foremost, I would like to express the deepest appreciation to my greatest supervisor, Professor Richard Body, who made this work possible. I could not finish my PhD without his support, guidance, knowledge and experience. He was my mentor and my friend during this long journey. I wished to have a good supervisor in the beginning of my study but I had the most wonderful supervisor. Thank you for everything.

I would also like to express my warmest thanks to my two co-supervisors: Dr Farzin Fath-Ordoubadi and Dr Reza Aghamohammadzadeh, for their support and for providing all the necessary information, knowledge and help that I needed.

Also, I am extremely grateful to the research nurses at Manchester Heart Centre for their help in the beginning of my study. My sincere thanks to all the staff in the Doctoral Academy for their support and help during my journey. They supported me with all the necessary documents, files and guidance that I have requested.

Last but not the least, I would like to thank my lovely family for all the support and sacrifices they made during my journey. Without you I would never have been able to finish my PhD. Nothing can express my deep and big gratitude for my father Haqi, for believing in me and for his prayers, emotional and financial support. I would also like to say thank you to my mother and brother Samah and Zaid, for their love, prayers and support during my journey. Also, my special thanks to my uncle

family doctor Audi and Farah, for all the support, they have been there for me from the first day I arrived in the UK.

would also like to express my love, gratitude, pride and thanks to my lovely wife Laween, to be there for me during my desperate and darkest days. You stood beside me when no one was there. Without you or without your love or support, I would not have been able to complete my journey. Thank you very much for all the sacrifices you made for me.

Chapter 1 : Background

1.1. Epidemiology of ischemic heart disease

Ischaemic heart disease (IHD) is the leading cause of death and disability worldwide, putting a significant economic and resource load on the global/public health system. It was responsible for about 8.5 million deaths in 2016 (1), and has been the most important cause of death in the last 15 years. In the present time the IHD burden affects all the world. We can see a decrease in death rates in developed countries, though rates are increasing in developing countries due to increases in risk factors like smoking, obesity and lifestyle changes. IHD causes about 3.8 million men and 3.4 million women globally to die every year. From 1990 statistics 3.5 million of the 6.2 million deaths from IHD were in developing countries. It is projected that death from IHD will account for about 7.8 million of 11.1 million deaths by 2020 in these countries (2). In spite of the fact that mortality for this disease has decreased in the last 10 years in western countries, it is still the main cause of death accounting for one third of all deaths in people more than 35 years (3). In Europe IHD is the leading cause of death. It is responsible for just over 1,740,000 deaths a year affecting around 860,000 males and 875,000 females and accounting for approximately 40% of all deaths in total. The statistics are similar within the confines of the European Union (EU). In the EU, IHD is the leading cause of death as it is in Europe, and is responsible for 353,000 deaths (14%) among males and more than 297,000 deaths (12%) among females (Table 1.1) (4). IHD is not only a major cause of death, but also a major cause of disability. This can be measured using disability-adjusted life year (DALYs), which is an aggregate of years of life lost due to premature death and years of healthy life lost due to disability (5). We can see in Europe from 2002 statistics that IHD was responsible for 11% of all DALYs. This is the same as cancer, however, totally cardiovascular disease (CVD) was responsible for 23% of all DALYs. On the other hand, IHD was responsible for 8% of all DALYs in the EU, while CVD as a whole was responsible for 22% of all DALYs in the EU (5). According to 2015 statistics, IHD was responsible for 13% of all DALYs in Europe, representing an increase in the disease burden. Similarly, the EU also had a slight rise in 2015, as IHD was responsible for 10% of all DALYs (4). In the United Kingdom (UK), IHD is the leading cause of death, and it is responsible for more than 65,000 deaths each year. According to the

latest statistics, about 180 people die every day, which amounts to approximately 1 death every 8 minutes (6).

Table 1.1: Total number of deaths from (IHD) for males and females in Europe and the EU

Countries	Year	Males	Females
Albania	2009	1,658	1,306
Armenia	2014	4,442	4,128
Austria*	2014	7,041	7,244
Azerbaijan	2007	3,380	2,695
Belarus	2014	24,096	24,881
Belgium	2013	4,783	3,444
Bosnia and Herzegovina	2014	2,082	1,800
Bulgaria	2013	6,944	5,734
Croatia	2014	4,912	5,919
Cyprus	2013	438	205
Czech Republic	2014	12,603	13,436
Denmark	2012	2,426	1,944
Estonia	2014	1,495	1,909
Finland	2014	5,532	4,805
France	2013	19,445	13,993
Georgia	2014	2,943	3,043
Germany	2014	64,467	56,699
Greece	2012	7,312	4,491
Hungary	2014	14,589	17,550
Iceland	2009	204	146

Ireland	2013	2,707	1,935
Israel	2013	2,179	1,786
Italy	2012	37,958	37,140
Kazakhstan	2015	7,218	5,355
Kyrgyzstan	2013	5,575	5,574
Latvia	2014	3,551	4,536
Lithuania	2014	6,248	8,481
Luxembourg	2014	205	119
Malta	2014	352	337
Montenegro	2009	256	161
Netherlands	2013	5,354	3,912
Norway	2014	2,220	1,872
Poland	2014	21,044	17,494
Portugal	2014	4,178	3,278
Republic of Moldova	2014	6,533	8,090
Romania	2014	25,174	25,496
Russian Federation	2011	268,484	299,698
San Marino	2000	3	4
Serbia	2014	5,382	4,589
Slovakia	2014	6,138	7,200
Slovenia	2010	1,108	943
Spain	2014	19,510	13,573
Sweden	2014	6,947	5,626
Switzerland	2013	4,097	3,682
TFYR Macedonia	2010	1,086	666

Turkey	2013	32,257	22,987
Turkmenistan	2012	3,545	3,121
Ukraine	2014	126,762	164,683
United Kingdom*	2013	43,056	29,968
Uzbekistan	2014	22,300	19,592
EU		335,517	297,411
Europe		862,219	877,216

In the United Kingdom (UK) IHD is the leading cause of death, and it is responsible for more than 65,000 deaths each year. From the latest statistics about 180 people die every day, which approximate 1 death every eight minutes (6).

1.2. Pathophysiology of Coronary Artery Disease

Our understanding of the pathophysiology of CAD has increased and developed in recent years. Previously the common concept of CAD was that this is a cholesterol storage disease, where lipids accumulate within the arterial wall. Subsequently our understanding has evolved from suggesting that CAD is a cholesterol disease to the latest research suggesting that the coronary atherosclerosis is an inflammatory condition, occurring in response to an injury or infection affecting the artery (7). Atherosclerosis is a very serious medical condition that occurs when arteries are stenosed with plaque or atheroma. This stenosis will decrease the amount of oxygen, blood and nutrition arriving at the heart and brain. The severity of atherosclerosis will depend on the plaque size and it is stability. From that concept we can classify the plaques in to two types: the stable plaques and the unstable plaques. Stable plaques are the cause of stable angina and can present with stable clinical features like retrosternal heaviness or pressure and chest pain that happens due to physical activities or stress and can be relieved by rest. These plaques have more stability than unstable plaques because they are less likely to rupture and cause acute coronary syndrome (ACS). Unstable plaques are responsible for

unstable angina and are prone to rupture, causing ACS. These two plaques have different pathological features.

To understand those features and the differences between stable and unstable plaque we must first understand how plaques form. Atherosclerotic plaque is a complicated process that takes place in the innermost layer of the artery called the tunica intima, between smooth muscle cells and endothelial cells of the vessel wall and the leukocytes (cells of the immune system) (8). Stable plaque consists of dysfunctional endothelium that covers the plaque with hard fibrous cap that is unlikely to rupture, unlike unstable plaque. Fibrous cap consists of smooth muscles, collagen and macrophages, these compounds are responsible for the cap's strength. Under these structures are foam cells and finally consist of new vessels at the sides of these plaques. Moreover, these plaques are the main cause of the fixed obstruction of arteries.

In contrast, unstable plaques consist of less extracellular matrix, foam cells, macrophages, more lipids and a thin fibrous cap. Unstable plaque is also called vulnerable plaque, meaning that this plaque is vulnerable to rupture or erosion, which can lead to an acute coronary syndrome (9). Stable plaques are more likely to cause arterial stenosis, whereas the unstable plaques cause less fixed stenosis as the plaques are softer and 'outwardly remodel'. In fact, two thirds of unstable plaques stenose the artery by less than 30% (10). Symptoms of stable angina are caused by the fixed plaques that block the artery. When the patient is at rest, the myocardium is receiving sufficient amount of blood. However, when the patient is undertaking exercise or during stress (emotional or non-emotional), the myocardium will require more blood and oxygen due to the increase in contraction rate and stroke volume. This demand cannot be met while the arteries are stenosed, which will lead to myocardial ischemia and chest pain. However, these symptoms will disappear after the exercise or the stress are finished and the myocardial oxygen demand will come back to it is baseline. Therefore, the current

approach to detection and diagnosis of stable coronary artery disease generally depends on the detection of coronary stenosis.

1.3. Clinical manifestations of IHD

IHD can present in a number of ways. Acutely, it may present as an acute coronary syndrome (ACS). ACS is a spectrum of conditions that includes acute myocardial infarction (AMI) and unstable angina.

1.3.1. Acute coronary syndrome (ACS)

ACS is caused by a reduction of blood flow and therefore the delivery of oxygen to the heart (11). ACS predominantly occurs due a build-up of fatty materials called plaque inside the walls of coronary arteries, which deliver oxygen and nutrients to the myocardium. A rupture or split occurring in a plaque leads to the formation of thrombus. These clots are responsible for restricting the flow of blood to the heart muscle (12). Many risk factors increase the probability of ACS occurring including diabetes, family history, high blood pressure, high cholesterol, obesity, smoking and other life style factors such as lack of physical activity. ACS has three clinical manifestations: ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infraction (NSTEMI) and unstable angina. STEMI occurs due to a sudden and complete occlusion of a coronary artery, whereas NSTEMI usually occurs due to the partial (but not complete) obstruction of a coronary artery. Although these two types are almost the same in terms of clinical presentation, they look different in diagnosis especially with regard to the electrocardiogram (ECG). Both STEMI and NSTEMI typically cause chest pain. The pain may radiate to one or both arms, to the shoulder(s), neck and/or jaw. There may be associated shortness of the breath, nausea and vomiting (13). STEMI is initially diagnosed on the ECG, whereas NSTEMI is mainly diagnosed by cardiac biomarkers. The biomarker of choice for diagnosing myocardial infarction is cardiac troponin. Analysing the ECG is key to differentiating between STEMI and NSTEMI, as there is a difference in the so-called ST segment. In STEMI, the ST segment is raised up above the baseline, which is called ST elevation. As the condition evolves over time, we usually see the development of Q waves following the complete blockage of a

coronary artery. On the other hand, in NSTEMI the ST segment may be depressed (so called ST depression) and there may be T wave inversion, caused by partial blockage of the coronary artery (14). The ECG may also appear normal in NSTEMI.

Unstable angina occurs when there is a plaque rupture or split that causes the patient to have symptoms but does not cause detectable injury to the myocardium. It typically manifests as a pain in the chest that appears with physical activity or physical and emotional stress or at rest. This is differentiated from stable angina (which is defined below) by the occurrence of increasing symptoms with reducing exercise tolerance or increasing severity and duration of symptoms. This happens due to blockages of blood flow in the arteries that supplies the heart. Unstable angina is considered a very serious medical condition requiring immediate treatment. If left untreated it can lead to acute myocardial infarction, heart failure and arrythmias which can be life threatening conditions. Unstable angina has two types of symptoms: typical and atypical complaints. Typical presentation has symptoms of chest pain, which can be described as retrosternal pain and can be present as squeezing, crushing, pressure-like, burning, tightening and sharping pain, and can radiate to both arms, back, shoulders, neck and jaw. Atypical presentation includes dyspnoea, nausea, sweeting, syncope, abdominal pain and epigastric pain. Atypical symptoms often occur in patients with chronic disease like renal failure, diabetes and dementia, and they are common in elderly patients (15). Some studies showed that women many times are having the clinical manifestation of ACS but without chest pain or discomfort (12).

1.3.2. Stable Angina

IHD may also present as stable angina: this is a chest pain or discomfort usually in the centre of the chest that happens after physical excretion or stress due to narrowing or blocking in the coronary arteries that prevents the myocardium getting enough blood and oxygen. This is transient and related to the oxygen demand placed on the myocardium. The major symptom of stable angina is chest pain, but the pain may differ from one patient to another. Pain can present as squeezing, burning, pressure, compressing and indigestion. The pain will usually only last for several minutes. During the episode, this pain can radiate to the shoulders, arms, neck, and jaw or can stay in the chest. In addition, other symptoms may be present, including fatigue, dizziness, dyspnoea, palpitation, sweeting and rapid breathing. This pain usually occurs with or after physical exertion, exercise or stress, such as sport, climbing stairs and emotional distress (16).

1.4. Approaches to the diagnosis of stable angina

1.4.1. Patient symptoms

Although the symptoms of stable angina are typically as described in the paragraph below, symptoms experienced by individual patients may vary widely. The chest pain may be classified into three types: atypical chest pain, typical chest pain and non-anginal pain as shown in (Table 1.2) (17).

Table 1.2: Traditional clinical classification of anginal chest pain (14)

Typical angina	*Substernal chest discomfort *Pain increased by physical exertion or emotional stress *Pain can be relieved by rest or nitrates
Atypical angina	Meets two of the above symptoms or characteristics
Non-anginal chest pain	Meets none of these characteristics

Furthermore, patients with stable angina may also have shortness of breath that may sometimes be difficult to differentiate from lung diseases (17). Duration of the pain in stable angina is not usually

more than 10 -15 minutes maximum, but in most cases the pain lasts only for several minutes. Patients' symptoms usually appear after exertion like walking especially in cold weather or climbing stairs. Symptoms may also increase after a heavy meal or waking in the morning. In addition, emotional stress is also a very important factor for increasing symptoms of stable angina. These symptoms usually disappear or are relieved after the exertion finishes, after taking a rest or after taking sublingual or buccal nitrates. In the presence of these typical symptoms, clinicians may immediately suspect the diagnosis of stable angina, but often patient symptoms alone are insufficient to confirm or refute the diagnosis. The clinicians also need to check the medical history of the patient, physical examination and electrocardiogram (ECG) to give the full and accurate diagnosis.

1.4.2. Electrocardiogram (ECG)

All patients that are suspected to have angina pectoris based on history and symptoms should have a resting 12-lead electrocardiogram (ECG) recorded. A normal ECG is common in patients with angina and does not exclude ischemia. ECG can help the clinician to determine the differential diagnosis, if the ECG was taken during the pain episode. During pain episodes the ECG can detect ST-segment changes while ischaemia is present. Often, evidence of existing coronary artery diseases (CAD) such as previous MI can be detected on the ECG. In addition, ECG can show other diseases like right bundle branch block (RBBB), left bundle branch block (LBBB), left ventricular hypertrophy (LVH) and arrhythmias (17). During periods of ischaemia, ECG changes in patients with stable angina include ST-segment depression and T-wave inversion. The resting ECG in patients with stable angina is not enough to spot every case and is not 100% accurate to give the final diagnosis, therefore other methods of diagnosis are needed. However, the resting ECG is considered a very important step for diagnosis after the patient's symptoms and medical history.

1.4.3. Echocardiography

Echocardiography is a non-invasive cardiac investigation that uses ultrasound waves to check the heart structure and assess it functions. Echocardiography can show the heart chambers, heart valve functions, blood flow and function of the heart muscle. There are several different types of echocardiography. The first is a transthoracic echocardiogram, which is the most frequently used by doctors. The second is called transoesophageal echocardiogram, this is used when the clinician could not get a clear image of the heart or requires more details. The third type is called Doppler echocardiogram, which is used to detect the speed and flow of the blood to the heart. The last type is called stress echocardiogram, which can be conducted with either an exercise bicycle or a treadmill. This type is preferable for diagnosing patients who can exercise (18). Alternatively, pharmacological stress can be used to provoke ischaemia, which is discussed further below. Echocardiography has many advantages including low cost, excellent availability and zero radiation. However, disadvantages may also be present but not with a huge effect on patients. These include discomfort during removal of electrodes from a patient's body, irritation, sore throat (for transoesophageal echocardiography) and irregular heart beat due to the exercise (or medications) that are used to raise a patient's heartbeat.

1.4.4. Dobutamine stress echocardiography (DSE)

DSE is a test that uses ultrasound waves to assess the heart function and structure during rest and exercise. During this test, the patient will be injected with a medication called dobutamine that increases the heart rate slowly and gradually. For 24 hours before the test patients will be asked to stop ingesting caffeine products such as coffee, cola and chocolate will be restricted as also for tobacco. This test is less expensive than alternative tests including MPI. Furthermore, the duration of the test is not more than one hour, which is generally acceptable for patients. The test does have some small risks including heart rhythm abnormalities, AMI, decreased blood pressure, shortness of

breath, nausea, dizziness, headache and fatigue. A study conducted the UK National Institute for Health and Care Excellence (NICE) showed that DSE has a sensitivity of 77% (95% confidence intervals [CI] 69% - 83%) for identifying 50% stenosis of the coronary arteries, with 86% specificity (95% CI 68% - 95%) (19). On the other hand, DSE has a sensitivity of 64% (95% CI 49% - 76%), with specificity of 90% (95% CI 86% - 93%) for 70% stenosis of the coronary arteries (19).

1.4.5. Exercise ECG

Exercise ECG Is used to assess the function of the heart in patients with suspected CAD, and it is done by an exercise or stationary bicycle or walking on a treadmill. It also can be called stress test or cardiac stress test, and it uses different treadmill speeds that increase gradually (e.g. through the 'Bruce protocol'). In this test a patient's vital signs including blood pressure and heart rate are tracked throughout, in addition to the electrical activity of the heart through an ECG. The most important abnormality that can be detected with exercise ECG is ST-segment deviation in one or more ECG leads. Such ECG changes usually represent significant myocardial ischaemia, although in approximately 13% patients these changes are considered less significant if they only appear during the recovery phase (17). Many studies have been conducted analysing the accuracy of exercise ECG in CAD diagnosis. These studies have reported that the sensitivity ranges between 23 and100% (mean 68%) and the specificity ranged from 17-100% (mean 77%) (17). The accuracy of the test will be decreased or become valueless in the presence of left bundle branch block (LBBB), paced rhythm and Wolff Parkinson white syndrome (WPW) (17). Furthermore, this test has been shown to be less accurate in women (20). Exercise ECG results can be affected by different medications like digoxin, beta-blockers, antihypertensive, vasodilators and nitrates (21).

1.4.6. Myocardial perfusion imaging (MPI)

MPI is a non-invasive cardiac imaging test that uses a radioactive substance called tracer, isotope or radionuclide to evaluate the blood flow to the myocardium, by using a device or a scanner called a gamma camera that detects the radio-active agent in the blood. This test is also known as a thallium scan, nuclear medicine scan or technetium scan. Usually, the test is conducted during physical exercise by the exercise bicycle or treadmill and at rest, so that the clinicians can see the changes in blood flow during exercise and rest. In patients who cannot exercise, the doctor may give them medication that increases the heart rate instead of exercise. Patients should have some preparations before conducting this test like telling the doctor about the medications they take, because some medications will affect the accuracy of the test. Furthermore, patients should not eat or drink anything containing caffeine (tea, coffee, chocolate, cola, etc.) 24 hours before the test, because caffeine products can affect the quality of the test. Also, female patients who are pregnant should tell the doctor immediately, because this type of test requires the use of a radioactive substance can affect the mother and the child. However, this test has many negatives like exposure to X-ray radiations that may cause cancer in future, high cost and the long duration of the test and time to result. A meta-analysis conducted by NICE identified that MPI has a sensitivity of 81% (95% CI 74% - 86%) for a 50% stenosis of the coronary arteries, with 78% specificity (95% CI 70% - 85%) (19). On the other hand, for identifying 70% stenosis MPI has a sensitivity of 76% (95% CI 44% - 93%), with 76% (95% CI 58% - 88%) specificity (19).

1.4.7. CT coronary angiography (CTCA)

CTCA is a test that uses X-ray imaging to examine the coronary arteries and detect any narrowing or plaque that may block the coronaries. In this test the patient will receive a contrast agent containing iodine that is given as an intravenous injection (IV). This contrast material gives a clear picture of the inside and outside of the vessels and arteries. This test will show the inside of the body as images or, moreover, these images can be performed as three-dimensional images or viewed on the computer

monitor. The doctor will ask the patient not to eat or drink for several hours before the test especially caffeinated products like tea, cola and coffee, because it will increase the heart rate and that affects the test accuracy. There are many benefits of this imaging modality. For example, CT scanning is able to give a clear image of patient bone, blood vessels and soft tissues at the same time as imaging the coronary arteries, therefore the doctor can diagnose other illnesses that may affect the heart or cause the patient to have symptoms. However, the test also has many risks like disturbance of the kidney function in individuals with kidney disease, and may cause damage to the nerves or blood vessels if the material leaks out from the vein. Rarely, patients may have an allergic reaction to the contrast material. A systemic review by NICE identified that CTCA has a sensitivity of 96% (95% CI 94% - 97%) for identifying 50% stenosis of the coronary arteries, with 79% specificity (95% 72% - 84%) (19). On the other hand CTCA has a sensitivity of 96% (95% CI 88% - 99%) for identifying 70% stenosis, with 72% specificity (95% CI 55% - 85%) (19).

1.4.8. CT calcium scoring

CT calcium scoring is a non-invasive cardiac test that detect and measures the amount of calcium in the calcified plaque that can build up in heart arteries. The main use of this test is to detect and quantify CAD. A review made by NICE showed that, using an Agatson score threshold of 0, CT calcium scoring had a sensitivity of 99% (95% CI 97% - 99%) for a 50% stenosis of the coronary arteries, with 49% specificity (95% CI 36% - 63%) (19). At a threshold of 400 with the Agaston score, CT calcium scoring has a specificity of 88% (95% CI 87% - 88%) for identifying 50% stenosis of the coronary arteries, with 54% sensitivity (95% CI 52% - 57%) (19). This investigation is most commonly used to calculate the 10-year probability of IHD or CVD in asymptomatic patients and used as a part of a risk stratification consultation.

1.5. Cardiac troponin (cTn)

Cardiac troponin (cTn) is a protein that is involved in muscle contractions. Troponin can be found in both cardiac and skeletal muscles, though there are cardiac-specific isoforms that are only contained in cardiac muscle. This protein is released to the blood after myocardial damage or injury that can help the doctors to detect the heart muscle damage in a simple blood test. There are three types of cardiac troponin: troponin C, troponin I, troponin T. Troponin T and I are the most popular types for diagnosing heart disease cases, unlike troponin C as there are no assays available (22). Previously doctors used different types of blood tests for diagnosing heart diseases, including creatine kinase (CK) MB isoenzyme (CK-MB). However, these enzymes were not very accurate because they were not sensitive and specific enough for AMI. Therefore, cardiac troponin is now the preferred test for doctors due to its high sensitivity and specificity (23). Cardiac troponin concentrations are elevated in AMI but also in patients with myocardial injury or other chest pain caused by different reasons. Cardiac troponin is mainly used to diagnose AMI. The fourth universal definition of myocardial infarction states that the diagnosis of AMI should be made with cTn. Patients with at least one cTn concertation exceeding the 99^{th} percentile upper reference limit (URL) for normal values is considered to have myocardial injury (24). Cardiac troponin can be detected not only in AMI but also in different diseases including severe tachycardia, heart failure, myocarditis, pericarditis and cardiomyopathy. Furthermore, cardiac troponin may increase in non-cardiac diseases such as sepsis, gastrointestinal bleeding, pulmonary embolism, chronic obstructive pulmonary disease (COPD) and end-stage renal disease. In order to make the diagnosis of AMI, clinicians must consider whether the myocardial damage has been caused primarily by CAD (type 1 myocardial infarction) or by an underlying condition that puts stress on the heart. The latter is called type 2 myocardial infarction if there is other evidence of myocardial ischemia, and myocardial injury if not. Myocardial injury may be labelled as acute or chronic. In acute myocardial injury, levels will change over time; whereas levels will remain relatively stable over time in a chronic myocardial injury. It is important to also note that, even in the absence of 'myocardial injury', cardiac troponin levels are predictive of cardiovascular risk.

Even when troponin levels are within the normal range, we know that higher levels predict the development of cardiovascular disease in future (25). Further, we also know that cardiovascular risk factors (such as hypertension, hyperlipidaemia or heart failure) lead to higher baseline cardiac troponin levels even in apparent health (26).

1.5.1. High-sensitivity cardiac troponin testing

High-sensitivity cardiac troponin (hs-cTn) assays help us to detect very low concentrations of cardiac troponin, therefore, the detection of AMI is improved and more accurate. An assay is only labelled as high-sensitivity if meets the following two criteria: first, the assay should have high precision, which can be measured with the so-called coefficient of variation (CV). The CV is calculated by measuring the sample several times then dividing the standard deviation by the mean result (22). So, the assay will only be labelled as high-sensitivity if the CV is less than 10% when the concentration of the cardiac troponin in the sample is equal to the 99th percentile upper reference limit. Second, the assay should be able to quantify the concentration of troponin (high analytical sensitivity) in at least 50% of apparently healthy people. Previous troponin assays were unable to detect the low concentration of troponin in healthy people but now these new hs-cTn assays are able to detect cTn in more than 50% of healthy people (22). The key advantage that hs-cTn assays provide is early diagnosis of ACS, decreasing the duration of patient stay in hospital, therefore, patients will be reassured early and the burden upon crowded hospitals will be decreased.

1.5.2. Cardiac troponin testing in stable angina

Cardiac troponin is a marker of myocardial injury. After an acute injury (such as a myocardial infarction), we would expect to see levels rise and fall. That would not be expected to occur in patients with stable angina. Some patients have a chronic myocardial injury, which means that they have raised cardiac troponin levels (above the normal range) on an ongoing basis. This can be caused

by chronic kidney disease or chronic heart failure, for example. With high-sensitivity cardiac troponin assays, we can measure cardiac troponin levels in the majority of apparently healthy individuals. Patients with cardiovascular disease, or with risk factors for cardiovascular disease, are known to have higher cardiac troponin levels at baseline, though they may still be within the normal range (27). This leads us to question whether patients with stable coronary artery disease may have higher baseline levels, and therefore whether symptomatic patients with very low baseline levels have such low probability of coronary artery disease that they do not require any imaging.

At the present time there is no clear evidence to support cardiac troponin in diagnosing stable angina. A study has been conducted on high-sensitivity cardiac troponin I in the detection of exercise induced myocardial ischemia in patients without known coronary artery disease. In this study 714 patients were included without any history of coronary artery disease, who were undergoing rest/stress myocardial perfusion single photon emission tomography and coronary angiography to diagnose possible myocardial ischaemia (28). In this study the cardiologist was able to access all the clinical information about the patients to assess the diagnosis of exercise induced myocardial ischemia by using a scale at two time points, before and after exercise testing. High-sensitivity cardiac troponin I was measured before the patient underwent the exercise test. In this study high-sensitivity cardiac troponin showed similar diagnostic accuracy to the clinical judgement of the treating clinician after myocardial perfusion imaging had been completed, and had superior accuracy to the clinical judgment of the treating clinician before imaging. Furthermore, high-sensitivity cardiac troponin beside the clinical diagnosis provide sensitivity of 98% and negative predictive value of 96% in diagnosing exercise induced myocardial ischemia (28). Therefore, in conclusion the high-sensitivity cardiac troponin I could potentially have ruled out stable coronary artery disease for some patients without the need for exercise test or myocardial perfusion single photon emission tomography.

1.6. Risk scores

Risk scores are a tools that made a major effect in assessing and predicting the outcomes in the healthcare field. These risk scores are used to check the probability of the conditions that are suspected by clinicians, and help the healthcare professionals to make decisions regarding a patient's diagnosis. The most important risk scores used in cardiovascular diseases are the Framingham risk score and Diamond-Forrester risk score. The Framingham risk score is used to determine the risk of cardiovascular disease in 10 years duration, this score is calculated based on patient risk factors like (age, cholesterol, blood pressure, smoking, sex and diabetic statues) (29) (30).

The Diamond-Forrester risk score is a pre-test probability tool that use to identify patients with CAD. This tool was developed in 1979 and it uses patients risk factors like age, sex, chest pain (typical, atypical and non-anginal). By combining these variables, the Diamond-Forrester risk score will estimate the probability of CAD and give a rational approach to address the need for further diagnostic tests (31,32). This is done by consulting with a look-up table, which provides pre-test probability for each combination of age, sex and chest pain typicality.

According to that the pre-test probability, the risk score has been used to guide the referral of patients for non-invasive cardiac imaging. However, many studies demonstrated that although the Diamond-Forrester risk score has played a significant role for estimating the need for cardiac imaging, it also overestimated the likelihood of CAD. For example in the study by Bittencourt MS et al, DF systematically overestimated the probability of CAD. For example, if DF calculated the probability of CAD to be 70%, the actual probability was approximately 30% as detected by coronary CTA. Moreover, DF gave low positive predictive value of 44% (180 of 410) compared to the both CAD consurtum model used in this study of 71% (17 of 24) and 79% (19 of 24) respectively, the use of DF led to higher proportion of patients with higher pretest probability of 18.0% versus 1.1% for both CAD consortium scores used in this study (33). This issue led to increased efforts to create new risk scores that will give a rational results for estimating the probability of CAD.

Therefore, three new risk scores were created. The updated Diamond-Forrester risk score (UDF) used age, sex and chest pain type. The original DF study included patients aged between 30 and 69 years old, and in this model they included patients of all ages and used age as a continuous predictor. The AUC was 0.79 (95% CI 0.77 – 0.81), which demonstrated a good performance for this model (34).

The coronary artery disease consortium (CAD-C) module, which is divided into three subtypes. The basic CAD-C module used age, sex and symptoms. The clinical module used the basic module plus cardiovascular risk factors (diabetes, hypertension, smoking, hyperlipidaemia and body mass index). And the extended module used the clinical module plus coronary artery calcium score. In this study they used the clinical model as the main predictor, and all the risk facors used in this model were assoisiated with obstructive CAD except the body mass index. Moreover, in comparison with the basic model, the clinical CAD-C model improved the prediction of CAD from 0.77 to 0.79 using cross validation C statistics. In terms of probabilities, CAD-C clinical model estimated that an 80 year old male with typical chest pain and risk factors would have a 91% CAD probability, whereas it estimated only 2% for a 50 years old women with non specific chest pain and without risk factors (35).

The CONFIRM (Coronary CT angiography evaluation for clinical outcomes: an international multicentre registry) registry score (CRS) used age, sex, symptoms and cardiovascular risk factors. This model was devided into two types: integer and best fit model. The integer model had a low AUC of 0.71 whereas the the fit model had the hughest AUC of 0.76. However, both models were superior to the DF, which had the lowest AUC of 0.64 (36).

There has been a comparison between these risk scores to determine which one is more accurate to identify patients with CAD. These risk scores are used in patients who had cardiac imaging like invasive coronary angiography and coronary computed tomography angiography (CCTA). Therefore, a study by Baskaran et al was conducted to compare these three risk scores. The study included patients who had symptoms of stable CAD with CCTA (37). In this study CAD-C model had the higest AUC of 0.790 (95% CI 0.768 to 0.811) compared to the UDF and CRS of 0.767 (95% CI 0.744 to 0.790)

and 0.749 (95% CI 0.726 to 0.771) respectively. The CAD-C model provided the best discrimination result for CAD and gave a best reclassification for low risk patients with CAD in compared with UDF and CONFIRM (37). This all showed that risk scores can give an accurate results by themselves, and areworthy of being evaluated as independent tools to diagnose patients with CAD.

1.7. Aims and objectives of this PhD

1.7.1 Aims

In my PhD I aimed to evaluate the diagnostic accuracy of high-sensitivity cardiac troponin assays for stable coronary artery disease. Specifically, my aim was to determine whether the diagnosis of stable coronary artery disease could be ruled out in symptomatic patients using a high-sensitivity troponin test, thus eliminating the need for imaging. The evidence to date therefore shows that hs-cTn has promise as a marker of stable CAD and could be used to avoid unnecessary imaging. We do not yet know if hs-cTn can safely rule-out CAD, if it is better than existing risk scores or if combining hs-cTn and risk scores may have any advantages.

1.7.2 Objectives

To address this aim, I had the following objectives:

- In Chapter 2, my objective was to systematically review the literature for existing evidence of the diagnostic accuracy of high-sensitivity troponin assays in suspected stable coronary artery disease.
- In chapter 4, my objective was to report overall recruitment and baseline charachteristics for the Manchester coronary artery disease study.
- In Chapter 5, my objective was to evaluate the accuracy of hs-cTnT in diagnosing patients with stable coronary artery disease.

- In Chapter 6, my objective was to use the available risk scores (Diamond Forrester, CAD-C model, Percentage probability, Pain typicality and Likert probability) in combination with hs-cTnT to rule-out patients with stable coronary artery disease.
- In chapter 7, my objective was to diagnose the accuracy of hs-cTnI in patients with stable coronary artery disease.
- In chapter 8, my objective was to check the accuracy of the combination of the available risk scores (Diamond Forrester, CAD-C model, Percentage probability, Pain typicality and Likert probability) and hs-cTnI in diagnosing patients with stable coronary artery disease.

Chapter 2 : Systematic review

2.1. Introduction

Before embarking on a new primary research project, it is important to know the existing literature in the field. Therefore, I aimed to systematically review the literature for evidence relating to the use of high-sensitivity cardiac troponin assays for the diagnosis of stable coronary artery disease (CAD) in outpatient settings.

Coronary artery disease (CAD) is one of the most common causes of death globally, and also creates a very high burden on the health system. Therefore, the early diagnosis of CAD is very important to avoid further risks and give good clinical outcomes for patients. In the meantime, the detection of CAD is mainly based on clinical decisions made by clinicians with reference to cardiac imaging. Better approaches to diagnosis are still required. Currently, the pre-test probability of CAD is low among patients who undergo imaging. Cardiac imaging has important limitations starting with the risk associated with unnecessary exposure to ionising radiation and ending with high costs. Therefore a new way of diagnosis is required to increase accuracy of the diagnosis. In that context, cardiac biomarkers may be useful as new diagnostic tools, and the most widely used for acute complication of CAD are cardiac troponin I and T. As technology developed in recent years, the assays have seen many improvements leading to development of high-sensitivity assays for cardiac troponin measurements. Therefore, these high-sensitivity assays have high sensitivity and specificity and with these good values will help us to detect CAD in shorter time and without the need of further cardiac imaging.

2.2. Methods

I used the following search strategy to screen for relevant papers, using the Ovid interface:

Database: Embase 1980 to 2020 Week 36, Ovid MEDLINE(R) 1946 to August Week 4 2020

Search Strategy:

1 stable angina.mp. (24243)

2 angina pectoris$.mp. (131776)

3 coronary artery disease.mp. (341447)

4 1 or 2 or 3 (444349)

5 sensitiv$.mp. (3566699)

6 troponin$.ti. (22415)

7 4 and 5 and 6 (1572)

8 7 (1572)

9 limit 8 to yr="2009 -Current" (1224)

10 diagnosis.mp. (8454969)

11 10 and 9 (735)

12 remove duplicates from 11 (624)

2.3. Identification of relevant papers

I reviewed the titles and abstracts of all papers identified, shortlisting potentially relevant titles. I reviewed the full text of potentially relevant papers, which was then checked by a second reviewer (Professor Richard Body, my primary supervisor). I included papers that evaluated patients presenting with suspected stable CAD in an outpatient setting (e.g. Rapid Access Chest Pain Clinic RACPC) who underwent high-sensitivity cardiac troponin testing as an index test; and imaging investigations as a reference method. The review intended to focus primarily on the diagnosis of anatomical CAD (to be consistent with NICE Clinical Guideline 95) but studies using functional imaging as a reference method were also considered for inclusion.

2.4. Assessment of bias and quality

All relevant studies were evaluated for risk of bias and methodological quality. For this purpose, I used a modified Quality Assessment for Diagnostic Accuracy Studies 2 (QUADAS-2) tool (Table 2.1), developed by two investigators (myself and my primary supervisor) to be specific to the review question. This tool consisted of 4 domains: patient selection, high-sensitivity troponin selection, coronary artery disease diagnosis allocation and flow and timing. Each section has questions that help us to reach a decision on the risk of bias.

2.5. Results

The search identified about 570 papers and articles. I reviewed the titles and abstracts of these papers to identify papers that are relevant to my study. I have identified the papers below that are relevant to my study and included them in this report. The assessment of study quality and bias is shown in (Table 2.1).

Table 2.1: QUADAS-2 assessment of included studies (✓Low Risk, ✗High Risk,? Unclear Risk).

Study	RISK OF BIAS				APPLICABILITY CONCERNS			OVERALL		
	PATIENT SELECTION	TROPONIN	CAD ALLOCATION	FLOW AND TIMING	PATIENT SELECTION	TROPONIN	CAD ALLOCATION	PATIENT SELECTION	TROPONIN	CAD ALLOCATION
[Walter et al], 2020	✓ A B	✓ A B	✓ A B	✓ A B C D E	✓	✓	✓	✓	✓	✓
[Cwikiall et al], 2019	✓ A B	✓ A B	✗ A B	✗	✓	✓	✓	✓	✓	?

				A B C D E						
[Adamson et al], 2018	✓ A B	✓ A B	✓ A B	✓ A B C D E	✓	✓	✓	✓	✓	✓
[Mueller et al], 2018	✓ A B	✓ A B	✓ A B	✓ A B C D E	✓	✓	✓	✗	✗	✓
[Orsini et al], 2018	✓ A B	✓ A B	✓ A B	✓ A B C D E	✓	✓	✓	✗	✓	✓
[Mouridsen MR et al]	✓ A B	✓ A B	✓ A B	✓ A B C D E	✓	✓	✓	✓	✓	✓
[Walter et al], 2018. [Abstr]	✓ A B	? A B	✓ A B	✓ A B C D E	✓	✓	✓	✓	?	✓

The study by [Walter et al] was conducted with 1896 patients included having CAD and possible symptoms of inducible myocardial ischaemia. In this study they used myocardial perfusion imaging with single photon emission computed tomography, as well as coronary angiography and fractional flow reserve measurements where available to confirm the presence of inducible myocardial ischaemia (38). Before making any tests, the clinicians recorded clinical assessments for the patients suspected to have inducible myocardial ischaemia with a special scale between 0% and 100%. High-sensitivity cardiac troponin T and I were measured before conducting any tests. The final diagnosis of inducible myocardial ischaemia was determined by two cardiologists, blinded to biomarker results. This study concluded that even with the use of very low high-sensitivity cardiac troponin results with concentration less than 2.5 ng/L (Abbott hs-cTnI) in patients with CAD, inducible myocardial ischaemia cannot be excluded (38).

The study by [Cwikial et al] has been conducted at Oslo University Hospital Ullevaal to see the value of adding high-sensitivity cardiac troponin T to an exercise stress test (EST) in diagnosing CAD. In this study, 297 patients with symptoms of CAD were included. All patients had EST and coronary angiography (39). The results of cardiac angiography were seen by one cardiologist and if there is any doubt in the results, another cardiologist was involved, blinded to the adjudication of the first one. This study reported that hs-cTnT had good accuracy in diagnosing CAD, it found that hs-cTnT were increased during rest and EST in patients with CAD diagnosed by angiography. Moreover, hs-cTnT when combined with EST gave a good diagnosing value than the EST alone in diagnosing CAD (39).

The third paper by [Adamson et al] reports on the findings of a study conducted in Scotland. In this study, 943 patients were included with suspected stable angina. All patients in the study had coronary computed tomographic angiography (CCTA). The blood samples for measuring high-sensitivity cardiac troponin I was obtain before CCTA was applied. In this study they used high-sensitivity single-

molecule counting assay on the Erenna platform (Singulex, Inc, Alameda, CA), which has a limit of detection of 0.1 ng/L, a limit of quantification (coefficient of variation, <10%) of 0.4 ng/L, and a 99th percentile upper reference limit of 10.9 ng/L for measuring hs-cTnI concertation. The authors concluded that using hs-cTnI in the RACPC could enhance the prediction of obstructive CAD in patients with suspected stable angina (40).

The study by [Mueller et al] was held at University Hospital Basel with 2062 patients who were suspected to have functionally relevant coronary artery disease (fCAD). In this study all patients had rest/stress myocardial perfusion single-photon emission computed tomography/computed tomography and coronary angiography to confirm the presence of fCAD(41). Blood samples were taken, and cardiac troponin was measured before stress testing. The measurement of hs-cTnT and hs-cTnI were done in a blinded manner. In this study hs-cTnI had sensitivity of 95% (93-96) and NPV of 89% (84-92), and for hs-cTnT the sensitivity was 95% (93-97%) with an NPV of 88% (84-92%). In conclusion for this study both hs-cTnT and hs-cTnI were found to be higher in patients with fCAD rather than patients without, moreover, both cardiac troponin assays showed good diagnostic accuracy (41).

In the fifth paper a study of 125 patients with suspected stable ischemic heart disease was conducted in Pisa, Italy. All patients had a stress test (electrocardiogram/echo exercise, echo dipyridamole and echo dobutamine tests). Blood was obtained before and after stress testing for hs-cTnT. In this study hs-cTnT was unable to detect the diagnosis of inducible ischaemia (42).

In the sixth paper a study was conducted with 157 patients, having suspected stable ischemic heart disease. In this study they used exercise testing to confirm the presence of ischemic heart disease. However, patients with a positive exercise test were referred for CT coronary angiography (CTCA). In

practice, 124 patients had CTCA (+/- coronary angiography) and 33 had coronary angiography. In this study high-sensitivity (Elecsys hs Troponin T, Roche Diagnostics A/S, Hvidovre, Denmark) with limit of blank 3.00 ng/L, a limit of quantification (coefficient of variation, 10%) of 13 ng/L and the 99th percentile in healthy individuals was 14 ng/L was used. It is found that the combination of exercise testing with cTnT had better results than exercise test by itself, and had higher sensitivity and negative predictive value.

In conclusion, when combined with other results, high-sensitivity cardiac troponin appears to improve sensitivity and specificity for CAD, thus adding clinical value for the diagnosis of stable coronary artery disease (43). Key results of the above studies can be found in Table 2.2 and Table 2.3.

Table 2.2: Basic description of studies.

Study	Study design	Country	Troponin assay studied	Definition of CAD
[Walter et al], 2020	Prospective diagnostic cohort study	Switzerland, university of Basel	hs-cTnI or hs-cTnT, Roche, Abbot, Singulex, Architect STAT High Sensitive Troponin-I assay (Abbott Laboratories)	Determined using stress and rest myocardial perfusion imaging with single photon emission computed tomography combined with computed tomography (MPI-SPECT/CT)
[Cwikial et al], 2019	Prospective diagnostic test accuracy study	Oslo, Norway	Troponin T, high-sensitivity cardiac troponin T hs-cTn T	Determined using exercise stress testing or coronary angiography
[Adamson et al], 2018	prospective, multicentre, randomized controlled study	Scotland, Edinburgh, United Kingdom	high-sensitivity single-molecule counting assay on the Erenna	Coronary Computed Tomographic Angiography, coronary artery calcium scoring

			platform (Singulex, Inc, Alameda, CA), hs-cTnI	
[Mueller et al], 2018	prospective diagnostic study	Basel, Switzerland	High-sensitivity cardiac troponin I and T, hs-cTnI, hs-cTnT	Rest/stress myocardial perfusion single-photon emission computed tomography/computed tomography and coronary angiography
[Orsini et al], 2018	Prospective diagnostic accuracy study	Pisa, Italy	High-sensitivity cardiac troponin T, Elecsys TNT hs STAT, Roche Diagnostics, hs-cTnT	Stress testing for myocardial ischaemia (either exercise stress testing or dobutamine/dipyridamole stress echocardiography)
[Walter et al], 2018. [Abstr]	Prospective cohort study	Basel, Switzerland	High-sensitivity cardiac troponin T, hs-cTnT in combination with the European Society of Cardiology (ESC) risk score	Expert adjudication of CAD based on myocardial perfusion single-photon emission tomography (MPI), coronary angiography and fractional flow reserve (whichever available)
[Mouridsen MR et al]	Prospective diagnostic study	Copenhagen, Denmark	Elecsys hs Troponin T, Roche Diagnostics A/S,	Exercise stress test

Table 2.3: Key results on diagnostic accuracy.

Study	Troponin assay and cut-off	Outcome measure	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Other key results
[Walter et al], 2020	Abbott hs-cTnI, 1.5ng/L	Any ischaemia	97% (96 – 98)	8% (6-9)	Not stated	76 (67-84)	
	Abbott hs-cTnI, 2.5ng/L	Any ischaemia	90% (88 – 92)	20% (17 – 22)	Not stated	70 (64 – 75)	
	Abbott hs-cTnI, 3.0ng/L	Any ischaemia	85% (83– 87)	26% (23 – 29)	Not stated	69 (63 – 72)	
	Abbott hs-cTnI, 1.5ng/L	≥10% ischaemia	98% (95 – 99)	6% (5 – 7)	Not stated	94 (88 - 98)	
	Abbott hs-cTnI, 2.5ng/L	≥10% ischaemia	92% (88 – 95)	16% (15 – 18)	Not stated	93 (89 – 95)	
	Abbott hs-cTnI, 3.0ng/L	≥10% ischaemia	88% (83 – 91)	22% (22 - 25)	Not stated	92 (89 – 94)	
[Cwikial et al], 2019	Roche hs-cTnT, 6ng/L (median in the study)	Angiographic coronary artery disease (≥75% stenosis)	Hs-cTnT - 71% Hs-cTnT + EST - 84%	Hs-cTnT - 63% Hs-cTnT + EST - 46%	Hs-cTnT - 71% Hs-cTnT + EST- 48%	Hs-cTnT - 63% Hs-cTnT + EST - 83%	
[Adamson et al], 2018	Singulex hs-cTnI	Angiographic luminal Cross-sectional area stenosis of ≥70% (approximating to a 50% diameter stenosis) in at least 1 major epicardial vessel or ≥ 50% in the left main stem.	-	-	-	-	Sensitivity and specificity not stated. Median in patients with CAD: 1.9 (IQR, 1.3–3.1) ng/L. Median in patients without CAD: 1.2 (IQR, 0.8–

							1.9) ng/L, P<0.001.
[Mueller et al], 2018	HS-cTnI, ≤ 1.3 ng/L	-	95% (93–96)	-	-	89% (84–92)	-
	Hs-cTnT, ≤ 3 ng/L	-	95% (93–97)	-	-	88% (84–92)	-
[Orsini et al], 2018	hs-cTnT measured pre-test and 6h post-test. A rise of ≥10% was considered significant	Inducible myocardial ischaemia	80%	32%	-	-	-
		Angiographic coronary artery stenosis (subgroup of 50 patients)	89%	47%			
[Walter et al], 2018. [Abstr]	Hs-cTnT in addition to the ESC risk score (new score derived and then validated)	Functionally relevant CAD	-	-	-	-	Derivation study: AUC for the original ESC risk score: 0.67, vs 0.72 for ESC score + hs-cTnT Validation study: AUC 0.69 vs. 0.73 ESC score + hs-cTnT
[Mouridsen MR et al]	Exercise stress test	Coronary stenosis ≥50	43.9%	76.7%	40.0%	79.5%	[Mouridsen MR et al]
	Hs-cTnT, 3ng/L	Coronary stenosis ≥50	70.7%	72.4%	47.5%	87.5%	
	Exercise stress test + hs-cTnT	Coronary stenosis ≥50	85.4%	54.3%	39.8%	91.3%	

	Exercise stress test	Coronary stenosis ≥70	60.0%	77.3%	33.3%	91.1%	
	Hs-cTnT, 3ng/L	Coronary stenosis ≥70%	68.0%	66.7%	27.9%	91.7%	

2.6. Discussion

In this systematic review I identified evedince for the use of hs-cTn assays to rule-out CAD in patients with stable chest pain. All of the papers identified patients with stable CAD using high-sensitivity cardiac troponin as a biomarker. Each of these papers suggests that hs-cTn had suboptimal diagnostic accuracy in diagnosing CAD. However, the evidence suggests that this biomarker gave a higher diagnostic sensitivity and more accurate result if it combined with the clinical assessments of the treating clinicians.

We can see in the study by Walter et al that using a low cut-off (1.5ng/L) with the Abbott hs-cTnI assay gave 97% sensitivity which seems acceptable. However, the NPV was only 76% (Table 2.3). This means that there is still a high probability (24%) that the patient has CAD after a negative result. Further, at that cut-off the test had only 8% specificity. Therefore, if a patient without CAD has only 8% chance of having a troponin less than 1.5 ng/L, meaning that use of the test would only lead to a small reduction in the use of imaging. At higher cut-offs the sensitivity was worse, so, in this study troponin by itself it is not enough to rule-out CAD. If a test could achieve sensitivity of this magnitude but with a much higher NPV and specificity, then many people who have not got CAD could have the diagnosis ruled out with one test.

Cwikial et al used a cut off that was probably too high to rule-out stable CAD (6ng/L). It seems unlikely that any cut-off above the limit of blank (3ng/L) will rule-out CAD. In that study, hs-cTnT had a sensitivity of 71%, which is again not sufficient to rule-out CAD. The NPV was 63%, which supports that conclusion. Even adding in an exercise stress test only achieved a sensitivity of 83%, which means that 17% of patients with CAD would not be correctly identified. Therefore, in this study troponin by itself cannot rule-out CAD.

The study by Adamson et al only tells us that the average hs-cTn concentration is higher in patients with CAD than in patients without CAD. From that it is helpful to tell us that patients with stable CAD do have higher troponin levels but it cannot tell us whether we can rule-out the diagnosis, limiting the practical value of that evidence.

In the study by Mueller et al, a low hs-cTnI cut-off was used (1.3ng/L), which achieved a sensitivity of 95%. While a sensitivity of 95% may seem high, the NPV of 89% means that if a patient tests negative there is still an 11% chance that they have CAD. The same was observed with troponin T. Patients have 88% chance that they do not have CAD if they test negative, but there is 12% chance patient will have CAD. Therefore, again neither biomarker was able to rule-out CAD by itself.

The study by Orsini et al only reports sensitivity and specificity for a change in troponin before and after stress testing, rather than for a single baseline measurement. The sensitivity of 80% means that we cannot use the troponin to rule-out inducible myocardial ischemia. If we do not see a rise in troponin there is still a high chance of observing inducible ischaemia.

For the study of Walter et al, the authors asked whether we could combine a new risk score with troponin to rule-out CAD. This was a big study with a large sample size, but the AUC was only 0.73 on validation. However, there is not enough detail presented to say whether we could actually rule-out CAD even when the ESC risk score was used in combination with troponin.

In the study by Mouridsen MR et al they used hs-cTnT with cut-off of 3ng/L in combination with an exercise test. In this study they had three different results with two types of coronary stenosis. The

first type of coronary stenosis was ≥50% stenosis, in this type they had three results: first, exercise testing had 43.9% sensitivity and 79.5% NPV, second hs-cTnT alone had 70.7% sensitivity and 87.5% NPV, and the third result was the combination of exercise test and hs-cTnT, which had 85.4% sensitivity and 91.3% NPV. The second outcome studied was coronary stenosis was ≥70%, and as for the first analysis three results were presented: first, the exercise test alone had 60.0% sensitivity and 91.1% NPV, second hs-cTnT had 86.0% sensitivity and 91.7% NPV, and the third result was the combination of exercise test and hs-cTnT with 92.0% sensitivity and 97.1% NPV. From these results we can see that hs-cTnT alone had low sensitivity and NPV for both types of stenosis, however, when we combined it with the results of exercise testing the sensitivity and NPV increased for both levels of stenosis. From that we can see that troponin alone cannot give good results for diagnosis coronary artery disease, but these results can be improved if we add another cardiac test or any risk factors as we saw when we combined cTnT with exercise test.

The evidence therefore shows that, using troponin by itself, stable CAD could not be ruled out because of a low NPV. However, there are a lot off weaknesses in the studies presented. Some of them used high cut-offs, some of them did not use a cut off at all just pre and post stress test, which is not we are looking at. Only one of them used a risk score as well as troponin, but did not present enough details for us to know sensitivity and specificity that help us to know whether we can rule-out CAD. So, the evidence we found essentially identifies that hs-cTn is higher in patients with stable CAD, but we require further research in order to determine whether, using low cut-offs and in combination with other clinical information, the test could be used to safely avoid the need for imaging.

The systematic review showed many gaps in order to understand the use of hs-cTn to rule-out stable CAD, and these gaps can be illustrated as follows:

In this systematic review all the studies have evaluated the use of hs-cTn assays in patients with stable CAD, many used high cut-offs which are above the limit of detection. Therefore, in future research,

they should use the lowest possible cut-offs to maximise sensitivity, which can be set around the limit of detection or limit of blank of the assays.

Adding the risk scores alongside hs-cTn assays can improve the sensitivity and NPV as reported in some studies in the systematic review. However, these evaluations have not been included in previous studies yet when using the risk scores with lowest possible hs-cTn cut-offs. Therefore, future studies are needed to address this issue.

2.7. Conclusion

This systematic review summarised the evidence for the use of hs-cTn to rule-out stable CAD. The level of evidence to back this use case for hs-cTn is relatively poor and it appears that by itself hs-cTn probably cannot rule-out CAD. We still need more consistent evidence with very low troponin cut-offs using highly sensitive assays, and we require more evidence with regard to using the combination of hs-cTn and clinical features to rule-out stable CAD.

Chapter 3 : Methods for the Manchester Coronary Artery Disease (MCAD) study

3.1. Background

Cardiovascular disease (CVD) is the most common cause of death worldwide (44). Early detection of coronary artery disease (CAD) and exercise induced myocardial ischemia is critical in the efforts of reducing serious downstream effects of CAD. Measurements of cardiac troponin I (cTnI) and T (cTnT) are a mainstay in the diagnosis and management of patients with suspected acute coronary syndrome (ACS) (45). In that context, high-sensitivity cardiac troponin (hs-cTn) assays have enabled the diagnosis of ACS to be 'ruled in' and 'ruled out' earlier than was previously possible, reducing hospital length of stay, enabling earlier reassurance for patients when safe and appropriate, and unburdening crowded hospitals. Numerous studies have now demonstrated that hs-cTn assays have a significant clinical utility in patient management outside of the acute setting to predict risk and stratify patients in the primary and secondary prevention setting (46–52). To date nevertheless hs-cTn has not been extensively used to rule-out the diagnosis of stable CAD. Functional and anatomical cardiac testing are used in the workup of patients with suspected CAD, however an overuse of testing is reported and technologies are limited by poor diagnostic yield, high costs, and/or radiation exposure (53–58). Therefore, additional more cost-effective methods are needed to improve patient selection for such diagnostic testing of patients with suspected CAD. Analytical sensitivity and precision of hs-cTn assays are constantly improving, such that cardiac troponin concentrations can now be detectable in 100% of apparently healthy individuals (59). With these advances, there is a great potential to use this biomarker to identify patients with non-acute symptoms having low or intermediate probability of CAD to rule-out cardiac ischemia and therefore obviate the need for further testing. Recent studies from Basel University Hospital demonstrated that patients who have hs-cTnI concentrations below an extremely low threshold, using the Singulex Clarity™ cTnI assay (limit of detection of 0.08 pg/mL), at a cut-off of 0.5 pg/mL, had 94% sensitivity and 91% negative predictive value for ruling out stress induced ischemia in patients suspected of having CAD but without a history of CAD (60,61). When combined with a clinical estimate of pre-test probability, the sensitivity improved to 97% and negative predictive value to 93% (61). Based upon these above findings, the Singulex Clarity™ cTnI assay was

recently CE marked with the indication to be used in conjunction with clinical evaluation for ruling out cardiac ischemia in patients suspected of having CAD (62). Clearly, using a highly sensitive cTnI in this chronic setting to rule-out stable CAD and to decrease and/or eliminate the usage of functional and/or anatomical cardiovascular diagnostic procedures could not only allow earlier reassurance for patients but also avoid performing unnecessary specialist referrals and tests, reduce exposure to ionizing radiation and intravenous contrast and yield cost savings for health services. While this evidence is promising, we now need further prospective evidence to determine the diagnostic accuracy of different hs-cTn assays for the diagnosis of stable CAD before considering their use in practice.

3.2. Objectives

This study aimed to determine whether hs-cTn assays (Roche Elecsys hs-cTnT and Abbott ARCHITECT hs-cTnI) can be used to rule-out stable coronary artery disease (CAD) based on the institution routine clinical practice for CAD assessment. Thus, hs-cTnT and hs-cTnI assays were tested in conjunction with the existing clinical protocol in use at Manchester University NHS Foundation Trust, to assess the presence of CAD and/or cardiac ischemia in patients suspected of having CAD.

3.3. Study Design

Prospective single arm observational diagnostic test accuracy study.

3.4. Governance and ethics

Approval was obtained from the Health Research Authority (HRA) and National Research Ethics Service, as well as local approvals before study activities took place. Manchester University NHS Foundation Trust sponsored the study. All participants provided written informed consent to be included in the study. Potential participants were identified from upcoming clinic lists for the Rapid Access Chest Pain

Clinic (RACPC). Participants were initially invited to participate either by post, telephone or face to face in the RACPC. Participants were contacted by post when the appointment letter for the Rapid Access Chest Pain Clinic (RACPC) was dispatched, or by telephone when a member of the research team contacted the potential participant to discuss the study before sending study information by post. This ensured that potential participants had an opportunity to consider their participation prior to arrival at the clinic when study procedures must be undertaken. For patients who had questions about the study in advance of their clinic appointment, a contact number and email address were provided in the participant information sheet. Some patients only received the study information face to face on arrival at their scheduled clinic appointment at the RACPC. In this case, the patients were approached immediately and provided with the Participant Information Sheet. The patient was given as much time as possible to consider the study and was given the opportunity to discuss the study with the research team and ask any questions they may have had. Although the patient was given as much time as possible before being asked to decide whether to take part, the written informed consent must have been provided on the same day in order for the study procedures to take place. It was made clear that the patient was under no obligation to take part, and their care would not be affected whether they decide to take part or not. The study was undertaken in accordance with the principles of ICH-GCP. The hs-cTn assays evaluated were CE marked.

3.5. Study Population

All consecutive adult patients referred from the community or hospital to a cardiologist outpatient clinic – the Rapid Access Chest Pain Clinic (RACPC) – suspected of having stable CAD, based on clinical symptoms, for further cardiology assessment. The RACPC evaluates outpatients with suspected CAD. All patients who do not have known CAD or valvular heart disease are evaluated in this clinic. The study aimed for inclusive recruitment, without discrimination or selecting specific patients from those who were referred to RACPC. Therefore, from that principle the population diversity was presented in this

study. All included participants had suspected stable angina. Therefore, any patients attended to the RACPC with signs of unstable angina were redirected to the emergency department or to the on-call cardiologist and were not eligible in this study.

3.6. Inclusion criteria

Adult patients (≥ 18 years) referred for diagnostic work-up with suspicion of stable CAD.

3.7. Exclusion criteria

- Pre-existing CAD

- Known valvular heart disease

- Clinical evidence of heart failure

- End stage renal failure (requiring haemodialysis)

- Unable or unwilling to provide written informed consent

3.8. Study procedures

All study procedures were undertaken when the patient attended the RACPC for their scheduled appointment. No study procedures were carried out prior to the participant providing written informed consent. Other than subsequent sample analysis and data collection to verify the outcome of relevant investigations, the patient's involvement in the study ended once the below study procedures had been completed.

3.9. Data collection

We collected data on patient demographics, past medical history, risk factors for CAD, the data required to calculate relevant risk scores (Diamond-Forrester score and CAD-C model) and clinician perceived probability of CAD (Table 10.1). We evaluated these risk scores and the clinician-estimated

clinical probability because of the previous data, suggesting that the sensitivity and NPV of hs-cTn may be improved when applied in patients with low pre-test probability of CAD.

We also asked patients to complete an EQ-5D-3L questionnaire (Table 10.2). All data was entered into an electronic database and anonymised for analysis. All identifiable data collected was stored securely with the research team. The database containing identifiable information was only accessible to members of the clinical team. The anonymized database was made available to investigators who are responsible for statistical analysis. Participants were identified using a unique study identification number. Only members of the clinical team were able to link this back to identifiable information, should the need have arisen. The original study database was stored on a secure NHS server, which is regularly backed up and stored in accordance with all of the relevant data protection requirements of the sponsor organisation. The information collected was kept confidential and used only for the purpose of this study. A full list of data collected is shown in the case report form in appendix 1 (Table 10.1) (Table 10.2).

3.10. Index test

The index test requires a single venepuncture. Venous blood (approximately 12.5ml) was drawn after written consent has been provided, during the patient's attendance at the RACPC but prior to any imaging. Blood was drawn into an EDTA vial and a serum-Gel vial. The serum-Gel sample was processed by local laboratories, who w measured hs-cTnT (Roche Diagnostics Elecsys 5th generation). The EDTA vial was centrifuged, and plasma was extracted and divided into 1ml aliquots. We originally planned for 1ml of EDTA plasma per patient to be analysed in batches for hs-cTnl (Singulex Clarity™cTnI) at Manchester Molecular Pathology Innovation Centre, located in CityLabs 1.0, Nelson Street, Manchester. Further, 1ml of EDTA plasma per patient was sent to The Royal Oldham Hospital, Rochdale Road, Oldham, for analysis using Abbott hs-cTnI. Samples were processed and analysed as per SOPs and manufacturer's instructions for use. All investigators and clinicians responsible for the care of the

patient were blinded to the results of investigational assays. However, if an investigational assay analysed at MFT produced a clinically significant result, this was referred back to the clinician in charge of the patient's care, in case they wished to advise the patient further. Results of blood tests carried out for the purpose of this research study were be used to make clinical decisions on a patient's care.

3.11. Reference standard

All patients included in the study underwent imaging investigations for suspected CAD in accordance with routine clinical practice. The choice of investigation and the decision to proceed to imaging was not influenced by the study protocol. Cardiac imaging included one of: (1) invasive angiography; (2) myocardial perfusion imaging (SPECT); (3) stress echocardiography; (4) CT coronary angiography; or (5) magnetic resonance coronary angiography. This cardiac imaging will form the reference standard for CAD. Imaging was considered to indicate CAD under the following conditions:

- Angiography (invasive or non-invasive): At least 50% stenosis of a major epicardial vessel
- SPECT or stress echocardiography: Any evidence of reversible myocardial ischaemia

All imaging was reported by a consultant radiologist or cardiologist, blinded to investigational assay results. The outcomes were adjudicated by two investigators (RB and RA) with reference to all imaging investigations but blinded to hs-cTn results and the outcome of relevant risk scores. For patients with inconclusive diagnosis based on the initial reference test, adjudication of cardiac ischemia/CAD was based on initial reference test interpretation combined with information from a second stress test, coronary CT angiography or coronary angiography when available as per routine clinical practice of the institution to further improve the accuracy of the reference test. The time interval between the index test (hs-cTn measurement) and performance of reference standard imaging investigations was specified and must not have been longer than three months. This was tracked by monitoring the date of investigations recorded in the time stamped electronic patient record.

3.12. Statistical Analysis Plan

The detailed statistical analysis plan for each analysis within the MCAD study is described within the individual chapters. As a basic principle we summarised baseline characteristics including patient demographics and past medical history using descriptive statistics. The proportion of patients who were confirmed to have CAD were reported. To evaluate the diagnostic accuracy of the hs-cTn assays we initially calculated the area under the receiver operating characteristic (ROC) curve (AUC) with 95% confidence intervals. We compared the AUC of the Roche hs-cTnT assay and Abbott hs-cTnI assay, and to the outcome of the Diamond-Forrester risk score. We constructed 2x2 tables. These were used to calculate sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratios and their respective 95% confidence intervals. For this analysis, a positive result was considered to be ≥0.5ng/L for Singulex Clarity hs-cTnI (based on the results of previous investigations), <3ng/L (the limit of blank of the assay) for Roche hs-cTnT, and >2ng/L (the limit of detection of the assay) for Abbott hs-cTnI. We also explored the diagnostic accuracy of additional cut-offs. Data was subset by sex for separate analysis, since many studies have reported lower hs-cTnI concentrations in women compared to men.

3.13. Power and Sample Size

The sample size calculations are based upon the lower 95% confidence limit of the clinical sensitivity from previous study results (63). The assumed prevalence of ischemia/significant CAD presence is approximately 20% for consecutive patients suspected of having CAD. In a previous study, the combination of Singulex Clarity hs-cTnI <0.5ng/L and an estimated probability of CAD <30% yielded 97% sensitivity and 9% specificity for CAD. On that assumption, a sample of 450 patients would detect a sensitivity of 97% with the lower bound of the 95% confidence interval being greater than 90%. In this study we expected loss to follow up to be minimal as all patients were undergoing imaging investigations by virtue of the inclusion criteria, and all study procedures were completed in a single clinic visit at the time written consent was obtained. We therefore aimed to recruit a sample of 450 participants.

Chapter 4 : Recruitment to the MCAD study

Recruitment to the MCAD study took place between June 2018 and May 2019. The recruitment of the patients took place in two sites: Manchester Royal Infirmary (MRI) and Wythenshawe Hospital. All the recruited patients were referred to the Rapid Access Chest Pain Clinic (RACPC) with symptoms of stable chest pain. The recruitment started by the research nurses first at MRI, then after two months recruitment commenced at Wythenshawe Hospital. All the recruited patients had to sign the consent forms before enrolling in the study. After the patients provided written informed consent, we took the blood from the patients and sent the samples to the lab for analysis.

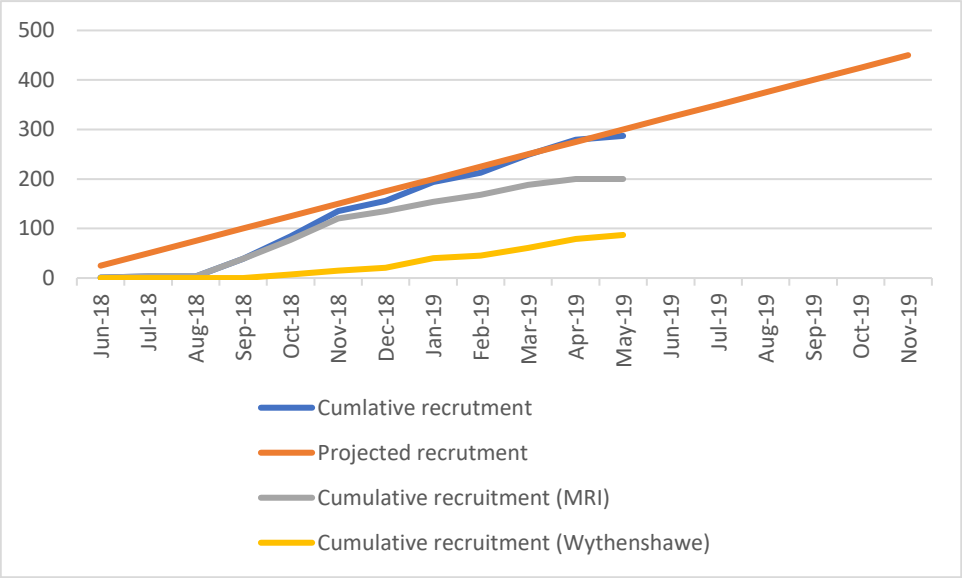

Figure 4.1: Cumulative and projected recruitment to the MCAD study.

This line graph (Figure 4.1) illustrates the recruitment progress at MRI and Wythenshawe Hospital. As demonstrated in the figure, we can see the initial recruitment rate was below target, but after a run-in period the rate increased since August 2018 at MRI. As for Wythenshawe Hospital, recruitment started in October 2018 at a lower rate than initially anticipated. This rate remained lower than that at MRI, which is perhaps explained by the fact that the study was less well supported by clinical research nurses at Wythenshawe and I supported the recruitment at MRI. However, overall recruitment between the two sites was generally on target.

Unfortunately, before the end of patient recruitment the funding company (Singulex) declared bankruptcy in May 2019. Therefore, recruitment was stopped. This problem affected our plans and the anticipated timeline for my PhD. However, after many meetings and discussions with my supervisors, we decided that although the sample size of the study was not reached the study will be continued as we have enough data to make full analysis. However, we accepted that the confidence intervals will be wider than originally anticipated with a full sample size. Moreover, a plan was initially made to continue patient recruitment. We managed to get the necessary finance by using my bench fees. However, the COVID-19 pandemic caused all recruitment to be paused for a substantial period of time, making it unfeasible to extend patient recruitment within this PhD.

Impact of terminating recruitment early:

This early termination led to several impacts which can be illusterated as :

- We were not able to recruit the full sample size of 450 patients, and we recruited a total of 306 patients.
- Not reaching the full sample size whoud be expexted to lead to wider 95% confidence intervals, including around the sensitivity. However, using the same assumptions that I made originally I re-calculated the sample size and refreshed the width of the CI based on the final sample size of 306 patients:
- Assuming we had a CAD prevelance of 20%.
- Assuming the sensitivity of hs-cTn should be 97%.
- Assuming the specificity of hs-cTn should be 9%.
- With these assumptions, We would achieve a lower bound of the 95% CI for sensitivity of 90.5%, which is still above 90% as our orginal target.

Looking to the results above the under-recruitment had only a minmal imact on the overall analyses.

4.1. Role of the PhD candidate in supporting data collection

Prior to commencing my PhD, I had been involved in discussions around the setup of the MCAD study. I was aware that this would form the basis for my PhD project. I was given the opportunity to review the protocol prior to commencing my PhD. Approvals were then obtained prior to my arrival. I was then able to support patient recruitment soon after starting my PhD.

I supported patient recruitment and data collection by liaising with the research nurses at MRI to discuss coverage of clinics. I attended clinics and supervised the informed consent process, blood sampling and handling and data collection. I was responsible for all data entry and compilation of the main study database. I handled all data queries and missing data. I undertook patient follow-up at three months, and I coordinated the adjudication of the diagnosis with my supervisors. In addition, I was responsible for maintaining the study database and for all statistical analysis.

4.2. Data collection/entry

One of the most important and challenging stages of my study was creating the electronic database and entering data for every patient recruited into this study. The database includes all information entered in the case report form designed for the Manchester Coronary Artery Disease (MCAD) study. After obtaining written informed consent from participants, the case report forms were completed by the research nurses. My role was to collect these forms and enter the data electronically to a database made especially for this study. I started entering the data in March 2019. In the beginning my progress was good and there was very little missing data. However, after a while my progression slowed due to the shortage of information in the forms. As result of the missing data, I had to retrieve the patients' clinical letters from a program called Chameleon which is used by Manchester Royal Infirmary (MRI). Chameleon stores relevant clinical information including past history, clinical letters, test results, and disease diagnosis. The program was very useful for me, it helped me to complete the

missing information and finish the database. Furthermore, Wythenshawe Hospital also participated in the study. Recruitment at Wythenshawe Hospital has progressed at a slower pace because there was only one nurse available to recruit participants. To mitigate for this, I assisted with recruitment at clinics without an available research nurse.

The risk scores evaluated in this PhD were the Diamond-Forrester score and the CAD-C model. We also evaluated the estimated probability of CAD in the opinion of the treating clinician using two different scales: a five-point Likert scale and an estimated probability of CAD expressed as a percentage. These data were recorded by the responsible clinician, who was a specialist nurse working in the RACPC. Data were entered directly into a custom-made database for Manchester Heart Centre and retrieved from there. The risk scores were calculated as reported in the original publications (REFs – Diamond-Forrester and CAD-C papers) and the rationale for the data collection on the risk scores can be found in chapter 6.

4.3. Adjudication of the diagnosis of coronary artery disease

After collecting the data from patients and completing recruitment, all relevant data were sent to three investigators for adjudication of the final diagnosis. The investigators were blinded to the results of hs-cTn assays and risk scores. Two cardiologists (RA and FO) and one emergency physician (RB) were involved in the adjudication process. This adjudication committee reviewed all imaging evidence undertaken within three months of initial recruitment. A diagnosis of sCAD was assigned if the patient had >50% stenosis of any major epicardial vessel on CTCA; if they had any confirmed perfusion defect on myocardial perfusion imaging or any new wall motion abnormality following pharmacological stress with dobutamine stress echocardiography. If the results of imaging were equivocal, alternative imaging was sought. If there was insufficient information to assign a diagnosis, the data were considered to be missing. We referred to NICE guidelines for stable chest pain. This

used two thresholds: 50% stenosis and 70% stenosis. As the aim of my research was to rule-out CAD, after discussion with my supervisors we felt that 50% was a more appropriate threshold to use.

A threshold of 50% stenosis was chosen after discussion with the research team. Based on NICE guidelines, there were two choices of 50% and 70% stenosis. We agreed that 50% stenosis will be more appropriate for several reasons:

- A 50% stenosis patients was felt likely to cause CAD symptoms if it is compared with patients with less stenosis.
- 50% stenosis has been used and still been used as reference standard in other studies.
- Selecting the lower threshold is a conservative approach, which seemed most appropriate.
- 50% stenosis allows for early symptom detection and intervention for cardiologists compared to 70% stenosis.

And why we used confirmed perfusion defect or wall motion abnormality with pharmacological stress as reference standard are:

- Tests like stress echocardiography or SPECT, generate stress on the heart muscle which increase heart muscles demands for nutrition, oxygen and blood in which this stress can reveal any CAD disease that not been found at rest.
- These tests have high sensitivity and specificity for detecting CAD.
- Pharmacological stress tests provide more evaluation of CAD by assessing both perfusion defects and wall motions abnormalities giving more comprehensive assessment of CAD.

While NICE recommends CTCA as the main investigation for CAD, in reality many patients still have pharmacological stress tests instead. It would not have been possible to change this for the study due to resource implications. Therefore, taken alongside the evidence for their accuracy, I considered pharmacological stress tests to be an acceptable reference standard (64).

Chapter 5 : High-sensitivity cardiac troponin T

5.1 Background

Cardiac troponin is a protein that released into the blood after myocardial damage or cardiac injury, and the biomarker of choice for diagnosing or excluding acute myocardial infarction (AMI). In recent years hs-cTn assays have been developed, which allow the detection of very low concentrations of cardiac troponin with high precision (65). There is now a large body of evidence to demonstrate that the use of hs-cTn assays can lead to accurate early diagnosis and 'rule-out' of AMI. Moreover, hs-cTn is a powerful prognostic marker in patients with ACS and in apparently healthy individuals (66).

5.2 Objectives

In this study I aimed to evaluate the diagnostic accuracy of an hs-cTn assay (hs-cTnT, Roche Diagnostics Elecsys 5th generation) in diagnosing patients who are suspected to have stable coronary artery disease.

5.3 Methods

5.3.1 Study design and settings

I undertook an analysis from the MCAD study, the methods for which have been described in detail in Chapter 3. The MCAD study was a prospective single arm diagnostic accuracy study including patients that referred to the Rapid Access Chest Pain Clinic (RACPC) suspecting of having stable coronary artery disease based on clinical evaluation. Recruitment of the patients took place in two sites, the first site Manchester Royal Infirmary (MRI) and the second site was Wythenshawe Hospital, which are part of Manchester University NHS Foundation Trust. All patients provided written informed consent for their participation in the study.

5.3.2 Index test

After providing written informed consent, all patients involved in the MCAD study were asked to provide a venous blood sample. This occurred during their attendance to the clinic but before any type of imaging. For this analysis, we tested a serum sample for high-sensitivity cardiac troponin T (Roche Diagnostics). The 99th percentile of the assay is 14ng/L, the limit of detection is 5ng/L and the

limit of blank is 3ng/L. The assay has a coefficient of variation <10% at a concentration of 6ng/L. Fresh samples were tested on site at Manchester Royal Infirmary. All clinicians involved in the study were blinded to the hs-cTnT results. Full details of the methods can be found in chapter 3.

5.3.3 Statistical analysis

I summarised baseline characteristics using descriptive statistics (numbers and percentages) (Table 5.1). Ages were summarised by means and standard deviations. Measures of diagnostic accuracy were calculated including sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively) and likelihood ratios, together with their respective 95% confidence intervals. Test characteristics were calculated using MedCalc (67). As we intended to use hs-cTnT as a 'rule-out' test for CAD and because previous reports suggest that a very low cut-off is required, we set the cut-off at the limit of blank (3ng/L) of the assay. We also reported diagnostic accuracy at a number of other potential cut-offs.

Finally, as a measure of overall diagnostic accuracy, I plotted receiver operating characteristic (ROC) curves and calculated the area under the curve with 95% confidence intervals using the non-parametric method, in SPSS (IBM, Chicago, Ilinois). ROC curve analysis was used to provide a measure of overall diagnostic accuracy, demonstrating the range of possible sensitivities and specificities at different troponin cutoffs.

5.4 Recruitment

As noted in Chapter 4, recruitment started in June 2018. After an initial run-in period, recruitment rate began to exceed projections in August 2018 at MRI (Figure 5.1). At Wythenshawe Hospital, recruitment started in October 2018 but the rate was lower than initially anticipated. The recruitment rate remained lower than MRI because that less clinical research nurse support was available at Wythenshawe, meaning that recruitment could proceed at fewer clinics. Working with the clinical research nurses, I managed to recruit 208 patients at MRI and 98 patients were recruited at

Wythenshawe hospital by the clinical research nurses at that site. Follow-up was completed for all recruited patients in both sites.

Recruitment was close to reaching the target sample size. However, due to an unanticipated interruption in the funding of the study (which had been funded via a research grant from Singulex, which went into liquidation), recruitment was paused prior to reaching the target. The funding issue was successfully addressed (through a proposed use of PhD bench fees) and a protocol amendment had been prepared, submitted and approved to allow recruitment to re-commence. However, the COVID-19 pandemic meant that all non-COVID research was paused. The decision was therefore taken to terminate recruitment early, prior to achieving the full pre-planned sample size.

By the time COVID-19 restrictions had been eased, I could not have continued patient recruitment because I was at the beginning of my final year, leaving insufficient time to complete the study. Therefore, analysis commenced after recruitment of a total of 306 patients across both sites (Figure 5.2).

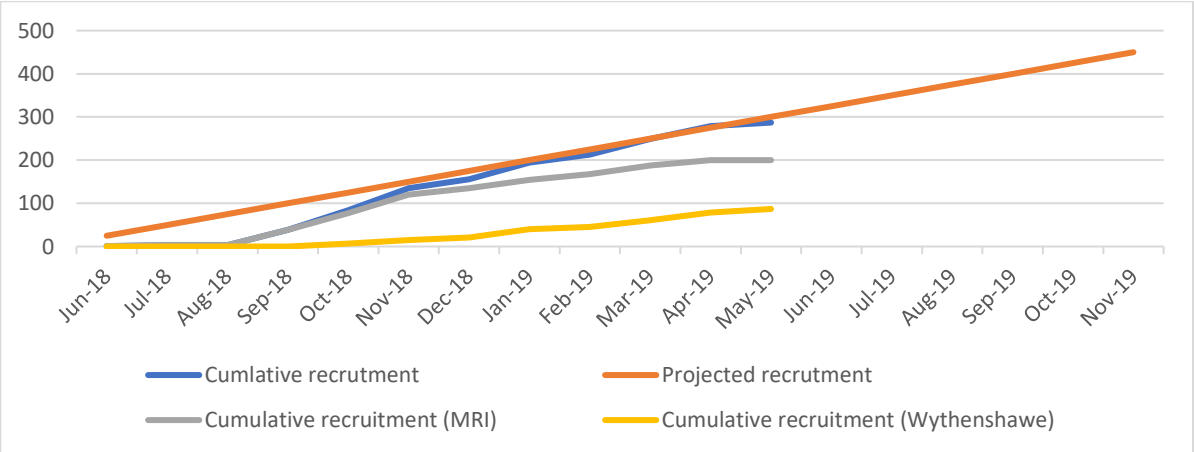

Figure 5.1: Cumulative and projected recruitment to the MCAD study.

5.5. Patient characteristics

Table 5.1 below show the baseline characteristics of the participants. As we can see in this table, patients were divided into two groups. The first group included all patients, and the second stratified patients by the final adjudicated diagnosis of CAD. From the table, we can see that patients with CAD had a higher prevalence of Framingham risk factors than patients without CAD.

Table 5.1: Baseline characteristics of the included participants.

	All patients, n=281	Patients with coronary artery disease, n=69	Patients without coronary artery disease, n=212
Age, years (mean, standard deviation)	56.4 (12.3)	55.6 (9.9)	55.4 (12.9)
Male sex	128 (45.6)	42 (60.9)	86 (40.6)
Hyperlipidaemia	109 (38.8)	39 (56.5)	134 (63.2)
Hypertension	94 (33.5)	27 (39.1)	141 (66.5)
Diabetes type 1	4 (1.4)	1 (1.4)	206 (97.2)
Diabetes type 2	36 (12.8)	14 (20.3)	187 (88.2)
CVA or TIA	8 (2.8)	4 (5.8)	207 (97.6)
PVD	12 (4.3)	2 (2.9)	200 (94.3)
Smoker	52 (18.5)	15 (21.7)	170 (80.2)
Heart failure	1 (0.4)	0 (0.0)	206 (97.2)
Family history	118 (42.0)	34 (49.3)	109 (51.4)

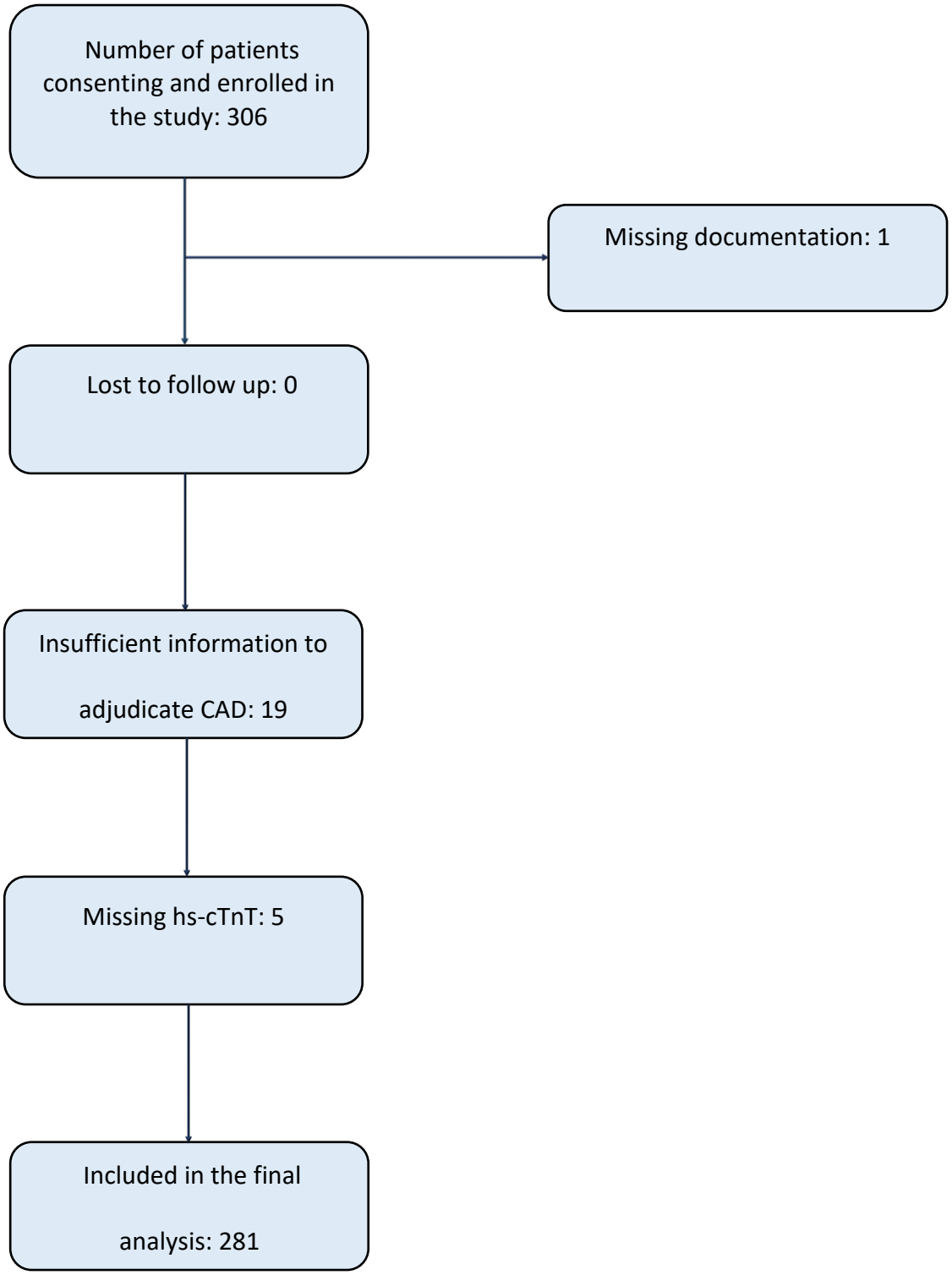

Figure 5.2: Participant flow diagram.

Figure 5.2 shows the flow of participants through the study, including the number who completed imaging and follow-up.

5.6. Diagnostic accuracy of hs-cTnT

The diagnostic accuracy of hs-cTnT was primarily calculated using the lowest possible cut-off of 3ng/L which is the limit of blank of the assay (the lowest concentration possible, aiming to achieve the highest possible sensitivity for CAD). In (Table 5.2) I have cross-tabulated the diagnosis of CAD (based on the adjudication of the expert cardiologists) versus hs-cTnT concentration at a cut-off of 3ng/L. As we can see in (Table 5.2), 57 patients had the troponin les then 3ng/l but had CAD, whereas 51 patients had no CAD. And with the cut-off of more than 3ng/L we had 12 patients with CAD, and with 161 patients with no CAD.

Table 5.2: Diagnostic accuracy of hs-cTnT at a cut-off of 3ng/L.

	Coronary artery disease present	Coronary artery disease absent
Hs-cTnT <3ng/L	57	161
HS-cTnT ≥3ng/L	12	51

Table 5.3 shows us show the global accuracy of troponin at the 3ng/L cutoff. Sensitivity was relatively low at 82.61%, NPV was also relatively low at 80.95%. This means there is a 20.05% probability of CAD even in patients with a hs-cTnT concentration <3ng/L. The specificity of hs-cTnT at this cutoff was also relatively low at 24.06%, as was the positive predictive value (26.15%), meaning that a hs-cTnT concentration ≥3ng/L does not 'rule-in' CAD.

Table 5.3: Test characteristics of hs-cTnT for CAD at a cut-off of 3ng/L.

Statistic	Value	95% CI
Sensitivity	82.61%	71.59% to 90.68%
Specificity	24.06%	18.47% to 30.39%
Positive Likelihood Ratio	1.09	0.95 to 1.24
Negative Likelihood Ratio	0.72	0.41 to 1.27
Disease prevalence	24.56%	19.64% to 30.02%
Positive Predictive Value	26.15%	23.68% to 28.78%
Negative Predictive Value	80.95%	70.68% to 88.23%
Accuracy	38.43%	32.72% to 44.40%

The ROC curve (Figure 5.3) shows poor overall diagnostic accuracy. Expanding upon that, Table 5.4 shows that the area under the curve (AUC) is 0.588 (95% CI 0.508 – 0.668). By chance alone, we would have expected to achieve an AUC of 0.50. The 95% confidence intervals for hs-cTnT cross 0.5, meaning that hs-cTnT (used alone) was not significantly better than chance alone for diagnosing stable CAD.

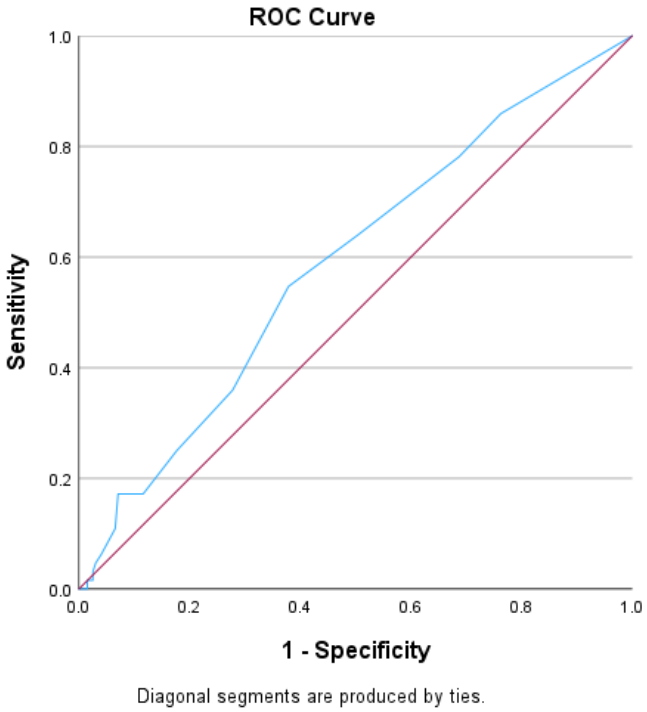

Figure 5.3: ROC curve for hs-cTnT and a diagnosis of CAD.

Table 5.4: Area under the ROC curve.

Test Result Variable(s): HS-cTnT (Roche)				
Area	Standard Error	P value (null hypothesis: AUC = 0.5)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.588	0.041	0.173	0.508	0.668

In Table 5.5 we can see that sensitivity decreased and specificity increased as the cut-off increased, which is to be expected for a diagnostic test. However, this table demonstrates that it was not possible to achieve a sensitivity of more than 85.9% at any cut-offs. At higher cut-offs, hs-cTnT had high specificity for the diagnosis of CAD but a very low sensitivity. This could help to rule-in stable CAD, though this would affect only a very small proportion of patients. Further, due to the prevalence of CAD, the positive predictive value at a cut-off of 10ng/L would be as low as 40.7% (95% CI 25.1 – 58.5%).

Table 5.5: Sensitivity and specificity of hs-cTnT at each possible cut-off.

Positive if Greater Than or Equal To	Sensitivity	1 - Specificity
2.50	85.9	23.7
3.50	78.1	31.3
4.50	64.1	49.5
5.50	54.7	62.1
6.50	35.9	72.2
7.50	25.0	82.3
8.50	17.2	88.4
9.50	17.2	92.9
10.50	10.9	93.4
11.50	6.3	96.0
12.50	4.7	97.0
13.50	3.1	97.5
15.00	1.6	97.5
17.00	1.6	98.0
19.00	1.6	98.5
21.00	0.0	98.5
26.50	0.0	99.0
32.50	0.0	99.5
35.00	0.0	100.0

5.7. Discussion

In this research I evaluated the use of hs-cTnT to diagnose patients with stable CAD, as adjudicated by two expert cardiologists. As we can see from the final results above, hs-cTnT had a low sensitivity of 82.61% (CI 95% (71.59% - 90.68%) and a specificity of 24.06% (CI 95% (18.47% - 30.39%) with 80.95% NPV. This suggests poor diagnostic accuracy and suggests that hs-cTnT cannot rule-out CAD alone as a single biomarker.

My systematic review above also suggested that hs-cTn alone cannot rule-out CAD due to the low NPV in the studies that were included. However, there were many weaknesses in the studies that were included in the systematic review, which means that there were remaining uncertainties about whether we can use hs-cTn rule-out CAD. Several of the studies identified in the systematic review had applied higher hs-cTnT cutoffs, which would be expected to yield lower sensitivity and NPV. However, the studies identified in the systematic review tended to show higher sensitivity than I observed in my study. However, that sensitivity was still not sufficient to rule-out CAD. Taken together with the findings of the systematic review, the new findings presented here strongly suggest that hs-cTnT cannot be used alone to rule-out the diagnosis of stable CAD.

From the results of the systematic review, the diagnostic accuracy of hs-cTn could be improved by adding in an estimate of pre test probability, such as a risk score or the clinical judgement of clinicians. The sensitivity of hs-cTnT alone may be insufficient to rule-out. However, if used in a population with low clinical probability of CAD then the sensitivity would be expected to increase. Therefore, there is still value in investigating the combination of hs-cTnT and clinical judgement. We also know that cardiac risk factors are more prevalent in patients with CAD. Therefore, we plan to evaluate the combination of hs-cTnT and cardiac risk factors.

5.7.1. Limitations

The limitations include the fact that patients underwent different types of imaging. This reflects current practice, though it would have been ideal to have a standard imaging procedure, such that every patient had the same reference standard (e.g. CTCA). Also, we did not achieve the full sample size due to the funder going bankrupt during the study. However, this did not affect the findings because the sensitivity was far from acceptable and could not have achieved an acceptable level even with further recruitment.

Also, there was some missing data. In 19 cases, the diagnosis of CAD could not be adjudicated. In six cases, there were no hs-cTnT results. However, this is a small proportion of the total sample size. (2% missing for hs-cTnT). Therefore, we felt it was reasonable to run a complete case analysis as this was deemed unlikely to affect our overall results.

5.7.2. Future work

One potential explanation for the lack of sensitivity for hs-cTnT observed in this research is that the hs-cTnT assay from Roche has relatively limited analytical sensitivity. Overall, 55% of apparently healthy individuals will have a cardiac troponin concentration above the limit of detection of the Roche hs-cTnT assay (68). Other assays have an apparently higher analytical sensitivity, as determined by the proportion of patients with detectable troponin. For example, with the Abbott ARCHITECT high-sensitivity troponin I (hs-cTnI) assay, 85% of apparently healthy individuals will have an hs-cTnI concentration above the limit of detection. A focus for future work, therefore, is to evaluate whether a high-sensitivity cardiac troponin assay with greater analytical sensitivity will achieve superior diagnostic sensitivity. This is the focus of a subsequent chapter in my thesis.

Finally I will evaluate the accuracy of troponin when adding other information like risk scores and clinician estimates of pre test probability. Ultimately, the study shows that troponin T by itself cannot

rule-out CAD even at very low cut-off. We need now to look at whether we can add risk scores to improve diagnostic accuracy. It will be also important to evaluate whather other troponin assays (e.g., high-sensitivity troponin I) may preform better. This is the focus for the next phase of the work.

5.8. Conclusion

In conclusion from the above results, hs-cTnT cannot rule-out CAD as a single biomarker. I now intend to evaluate whether an alternative troponin assay with apparently greater analytical sensitivity (Abbott ARCHITECT hs-cTnI) can achieve greater diagnostic sensitivity. Further, I will evaluate whether the diagnostic accuracy of hs-cTnT can be improved by accounting for other information including the number of Framingham risk factors that a patient has, and the clinical judgement of the treating clinician.

Chapter 6 : Using structured risk scores in combination with cardiac troponin T to diagnose patients with stable coronary artery disease

6.1. Background

Angina and chest pain are the most common clinical manifestation in patients with coronary heart disease, and represent about 1% of the yearly health expense in the UK (69). Rapid Access Chest Pain Clinics (RACPCs) were set up to provide clinical assessments for patients with suspected stable angina in the community. RACPCs will assess the patients with chest pain by taking the history of the symptoms, checking the blood pressure and recording electrocardiogram (ECG). Assessments are made by specialist nurses, doctors and consultants. After assessing the patients in RACPCs, clinicians will typically send the patients for more investigations including Cardiac imaging (70).

The latest NICE guidelines for patient with suspected stable cardiac chest pain states that cardiac imaging with computed tomography coronary angiography (CTCA) is the first line investigation for these patients. However, this imaging has disadvantages that can affect patients. One of the most known disadvantages is the radiation exposure, which can affect the patients in the long term. Moreover, administration of iodine contrast agent with CT imaging can cause many reactions to the patients such as anaphylaxis and nephrotoxicity (71). Therefore, we always try to reduce the use of cardiac imaging when diagnosing patients with chest pain.

In patients with acute chest pain, the use of high-sensitivity cardiac troponin assays has enabled the diagnosis of acute myocardial infarction to be rapidly ‘ruled out’ or ‘ruled in’. This is possible because hs-cTn assays can detect very low concentrations of cardiac cTn. Patients with concentrations below a very low cutoff can now have AMI immediately ‘ruled out’. Outside of the context of acute chest pain, patients hs-cTn concentrations even within the normal range have been shown to predict long-term cardiovascular prognosis (72). Further, patients with risk factors for cardiovascular disease have been shown to have higher baseline hs-cTn concentrations than apparently healthy individuals without risk

factors (27). This has led many to question whether very low hs-cTn concentrations could be used to 'rule-out' stable angina in patients with stable chest pain.

However, the results of the systematic review (Chapter 2) show that the sensitivity and negative predictive value of hs-cTn alone was insufficiently high to allow safe 'rule-out' of stable angina. The systematic review found that previous studies often used hs-cTn cut-offs that were above the limit of detection of the assay. Therefore, in the previous chapter we evaluated the use of hs-cTn alone as a 'rule-out' test for stable angina. The findings confirmed that neither hs-cTnT (manufactured by Roche Diagnostics) nor hs-cTnI (manufactured by Abbott Laboratories) can rule-out stable CAD when used alone.

There are risk scores available to help clinicians to estimate the probability of stable coronary artery disease. These risk scores are different, but they are all help the clinicians to give a better diagnosis to the stable CAD. Potentially, the use of these risk scores alongside hs-cTn could improve sensitivity and negative predictive value for stable angina, thus limiting the use of cardiac imaging and reducing the exposure of patients to unnecessary imaging studies involving ionising radiation, reducing cost and reducing waiting times for patients.

Several different risk scores have been described in this context. These include the Diamond-Forrester risk score and the Coronary Artery Disease Consortium (CAD-C) risk score. The Diamond-Forrester risk score is a tool has been developed to evaluate the pre-test probability of coronary artery disease in patients with chest pain. This tool has the advantage of being easy to use at the point of care, utilising routinely available data such as age, sex and symptoms. This approach adds no extra cost (73).

6.2 Objectives:

In this chapter I will use risk scores in combinations with high-sensitivity cardiac troponin T to check the sensitivity, specificity, negative predictive value and positive predictive value of this combination to rule-out patients with stable coronary artery disease.

6.3 Methods

This is a sub study of MCAD study, a prospective diagnostic test accuracy study. The full methods of the study are reported in Chapter 3. In brief, all patients included in the study were referred to RACPCs at two hospitals (Manchester Royal Infirmary and Wythenshawe Hospital) with suspicion of stable CAD based on clinical assessments and symptoms after giving written informed consent. We obtained all the necessary approvals for this study. The research nurses drew blood from participants when they attended RACPC. Blood samples were analysed immediately for hs-cTn concentrations using the Roche Elecsys high-sensitivity cardiac troponin T assay (99th percentile 14ng/L, limit of detection 5ng/L, limit of blank 3ng/L, coefficient of variation <10% at 13ng/L).

The data has been collected based on patients information, we used patients demographics, past medical history and risk scores. The data required to calculate the outputs of the Diamond-Forrester score and CAD-C model were also collected. The demographics information that was collected included patient age, name, date of birth and gender. In addition to collecting data for the Diamond-Forrester score and the CAD-C model, we also collected data on chest pain typicality, the estimated percentage probability of CAD and the probability of CAD using a Likert scale (both in the opinion of the treating clinician). Pain typicality has been coded into three types (typical, atypical and non-anginal pain) (74), Chest pain typicality was assessed by how many of the following factors were present: substernal chest pain; exertional chest pain; chest pain relieved by rest. ‘Typical’ chest pain is defined as patients with all three factors; pain is labelled as ‘atypical’ if two of the three features are

present; and ‘non-anginal’ if only one factor is present. The Likert probability has been calculated using a 5-point Likert scale (‘definitely’, ‘probably’, ‘could be’, ‘probably not’ and ‘definitely not’). This was recorded by the treating clinician (usually a specialist nurse) in the RACPCs. The percentage probability has been calculated depending on a designed tool that used different information to have the results (75). Data collection was overseen by the research nurses when the patients attended their RACPC appointments.

The outputs of the Diamond-Forrester and CAD-C risk scores were calculated as specified in the original publications, which is summarised in Table 6.1 (Diamond-Forester) and Table 6.2 (CAD-C). For the CAD-C model, the original publication reports three variations of the model (basic, clinical and clinical + CT calcium score). I used the clinical score for this evaluation.

Table 6.1: Diamond-Forrester risk score (70).

Number of typical features*	Age (years)	Men	Women
Typical	30-39	70%	26%
	40-49	87%	55%
	50-59	92%	79%
	≥60	94%	91%
Atypical	30-39	22%	4%
	40-49	46%	13%
	50-59	59%	32%
	≥60	67%	54%
Non-anginal	30-39	5%	1%
	40-49	14%	3%
	50-59	22%	8%

	≥60	28%	19%

* Number of typical features out of substernal location (yes/no); exertional nature (yes/no); relief with rest or nitrates (yes/no). NB, the original Diamond-Forrester score had no category for patients aged >69 years. We modified this to extend the age category from ’60-69 years’ to ‘≥60 years’.

Table 6.2: CAD-C module.

Variable	Beta coefficient
Constant	-7.539
Age	0.062
Male sex	1.332
Atypical chest pain	0.633
Typical chest pain	1.998
Diabetes mellitus	0.828
Hypertension	0.338
Hyperlipidaemia	0.422
Smoking	0.461

* The probability of sCAD was calculated as: p = 1 / 1 + exp(-(constant + (beta-coefficient * variable))), including each beta coefficient and variable listed above

The primary outcome was a diagnosis of stable coronary artery disease, which was based on cardiac imaging that has been taken over three months. The cardiac imaging was taken under clinician follow up, adjudicated by two investigators, blinded to hs-cTn and risk scores results. The full information on the adjudication process can be found in chapter 3.

6.3.1. Statistical analysis

We dichotomised the outputs of risk scores by saying that anyone with calculated probability of CAD >10% was 'positive' (and needs a scan), based on the assumption that the negative predictive value of the test must be >90% (i.e., the post-test probability must be <10%) in order to be clinically useful. We then combined risk scores with hs-cTnT by saying that patients tested 'positive' (i.e. would need a scan) if hs-cTnT >3ng/L OR they were positive for the relevant risk score (>10% probability).

For overall diagnostic accuracy of risk scores themselves we created ROC curves and calculated AUC with 95% CI. In addition to ROC curves and AUC, logestic regression has been used to evaluate whether hs-cTnT and risk scores give diagnostic information that is independed of each other. This analysis included both dependent (final diagnosis of CAD) and independent (hs-cTnT and risk scores) variables. In this analysis odds ratio and 95% CI were calculated using SPSS version 23.0.

6.3.2. Sensitivity analyses for missing data

I explored the potential impact of missing data on my findings. To do this, I first examined whether the missing data were missing completely at random (MCAR). This was done using Little's MCAR test using each risk score and the adjudicated diagnosis of sCAD as variables. As this revealed no statistically significant evidence to reject the hypothesis that the data were MCAR (p=0.080), I used multiple imputation to explore the potential impact of missing data. I created five imputed datasets with complete data for each risk score. I re-ran the main analysis for each risk score in combination with hs-cTnT using 2x2 tables, in each imputed dataset. The test characteristics were calculated in the same way as above and compared to the original findings without imputation.

6.4 Results

Descriptive statistics have been used to describe baseline characteristics as number and percentage (Table 6.3). Of the 306 patients included in the study, there were 280 patients with sufficient data to be included in the evaluation of chest pain typicality, 271 for the Diamond-Forrester score, 259 patients for the CAD-C module, 270 for estimated probability of sCAD and 281 for the estimated likelihood of sCAD using a Likert scale ().

Table 6.3: Baseline characteristics of the included participants.

	All patients, n=281	Patients with coronary artery disease, n=69	Patients without coronary artery disease, n=212
Age, years (mean, standard deviation)	56.4 (12.3)	55.4 (12.9)	59.6 (9.9)
Male sex	128 (45.6)	42 (60.9)	86 (40.6)
Hyperlipidaemia	109 (38.8)	39 (56.5)	134 (63.2)
Hypertension	94 (33.5)	27 (39.1)	141 (66.5)
Diabetes type 1	4 (1.4)	1 (1.4)	206 (97.2)
Diabetes type 2	36 (12.8)	14 (20.3)	187 (88.2)
CVA or TIA	8 (2.8)	4 (5.8)	207 (97.6)
PVD	12 (4.3)	2 (2.9)	200 (94.3)
Smoker	52 (18.5)	15 (21.7)	170 (80.2)
Heart failure	1 (0.4)	0 (0.0)	206 (97.2)
Family history	118 (42.0)	34 (49.3)	109 (51.4)

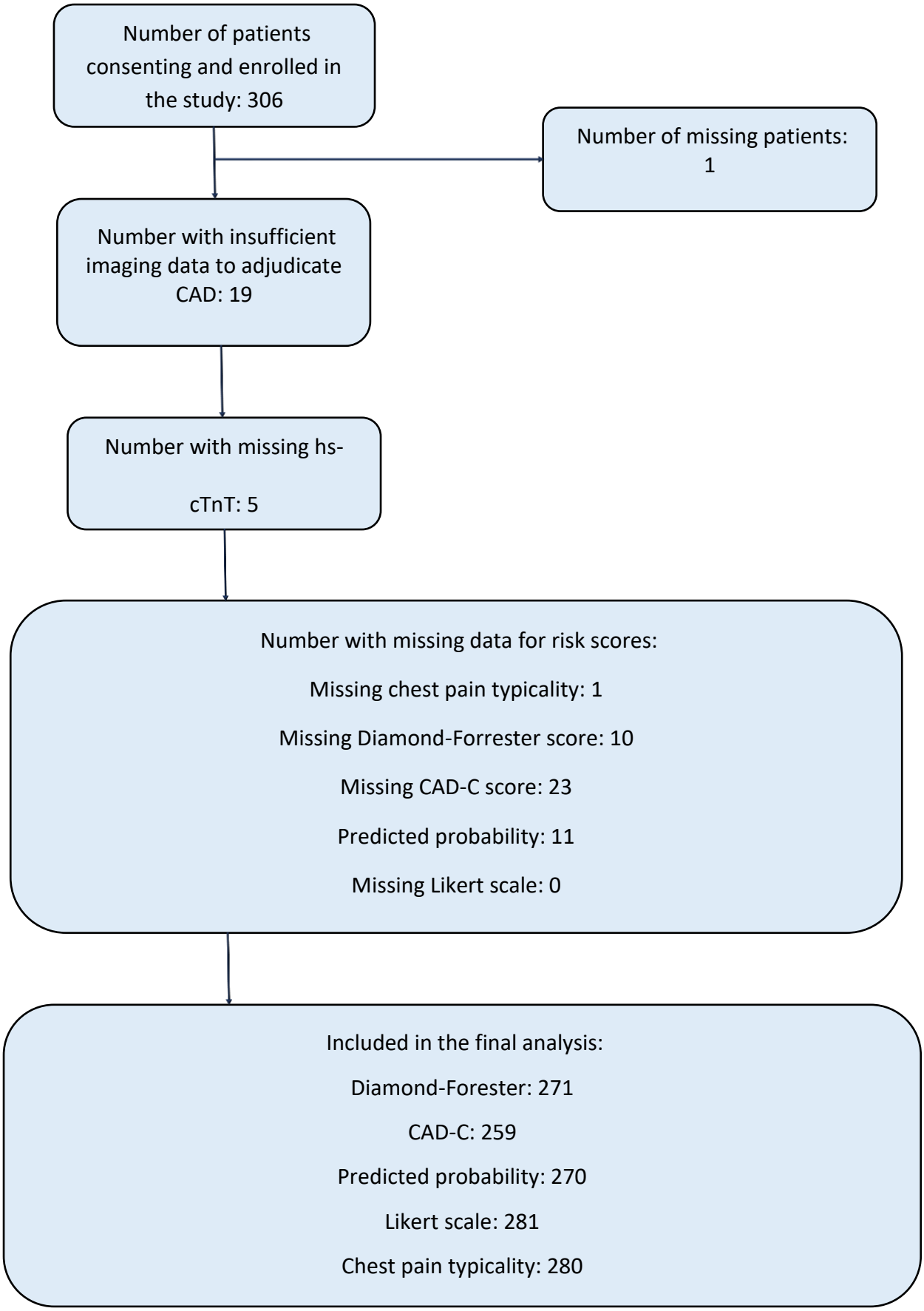

Figure 6.1: Participant flow diagram.

We cross-tabulated the data in two ways: first, we cross-tabulated risk scores with sCAD; and second we cross-tabulated the combination of risk scores plus hs-cTnT with sCAD (Appendix 3. We then calculated the test characteristics of each risk score to check sensitivity, specificity, NPV and PPV as can be seen in (Table 6.4). We can see that the sensitivity of the Diamond-Forrester score is low at 95.5% with low specificity and NPV at 19.0% and 92.9%, respectively. However, when Diamond-Forrester was combined with hs-cTnT the sensitivity and NPV increased to 98.6% and 95.8%, respectively. Similarly, the CAD-C model had a low sensitivity when used alone at 73.8%. However, when combined with hs-cTnT the sensitivity and NPV increased to 91.4% and 80.5%. As demonstrated in the table below this pattern was repeated across other risk scores.

Table 6.4: Test characteristics of the risk scores evaluated.

Rule-out strategy	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Chest pain 'atypical' or 'non-anginal'	28.6 (18.4-40.6)	77.7 (71.5-83.1)	29.4 (21.1-39.4)	77.0 (73.9-80.0)	1.28 (0.82-2.00)	0.92 (0.78-1.08)
Chest pain 'atypical' or 'non-anginal' and hs-cTnT <3ng/L	83.0 (69.2-92.4)	25.5 (20.5-32.0)	18.1 (16.0-20.4)	89.0 (78.0-94.0)	1.12 (096-0.30)	0.66 (0.34-1.28)
Diamond-Forrrester score <10%	95.5 (87.3-99.1)	19.0 (13.9-25.1)	27.5 (25.9-29.2)	92.9 (80.6-97.6)	1.18 (1.08-1.28)	0.24 (0.08-0.75)
Diamond-Forrester score <10% AND hs-cTnT <3ng/L	98.6 (92.3-100)	10.7 (6.9-15.5)	26.3 (25.3-27.4)	95.8 (76.0-99.4)	1.10 (1.05-1.16)	0.13 (0.02-0.98)
CAD-C score <10%	73.8 (61.5-84.0)	57.1 (49.9-64.1)	36.1 (31.3-41.2)	86.9 (81.3-91.5)	1.72 (1.39-2.14)	0.46 (0.30-0.70)
CAD-C score <10% AND hs-TnT <3ng/L	91.4 (82.3-96.8)	17.2 (12.4-22.9)	26.4 (24.7-28.3)	80.5 (73.1-93.3)	1.10 (1.01-1.21)	0.50 (0.22-1.13)

Likert scale 'probably not' or 'definitely not' sCAD	67.1 (54.9-77.9)	55.4 (48.5-62.2)	33.1 (28.4-38.2)	83.7 (78.2-88.0)	1.51 (1.12-1.88)	0.59 (0.42-0.85)
Likert scale 'probably not' or 'definitely not' sCAD AND hs-cTnT <3ng/L	94.3 (86.0-98.4)	14.8 (10.4-20.3)	26.4 (24.9-28.0)	88.9 (74.6-95.6)	1.11 (1.02-1.20)	0.39 (0.14-1.05)
Estimated probability of sCAD <10%	91.4 (82.3-96.8)	18.5 (13.6-24.4)	26.7 (24.8-28.6)	87.0 (74.7-93.8)	1.12 (1.02-1.23)	0.46 (0.20-1.05)
Estimated probability of sCAD <10% AND hs-cTnT <3ng/L	91.4 (82.3-96.8)	18.5 (13.6-24.4)	26.7 (24.8-28.6)	87.0 (74.7-93.8)	1.12 (1.02-1.23)	0.46 (0.20-1.05)

ROC curves were constructed for each risk score that had a continuous variable as an output. The ROC curves for each risk score, when used alone (without hs-cTnT) are shown in (Figure 6.2). Overall, diagnostic accuracy was poor for the risk scores without troponin. The area under the curve (AUC) for each risk score is shown in (Table 6.5). Again, the AUC was low for each risk score at (0.642, 0.704 and 0.713), demonstrating that the risk scores alone could not diagnose or rule-out sCAD.

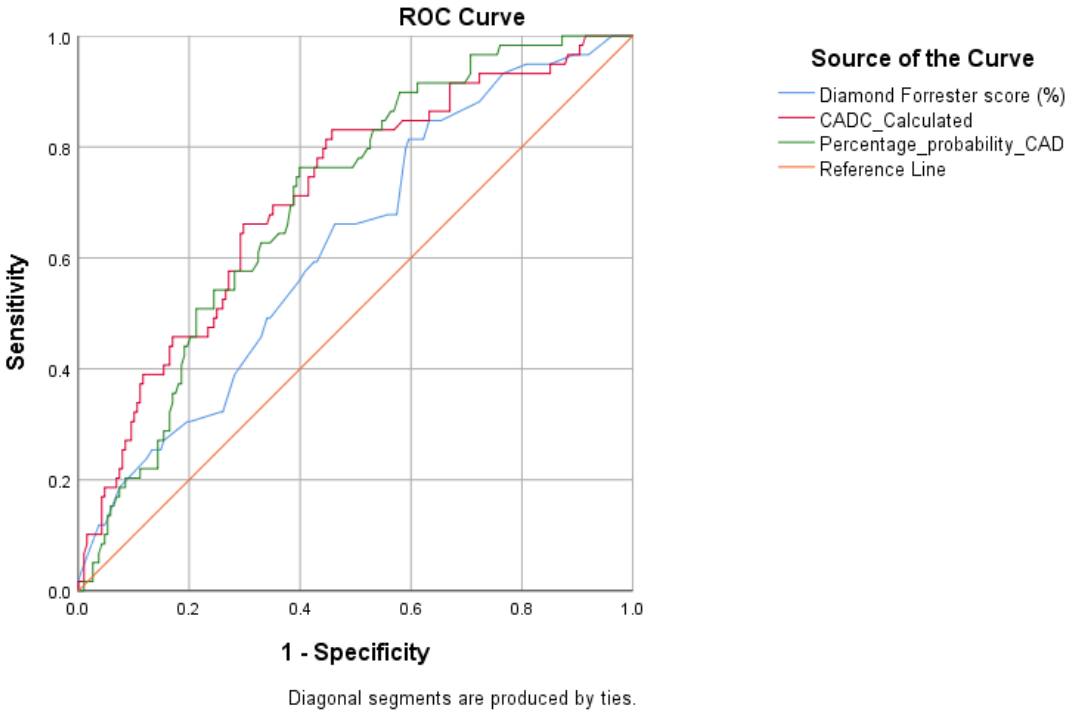

Figure 6.2: ROC curve for each risk score that had a continuous variable as an output.

Table 6.5: AUC for each risk score (only included risk scores with outputs that are continuous variables).

Risk score	AUC	95% CI	P value (AUC >0.5)
Diamond-Forrester score	0.624	0.545 -0.703	0.004
CAD-C	0.713	0.639 – 0.787	<0.001
Percentage probability of CAD	0.704	0.634 – 0.773	<0.001

ROC curves for the risk scores when combined with hs-cTnT are shown in (Figure 6.3). The AUC for hs-cTnT alone was 0.57. The AUC for the estimated probability of sCAD (by the treating clinician) was 0.71. When both parameters were combined, the AUC was 0.68, which is lower than that for estimated probability alone. Therefore, adding troponin was not helpful as there are no incremental value (Table 6.6). As for other risk scores the findings were broadly the same. The AUC for the combination of hs-cTnT and the risk score did not increase when compared to the AUC for the risk score alone, as shown in the Appendix 3.

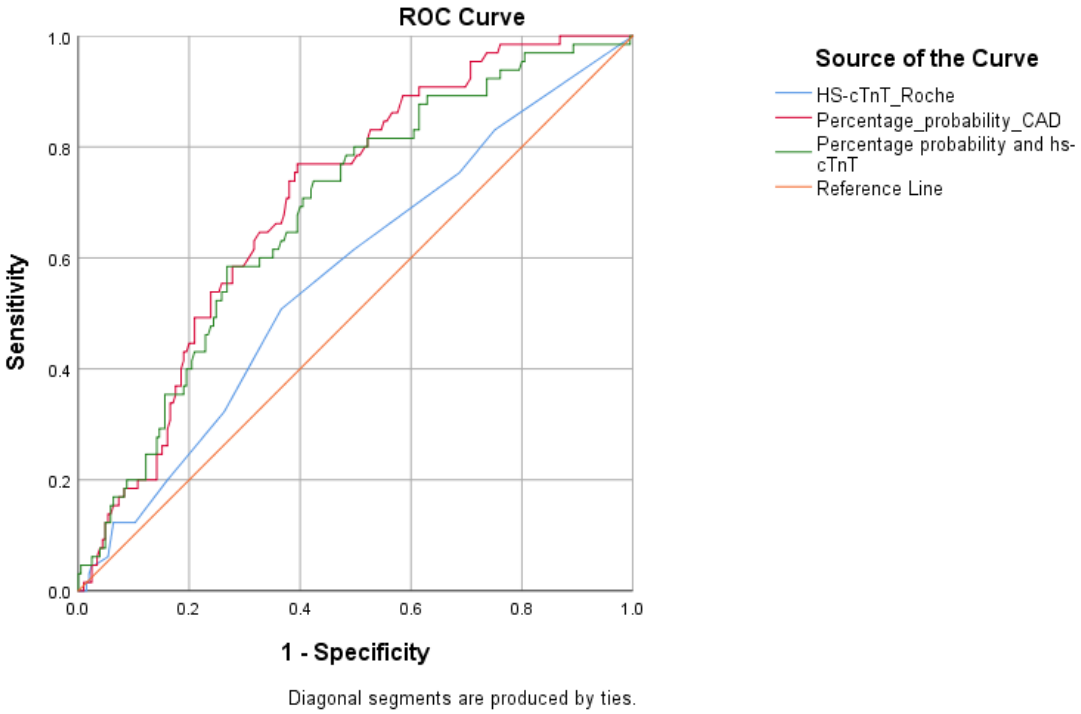

Figure 6.3: ROC curves for the risk scores (Percentage probability) when combined with hs-cTnT.

Table 6.6: AUC for percentage probability of CAD when combined with hs-cTnT.

Risk score	AUC	95% CI	P value (AUC >0.5)
Percentage probability of CAD	0.705	0.639 – 0.771	<0.001
Hs-cTnT Roche	0.568	0.489 – 0.648	0.096
Percentage probability and hs-cTnT	0.683	0.612 – 0.755	<0.001

To further explore the additive value of hs-cTnT used with risk scores, we calculated odds ratios for multivariate models including hs-cTnT and each risk score. The lower bound of the 95% confidence interval for the odds ratio is above 1 for the CAD-C module, indicating that patients with higher scores are more likely to have sCAD. However, the odds ratio for hs-cTnT was almost 1 with 95% CI crossing 1. This means that, once the value of the risk score is known, hs-cTnT results did not affect the likelihood of sCAD (Table 6.7).

Table 6.7: The odds ratios for CAD-C module and hs-cTnT.

Variable	Beta coefficient	Odds ratio (95% CI)
CAD-C	4.129	62.114 (9.291 – 415.260)
HS-cTnT	-0.039	0.962 (0.887 – 1.042)
Constant	-1.637	

Table 6.8: The odds ratios for Diamond Forrester risk score and hs-cTnT.

Variable	Beta coefficient	Odds ratio (95% CI)
Diamond-Forrester risk score	0.015	1.015 (1.005 – 1.025)
HS-cTnT	0.011	1.011 (0.944 – 1.084)

Constant	-1.901	

Table 6.9: The odds ratios for Predicted probability and hs-cTnT.

Variable	Beta coefficient	Odds ratio (95% CI)
Predicted probability	0.059	1.061 (1.030 – 1.093)
HS-cTnT	-0.059	0.943 (0.866 – 1.027)
Constant	-1.719	

Table 6.10: The odds ratios for Likert probability and hs-cTnT.

Variable	Beta coefficient	Odds ratio (95% CI)
Likert probability	0.750	2.116 (1.427 – 3.140)
HS-cTnT	-0.007	0.993 (0.925 – 1.066)
Constant	-3.094	

6.5. Sensitivity analyses: Missing data

Using five imputed datasets, the findings for the combination of each risk score and hs-cTnT were as follows:

- Diamond-Forester: No change in any of the values for any test characteristic across any of the five imputed datasets
- CAD-C: No change in any of the values for any test characteristic across any of the five imputed datasets
- Predicted probability: No change in any of the values for any test characteristic across any of the five imputed datasets

As there were no missing data for the Likert scale and only one missing value for chest pain typicality, multiple imputation was not used for these variables.

6.6. Discussion

In this analysis, the risk scores alone have demonstrated only moderate diagnostic accuracy for sCAD. There was fairly wide variation in sensitivity. Chest pain typicality had only 28.6% sensitivity whereas the Diamond-Forrester score had a sensitivity of 95.5%. Without troponin, only the Diamond-Forrester score had an NPV above 90%. The low numbers for the other risk scores tell us that they cannot be used alone to rule-out sCAD in patients with stable chest pain. With an NPV of 93%, even the Diamond-Forrester score is likely to be insufficiently sensitive to rule-out sCAD.

However, the accuracy increased when risk scores were added to hs-cTnT. The highest sensitivity we had is 98.6% for the Diamond Forrester-risk score, showing improved sensitivity compared with the risk score when used alone. The NPV also increased to 95.8% for the Diamond Forrester risk score when combined with hs-cTnT. This may be considered sufficient to rule-out sCAD.

This combination could help us to identify patients who are at low risk of sCAD, thus eliminating the need for imaging in some patients. However, it is also important to note that the actual value of hs-cTnT seems to add little new information once the risk score has been taken into account, i.e., sCAD does not become more likely the higher the value of hs-cTnT. This is demonstrated by the logistic regression analysis, which showed that hs-cTnT did not significantly predict sCAD once the Diamond-Forrester score had been taken into account.

Our findings here agree with Mueller et al (76), they also found with low pre-test probability and low troponin level that you can rule-out sCAD with reasonably high NPV. Using the same principle ,we found that combining troponin with risk scores can improve accuracy. Moreover, a study held in Scotland by Adamson et al (40) examined the Coronary Artery Disease Consortium Model (CADC) with Troponin, in which they found that adding troponin to the CAD-C model increased overall sensitivity and NPV.

6.7. Limitations

We had missing data for some of the risk scores. We did a complete case analysis because it was the most reasonable thing to do with these missing data, and there was no reason to believe that the data was systematically different for the patients who had missing values. The total proportion of missing data was relatively small (<10% for the risk score with most missing data). However, I examined the impact of this using multiple imputation and there was no difference from the original findings. Therefore, this is unlikely to have affected the overall conclusions.

6.8. Future directions

In this study we had a relatively small sample size, which was largely due to many problems we faced in the beginning of the study. However, it seems unlikely that a larger sample size will change the conclusion that hs-cTnT is not a strong predictor of sCAD. There was little evidence from this analysis that hs-cTnT could either rule-out sCAD when used alone, nor could it add substantial value to commonly used risk scores. Future work may therefore focus on Diamond-Forester, potentially in combination with hs-cTnT. However, it may be more prudent to focus on other predictors or biomarkers combined with risk scores.

6.9. Conclusions

The study showed that risk scores alone are not sufficient to rule-out sCAD, because the sensitivity and NPV were quite low. When combined with troponin, the sensitivity and NPV were improved especially for the Diamond Forrester with 98.5% sensitivity and 95.8% NPV, which can rule-out sCAD. However, the incremental value of adding hs-cTnT to risk scores was marginal.

Chapter 7 : High sensitivity cardiac troponin I

7.1. Introduction

Cardiac troponin I (cTnI) is a protein that is found in the heart muscle. It is released into the blood stream after an injury to the myocardium such as a heart attack or myocardial infarction. High sensitivity cardiac troponin I (hs-cTnI) assays can detect very low levels of cTnI in blood with high precision. In the previous two chapters we evaluated the use of high-sensitivity cardiac troponin T (hs-cTnT) as a biomarker to rule-out stable coronary artery disease (sCAD). However, the results were not promising, and we found that hs-cTnT had only moderate accuracy to rule-out sCAD. In this chapter we will evaluate an hs-cTnI assay (Abbott ARCHITECT hs-cTnI). According to the International Federation of Clinical Chemistry Committee for Cardiac Biomarkers (IFCC-CCB), this assay detects cardiac troponin in 85% of apparently healthy individuals, whereas the hs-cTnT assay evaluated in previous chapters detected troponin in only 57.4% (Table 7.1) (68). And as the table below shows, the limit of blank (LoB) and limited of detection (LoD) are lower for this hs-cTnI assay than the Hs-cTnT assay. Taken together, these findings suggest that the hs-cTnI assay may help us to achieve higher sensitivity to rule-out sCAD.

Table 7.1: Analytical characteristics of hs-cTn I and T assays by (IFCC-CCB).

Assay	Limet of blank LoB (ng/L)	Limit of detection LoD (ng/l)	Coefficient of variation (CV) at 99th percentile	Present normal measures ≥ LoD Overall M / F
Abbott high-sensitivity troponin I, Abbott ARCHITECT	1.0	1.6	Overall: 26.2 F: 15.6 M: 34.2	Overall: 85% F: 78% M: 92%
High-sensitivity troponin T, Roche Elecsys	2.53; (1.58 for e411)	3.16; (2.54 for e411)	Overall:14 F: 9 M:1 7	Overall: 57.4 %

7.2. Objectives

In this study I aimed to evaluate the diagnostic accuracy of an hs-cTn assay (hs-cTnI, Abbott Diagnostics ARCHITECT) in diagnosing patients who are suspected to have stable coronary artery disease.

7.3. Methods

7.3.1. Study design and settings

This is a secondary analysis from the MCAD study. In this study all included patients had been referred to the Rapid Access Chest Pain Clinic (RACPC) suspected of having stable coronary artery disease. The study has been designed as prospective single arm observational diagnostic accuracy study. We recruited the patients in two different sites (Manchester Royal Infirmary [MRI] and Wythenshawe Hospital). We obtained all the necessary approvals for the study (Research Ethics Committee reference 18/EM/0124) on 24/April/2018. All the recruited patients provided written informed consent to participate in the study, and all the blood samples were collected after taking the written consent.

7.3.2. Index test

All participants had a blood sample drawn during their attendance to RACPC, before having any cardiac imaging. We tested the samples for hs-cTnI, Abbott Diagnostics ARCHITECT. The assay has a LoD of 1.6ng/L but results are reported in integers down to 2ng/L. Samples were centrifuged and serum was stored at -20^{o}C or below within 4 hours of collection and at -70^{o}C or below within 72 hours of collection. Previously unthawed samples were subsequently transferred to Pennine Acute NHS Trust in dry ice for batch analysis.

7.3.3. Statistical analysis

Descriptive statistics were used to summarise the patients' baseline characteristics. We measured the sensitivity, specificity, negative predictive value NPV and positive predictive value to calculate the diagnostic accuracy of hs-cTnI. We used MedCalc to calculate the test characteristics with exact Clopper-Pearson 95% confidence intervals (67). Finally, as a measure of overall diagnostic accuracy, I plotted receiver operating characteristic (ROC) curves and calculated the area under the curve with 95% confidence intervals using the non-parametric method, in SPSS (IBM, Chicago, Ilinois).

7.3.4. Sensitivity analysis for missing data

To check the effect of missing data on my findings, I made another analysis of missing data. In this analysis I used the same method of Little's missing completely at random (MCAR) test for each risk score and the adjudicated sCAD as variable. This method returned with a P value of 0.930, thus providing no evidence to reject the hypothesis that the data were MCAR. For that reason, multiple imputation analysis has been used to check the impact of missing data.

7.4. Patient characteristics

The flow of participants through the MCAD study is described in detail in (Chapter 4). Figure 7.1 illustrates the flow of patients included in the MCAD study. Of the 306 participants in the MCAD study, 286 had sufficient data to adjudicate sCAD and 266 patients had a sample available for hs-cTnI analysis and were included in the evaluation presented in this chapter. Baseline characteristics of included participants are reported in (Table 7.2).

Table 7.2: Baseline characteristics of included participants.

	All patients, n=266	Patients with coronary artery disease, n=65	Patients without coronary artery disease, n=201
Age, years (mean, standard deviation)	56.4 (12.3)	59.4 (10.0)	55.4 (12.9)
Male sex, n (%)	125 (47.0)	40 (61.5)	85 (42.3)
Hyperlipidaemia, n (%)	102 (38.3)	36 (55.4)	126 (62.7)
Hypertension	91 (34.2)	26 (40.0)	131 (65.2)
Diabetes type 1	4 (1.5)	1 (1.5)	195 (97.0)
Diabetes type 2	35 (13.2)	14 (21.5)	177 (88.1)
CVA or TIA	8 (3.0)	4 (6.2)	196 (97.5)
PVD	11 (4.1)	2 (3.1)	189 (94.0)
Smoker	50 (18.8)	15 (23.1)	161 (80.1)
Heart failure	1 (0.4)	0 (0.0)	194 (96.5)
Family history	113 (42.5)	34 (52.3)	104 (51.7)

* This table reports data for all participants who had sufficient data to adjudicate sCAD and a valid hs-cTnI measurement

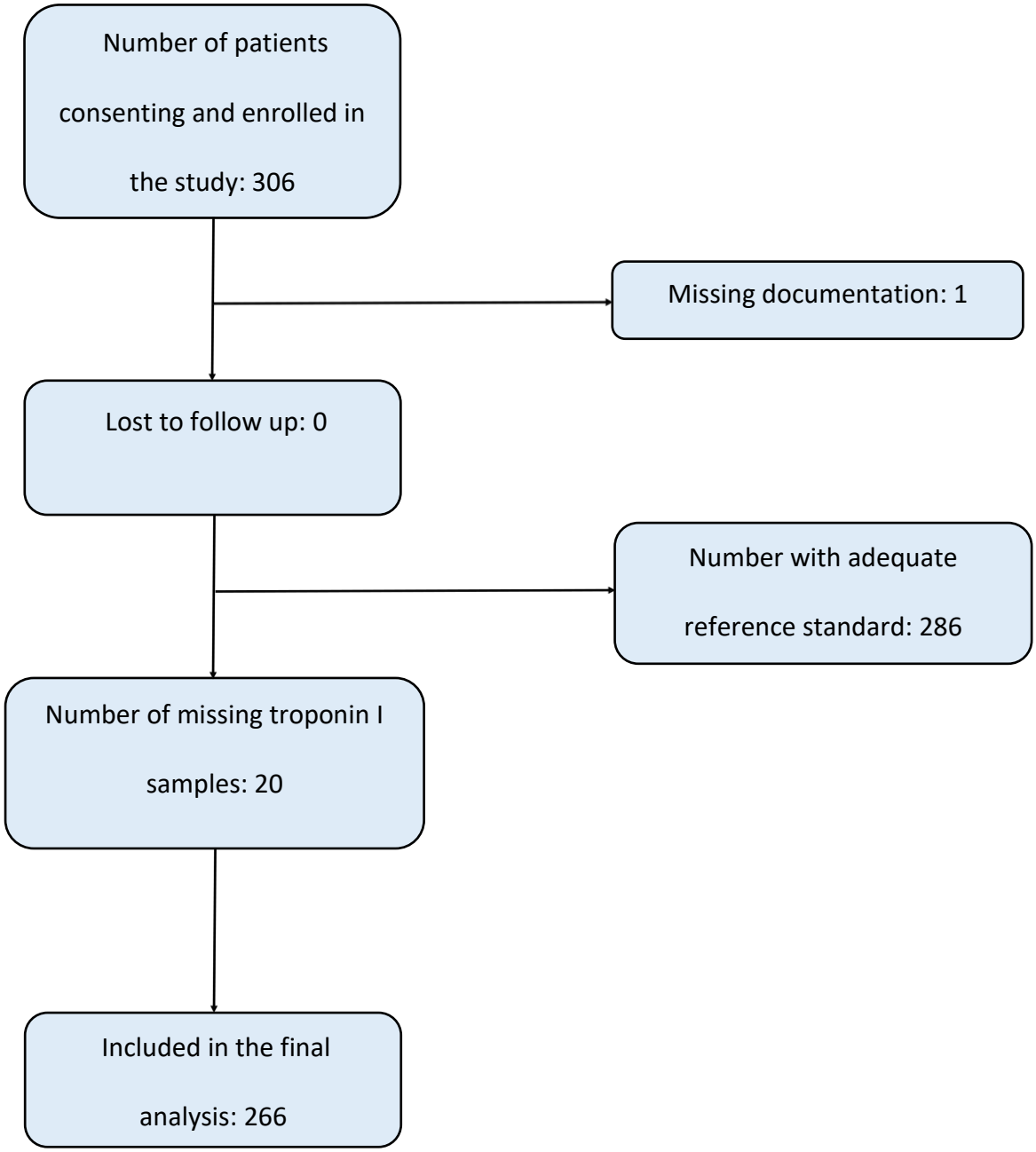

Figure 7.1: Flow diagram of included Participants.

7.5. Diagnostic accuracy of hs-cTnI

The accuracy of hs-cTnI was calculated by using the lowest possible cut-off which is 2ng/L (the lowest concentration possible, aiming to achieve the highest possible sensitivity for CAD). To achieve that I produced two by two tables to cross tabulate the diagnosis of CAD (based on the adjudication of the expert cardiologists) versus hs-cTnI concentration at a cut-off of 2ng/L. (Table 7.3) below shows that 43 patients with hs-cTnI less than 2ng/L had sCAD, whereas, 94 patients with troponin more than 2ng/l had sCAD. And 107 patients with troponin less than 2ng/l had no CAD, while 22 patients with troponin more than 2ng/l also had no sCAD.

Table 7.3: 2x2 table of high sensitivity cardiac troponin I (hs-cTnI) at a cut-off of 2 ng/L versus a diagnosis of sCAD.

Hs-cTnI	Coronary artery disease present	Coronary artery disease absent
Hs-cTnI <2ng/L	43	22
Hs-cTnI ≥2ng/L	94	107

In a post-hoc exploratory analysis to determine whether hs-cTnI could be used to 'rule-in' sCAD or identify a high-risk group, I explored the use of higher hs-cTnI cut-offs, set at 5 and 10 ng/l, as shown in (Table 7.4) and (Table 7.5). Table 7.9 below shows the accuracy with a cut-off of 5/ng/l.

Table 7.4: 2x2 table cross-tabulating of high sensitivity cardiac troponin I (hs-cTnI) at a cut-off of 5 ng/L versus a diagnosis of sCAD.

	Coronary artery disease present	Coronary artery disease absent
Hs-cTnI <5ng/L	14	51
Hs-cTnI ≥5ng/L	12	189

Table 7.5: 2x2 table showing a cross-tabulation of high sensitivity cardiac troponin I (hs-cTnI) at a cut-off of 10 ng/L versus a diagnosis of sCAD.

	Coronary artery disease present	Coronary artery disease absent
Hs-cTnI <10ng/L	6	59
Hs-cTnI ≥10ng/L	3	198

(Table 7.6) below demonstrates diagnostic accuracy at the 2 ng/L troponin I cut-off. The sensitivity is 66.1% with an NPV of 83.0%. Thus, 17.0% of patients with hs-cTnI <2ng/L had sCAD. Moreover, the specificity and PPV were also low at 53.2% and 31.4%, respectively, at a cut-off of ≥2ng/L.

Table 7.6: Test characteristics of hs-cTnI at a cut-off of 2ng/L.

Statistic	Value	95% CI
Sensitivity	66.1%	53.3% to 77.4%
Specificity	53.2%	46.1% to 60.3%
Positive Likelihood Ratio	1.41	1.13 to 1.78
Negative Likelihood Ratio	0.64	0.44 to 0.91
Disease prevalence (*)	24.4%	19.4% to 30.1%
Positive Predictive Value (*)	31.4%	26.7% to 36.5%
Negative Predictive Value (*)	83.0%	77.2% to 87.5%
Accuracy (*)	56.4%	50.2% to 62.4%

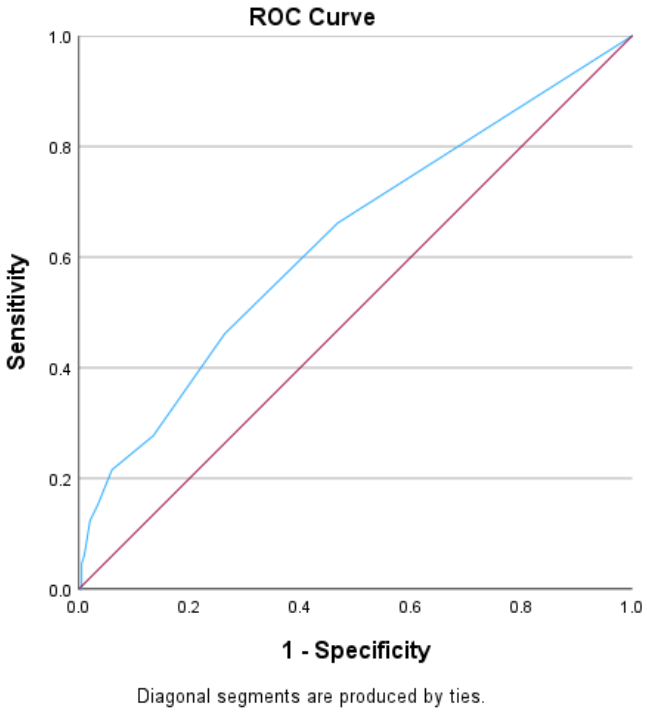

Figure 7.2: ROC curve for hs-cTnI.

Table 7.7: Area under the ROC curve for hs-cTnI for an adjudicated diagnosis of sCAD.

Test Result Variable(s): HS-cTnI (Abbot)				
Area	Standard Error	P value (null hypothesis: AUC = 0.5)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.631	0.041	0.173	0.550	0.712

Table 7.8 shows the sensitivity and the specificity at every possible cut-off. As noted above, even at the lowest possible cut-off, we could not achieve a sensitivity more than 66.2%.

Table 7.8: Sensitivity and specificity of hs-cTnI at every possible cut-off.

Positive if Greater Than or Equal To	Sensitivity	Specificity
1.50	66.2	53.2
2.50	46.2	73.6
3.50	27.7	86.6

4.50	21.5	94.0
5.50	15.4	96.5
7.00	12.3	98.0
9.00	9.2	98.5
11.00	6.2	99.0
13.00	4.6	99.5
16.00	3.1	99.5
21.00	1.5	99.5
47.00	0.0	99.5
73.00	0.0	100.0

Further I explored if Troponin I could rule-in CAD at selected cut-offs, I measured the cut-offs of 5ng/l (Table 7.9) and 10ng/l (Table 7.10). Table 7.9 shows that when the cut-off increased the specificity increased, which means that this group is identified as a high risk for CAD.

Table 7.9: Test characteristics of hs-cTnI at a cut-off of 5ng/L.

Statistic	Value	95% CI
Sensitivity	21.5%	12.3% to 33.5%
Specificity	94.3%	89.8% to 96.9%
Positive Likelihood Ratio	3.61	1.76 to 7.40
Negative Likelihood Ratio	0.83	0.73 to 0.95
Disease prevalence (*)	24.4%	19.4% to 30.1%
Positive Predictive Value (*)	53.8%	36.2% to 70.5%
Negative Predictive Value (*)	78.7%	76.5% to 80.9%
Accuracy (*)	76.3%	70.7% to 81.3%

In Table 7.10 I used a cut-off of 10ng/l, the table shows a high specificity of 98.5%. However, this still gave a relatively low PPV at 66.7%.

Table 7.10: Test characteristics of hs-cTnI at a cut-off of 10ng/L.

Statistic	Value	95% CI
Sensitivity	9.2%	3.5% to 19.0%
Specificity	98.5%	95.7% to 99.7%
Positive Likelihood Ratio	6.18	1.59 to 24.03
Negative Likelihood Ratio	0.92	0.85 to 1.00
Disease prevalence (*)	24.4%	19.3% to 30.1%
Positive Predictive Value (*)	66.7%	34.0% to 88.6%
Negative Predictive Value (*)	77.0%	75.6% to 78.4%
Accuracy (*)	76.7%	71.1% to 81.6%

7.6. Sensitivity analysis: missing data

To evaluate the potential impact of missing data, I first tested the hypothesis that the missing data were missing completely at random (MCAR). Little's MCAR test returned a p value of 0.930, suggesting no evidence to reject the hypothesis that the data were MCAR. Table 7.11 shows the diagnostic accuracy of hs-cTnI using imputed data. The results of this analysis were essentially the same as the complete case analysis.

Table 7.11: Results of cross-tabulation using an imputed dataset (five imputations, pooled data).

Statistic	Value	95% CI
Sensitivity	65.7	53.4 – 76.7
Specificity	54.2	47.3 – 60.9
Positive Likelihood Ratio	1.43	1.15 – 1.79
Negative Likelihood Ratio	0.63	0.45 – 0.90
Disease prevalence (*)	24.5	19.6 – 29.9
Positive Predictive Value (*)	31.7	27.1 – 36.7

Negative Predictive Value (*)	83.0	77.5 – 87.3
Accuracy (*)	57.0	51.0 – 62.8

7.7. Discussion

In this chapter I evaluated the accuracy of an hs-cTnI assay to rule-out sCAD in patients attending RACPC. The expectation of this study was that hs-cTnI may rule-out sCAD as this assay has a low limit of detection (2 ng/L) and has greater analytical sensitivity than the hs-cTnT assay originally evaluated, detecting troponin in a higher percentage of apparently healthy individuals. However, hs-cTnI could not rule-out nor rule-in sCAD. There are some possible reasons that may illustrate why hs-cTnI could not rule-out sCAD:

In a previous chapter, I explored the diagnostic accuracy of hs-cTnT (Roche Diagnostics) at the LoB of the assay, whereas in this chapter I used the LoD of an hs-cTnI assay. It is therefore important to clarify the distinction between the LoD and LoB. LoB is the maximum concentration that is reasonably expected to be found in a sample that contains no element. The LoD is the lowest concentration that can be measured in the sample and is always higher than the LoB (77). The LoD for the hs-cTnI assay studied here was 2ng/L, which appears low. The manufacturer does not report an LoD and therefore concentrations are only reported down to the LoD. However, the diagnostic sensitivity at the LoD was low at 66.1% to rule-out sCAD. In the previous chapter, for hs-cTnT we had an LoD of 5ng/L and the LoB was 3ng/L for the assay, achieving a sensitivity of 82.6% at the LoB cut-off. This difference in sensitivity may potentially be because results are only reported down to the LoD (rather than the LoB) with the hs-cTnI assay, based on the manufacturer's guidelines.

Although troponin T and troponin I are generally similar for diagnosing AMI, it may be that there are differences for prediction of cardiovascular disease in stable patients. Troponin is released into the blood stream after a myocardial injury, regardless of the type. However, it is possible that, in the context of sCAD, troponin T may be released in a higher amount than Troponin I, which can be highly detected. This may explain the reason why we had a higher sensitivity of troponin T than troponin I.

Finally, we need to consider whether the antibodies used in the different tests could have influenced our results. Troponin T and I assays have their own antibodies that bind to a certain area of the relevant proteins. It is possible that the sensitivity of troponin T was higher because the antibodies used in the test were more specific at binding with fragments of troponin T proteins that were circulating in patients with sCAD than the antibodies used in the troponin I test.

7.8. Limitations

We had a relatively small sample size in this analysis. And when the sample size is small, the 95% confidence intervals will tend to be wider, particularly for sensitivity when the prevalence of the target condition is relatively low. This means it is possible that the true sensitivity of hs-cTnI for sCAD could lie in a relatively wide range of values. Therefore, when the sample size increase, the confidence interval become narrower, giving more precise estimates of sensitivity. However, it is important to note that the upper bound of the 95% CI for sensitivity was 76.9%. Even at this level, hs-cTnI would not 'rule-out' sCAD when used alone.

The reference standard was also different between patients. Some patients had CTCA, some had Myoview/thallium scans, some DSE. However, this reflects current practice and is therefore pragmatic.

7.9. Conclusions

From the results above we can see that hs-cTnI cannot rule-in or rule-out sCAD as a single biomarker due to low sensitivity and NPV. Moving on, I will evaluate the accuracy of hs-cTnI in combination with risk scores to determine whether this can achieve higher sensitivity.

Chapter 8 : Using structured risk scores in combination with high-sensitivity cardiac troponin I to rule-out stable coronary artery disease

8.1. Background

In the previous chapters I evaluated the accuracy of high-sensitivity cardiac troponin T (hs-cTnT) alone as a single biomarker and the accuracy of hs-cTnT in combination with risk scores. Hs-cTnT alone was not accurate enough to rule-in and rule-out patients with stable coronary artery disease (sCAD). We found a sensitivity of 82.6%, which was low to rule-out sCAD, with low specificity at 24.1% to rule-in sCAD. These findings seemed consistent with previous literature. Because of that, I also used the available risk scores in combination with hs-cTnT hoping to achieve higher accuracy. This improved sensitivity, though the added value was very small.

In the previous chapter I evaluated the accuracy of an hs-cTnI assay to rule-out sCAD. I used the lowest possible cut-off, set at the limit of detection of the assay (2ng/L). The sensitivity was low at 66.1% but I found quite high specificity at 53.2%. Therefore, this suggested that I may potentially rule-in sCAD if I increase the troponin cut-off. In exploratory analyses, I started with a cut-off of 5ng/L, which gave a specificity of 94.3%. It was not bad but still not likely to be enough to rule-in sCAD. Therefore, I increased the cut-off to 10ng/L, which gave a specificity of 98.5%. However, due to the low prevalence this still gave a relatively low PPV at 66.7%.

Although I found that hs-cTnI had insufficient sensitivity to rule-out sCAD when used alone, the most promising approach described in the literature has combined high-sensitivity cardiac troponin with a means of identifying patients with low pre-test probability of sCAD. Therefore, in this chapter I will evaluate whether sCAD could be ruled out by combining hs-cTnI with risk scores that select patients who have a low pre-test probability of sCAD..

8.2. Objectives

To determine the diagnostic accuracy of an hs-cTnI assay when combined with commonly used risk scores for the diagnosis of stable coronary artery disease.

8.3. Methods

This is pre-planned secondary analysis within the MCAD study, which was a prospective single arm diagnostic accuracy study. The full description of the methods for the MCAD study can be found in Chapter 3. All selected patients in the study had been referred to the RACPC with suspicion of stable coronary artery disease. Patients were recruited at two centres (Manchester Royal Infirmary and Wythenshawe Hospital). Moreover, all included patients had available samples to measure hs-cTnI concentrations from the time of their RACPC attendance, and had full data for the risk scores of interests. All included patients provided written informed consent before giving blood.

Venous blood samples (approximately 4ml) were collected into EDTA vials by trained staff when patients attended the RACPC. Samples were transferred to the laboratory, centrifuged and plasma was separated. The samples were then stored at -80^{o}C pending subsequent transfer to a central laboratory (Royal Oldham Infirmary) for analysis of hs-cTnI (Abbott Diagnostics ARCHITECT, LoD of 1.6ng/L, rounded to 2ng/L because results are reported in integers). Samples were transported in dry ice and analysis was undertaken on the same day using previously unthawed samples.

Data were collected about patients' information, including demographics and data required to calculate the risk scores of interest, which has been explained in detail in Chapter 6. This information was collected by the specialist nurses, when the patient attended RACPC. Data required for calculation of the following risk scores were collected: chest pain typicality (typical, atypical or non-

anginal in nature), estimated probability of sCAD in the opinion of the treating clinician (expressed as a percentage), estimated probability of sCAD expressed using a five-point Likert scale (definitely sCAD, probably sCAD, could be sCAD, probably not sCAD or definitely not sCAD), Diamond-Forrester risk score, and CAD-C risk score.

8.3.1. Statistical analysis

In this analysis I used descriptive statistics, reporting numbers and percentages to summarise the participants' baseline characteristics. When applying the relevant risk scores, individuals with a calculated probability of CAD greater than 10% were considered "positive", implying that they would require imaging. When considering the accuracy of risk scores and hs-cTnI in combination, the focus was on 'ruling out' sCAD. Therefore, patients were considered to have a 'positive test' (requiring a scan) if the hs-cTnI concentration was at least 2ng/L (the LoD) or the risk score was positive (>10% probability, as stated above). For chest pain typicality, patients were considered to have a 'negative' test if the hs-cTnI was <2ng/L and the symptoms were regarded to be 'non-anginal' in nature. For Likert scale probability, patients were considered 'negative' (no scan required) if the hs-cTnI was <2ng/L and the clinician considered that the diagnosis was either 'probably not sCAD' or 'definitely not sCAD'. I used Medcalc (67) software for calculating test characteristics with Clopper-Pearson 95% confidence intervals were calculated using the exact method.

To illustrate the overall diagnostic accuracy of hs-cTnI and risk scores in combination, I created models using logistic regression. The risk score and hs-cTnI were entered into a logistic regression analysis with sCAD as the dependent variable. This allowed calculation of odds ratios and 95% CI for hs-cTnI and the relevant risk scores. The probabilities calculated as outputs from those models were then used to create ROC curves. ROC curves were plotted for hs-cTnI alone, each respective risk score alone and the combination (calculated using the logistic regression model). This provided a visual representation of the potential additive effect of using the two variables in combination (78). For

more accurate analysis, I used McNemar test to compare sensitivities and specificities of paired categorical data.

8.3.2. Sensitivity analysis for missing data

As in previous chapter for hs-cTnT plus risk scores I explored the potential impact of missing data on my findings. I used the same method by using Little's missing completely at random (MCAR) test for each risk score and the adjudicated sCAD as variable. Little's test returned a p value of 0.889, thus providing no evidence to reject the hypothesis that the data were MCAR. Therefore, I used multiple imputation analysis to check the potential effects of missing data. For that, as in previous chapter five, an imputed dataset was created. This created five imputations with no missing data for the relevant risk scores. The dependent variable (an adjudicated diagnosis of sCAD) was used as a predictor to impute values for risk scores, but missing values for sCAD were not imputed. I re-ran the full analysis for each risk score by making 2x2 tables in combination with hs-cTnI. I then repeated the original analyses for diagnostic accuracy, using pooled data from across the five imputed datasets. The results of this analysis were compared to the original database findings without imputation.

8.4 Results:

A total of 306 patients were included in the study, of which 271 had full data for the adjudicated diagnosis of sCAD and an available hs-cTnI concentration, meaning that they were eligible for inclusion in the final analysis (Figure 8.1). One patient had missing data for chest pain typicality, 9 patients for Diamond-Forrester score, 9 patients for the CAD-C module, 2 patients for estimated likelihood of sCAD using a Likert scale and 10 patients for estimated probability of sCAD. Baseline characteristics of the participants are shown in (Table 8.1)**Error! Reference source not found.**

Table 8.1: Patient baseline characteristics.

* This table reports data for all participants who had sufficient data to adjudicate sCAD and a valid hs-cTnI measurement.

	All patients, n=266	Patients with coronary artery disease, n=65	Patients without coronary artery disease, n=201
Age, years (mean, standard deviation)	56.4 (12.3)	59.4 (10.0)	55.4 (12.9)
Male sex,	125 (47.0)	40 (61.5)	85 (42.3)
Hyperlipidaemia,	102 (38.3)	36 (55.4)	126 (62.7)
Hypertension	91 (34.2)	26 (40.0)	131 (65.2)
Diabetes type 1	4 (1.5)	1 (1.5)	195 (97.0)
Diabetes type 2	35 (13.2)	14 (21.5)	177 (88.1)
CVA or TIA	8 (3.0)	4 (6.2)	196 (97.5)
PVD	11 (4.1)	2 (3.1)	189 (94.0)
Smoker	50 (18.8)	15 (23.1)	161 (80.1)
Heart failure	1 (0.4)	0 (0.0)	194 (96.5)
Family history	113 (42.5)	34 (52.3)	104 (51.7)

In this study we cross tabulated risk scores plus hs-cTnI with sCAD (Appendix 4). Then we calculated the sensitivity, specificity, positive predictive value and negative predictive value for each risk score. The combination of chest pain typicality with hs-cTnI gave the lowest sensitivity at 72.3% with an NPV of 82.0%. However, the combination of Diamond-Forrester risk score and hs-cTnI yielded the highest sensitivity and NPV at 96.8% and 92.6%, respectively. This combination had a specificity of 12.8% (Table 8.2).

Table 8.2: Baseline characteristics of the included participants.

Rule-out strategy	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Chest pain 'atypical' or 'non-anginal' and hs-cTnI <2ng/L	72.3 (59.8-82.7)	41.0 (34.1-48.2)	28.5 (24.8-32.5)	82.0 (74.8-87.5)	1.23 (1.01-1.48)	0.68 (0.44-1.03)
Diamond-Forrester score <10% AND hs-cTnI <2ng/L	96.8 (88.8-100)	12.8 (8.5-18.3)	26.1 (24.7-27.5)	92.6 (75.3-98.1)	1.11 (1.03-1.19)	0.25 (0.06-1.03)
CAD-C model <10% AND hs-TnI <2ng/L	86.9 (75.8-94.2)	33.3 (26.5-40.7)	30.3 (27.4-33.3)	88.4 (79.5-93.8)	1.30 (1.13-1.50)	0.39 (0.20-0.77)
Liker scale 'probably not' or 'definitely not' sCAD AND hs-cTnI <2ng/L	86.1 (75.3-93.5)	29.1 (22.9-36.0)	28.4 (25.8-31.2)	86.6 (77.2-92.5)	1.22 (1.07-1.39)	0.48 (0.25-0.90)
Estimated probability of sCAD <10% AND hs-cTnI <2ng/L	85.2 (73.8-93.0)	31.8 (25.3-38.8)	28.1 (25.3-31.1)	87.3 (78.5-92.9)	1.25 (1.08-1.44)	0.46 (0.25-0.88)

To further explore the additive value of hs-cTnI used with risk scores, we calculated odds ratios for multivariate models including hs-cTnI and each risk score. In Table 8.3 below we can see the output of the logistic regression analysis for the Diamond-Forrester score. This shows that the odds ratio for the

Diamond-Forrester score was above 1 and the 95% confidence intervals did not cross 1. Therefore, as the Diamond-Forrester score increases, the probability of sCAD also increases. The odds ratio shows that for every 1-point increase in Diamond-Forrester score the odds of sCAD goes up by 1.013. The lower bound of the 95% CI is 1.004 which does not cross 1, therefore higher the Diamond-Forrester score can be seen as a significant predictor of sCAD. However, while hs-cTnI also had an odds ratio above 1, the 95% confidence interval overlapped 1. This means that hs-cTnI was not a significant predictor of sCAD once the Diamond-Forrester score had been taken into account.

Table 8.3: The odds ratios for Diamond Forrester risk score and hs-cTnI.

Variable	Beta coefficient	Odds ratio (95% CI)
Diamond-Forrester risk score	0.013	1.013 (1.004 – 1.023)
HS-cTnI	0.016	1.016 (0.963 – 1.072)
Constant	-1.800	0.165

For further exploration we used the ROC curve for each risk score combined with troponin I. In the first ROC curve we used the Diamond-Forrester risk score in combination with hs-cTnI (Figure 8.1). The expectation was that adding troponin and Diamond-Forrester score as a combination will help us to identify more patients with sCAD than either troponin I or the Diamond-Forrester score by itself. However, the ROC curve shows that the combination of hs-cTnI and the risk score is not different from troponin I and the risk score alone. This suggests that the combination has little value for identifying new patients with sCAD. Moreover, from looking to the area under the curve (AUC) in (Table 8.4), we can see that the Diamond-Forrester score by itself had an AUC of 0.626 versus 0.621 for Troponin I. The combination had an AUC of 0.634, which is lower than Diamond-Forrester when used alone. This suggests that, in terms of overall diagnostic accuracy, there is no added value when we combined Diamond-Forrester with troponin I.

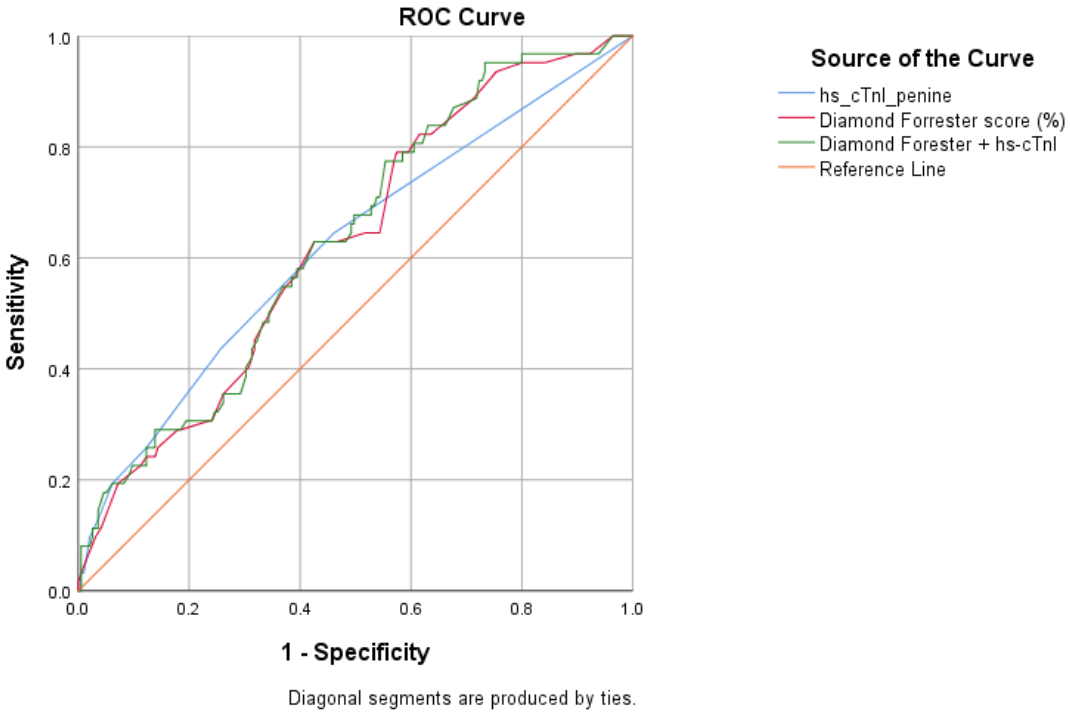

Figure 8.1: ROC curve for the risk scores (Diamond Forrester risk score) when combined with hs-cTnI.

Table 8.4: AUC for Diamond Forrester risk score of CAD when combined with hs-cTnI.

Risk score	AUC	95% CI	P value (AUC >0.5)
Diamond-Forrester risk score.	0.626	0.550 – 0.703	0.003
Diamond-Forrester and hs-cTnI.	0.634	0.558 – 0.710	0.001
Hs-cTnI	0.621	0.538 – 0.703	0.004

We also took another analysis using the McNemar test to compare the sensitivity of the Diamond-Forrester score by itself and Diamond-Forrester with troponin I, and to do that we only included the patients who had sCAD. In Table 8.5 we can see that the p value of the test is 1.000, which means there is no difference in sensitivity between Diamond-Forrester alone and with Troponin I. This also meant that adding troponin did not change anything including the sensitivity. However, when we compared specificity in patients who did not have sCAD, we found that the specificity was reduced when hs-cTnI was added to Diamond-Forester. As shown in Table 8.5, the p value for comparison of specificity was 0.01, which is less than 0.05.

Table 8.5: Results of McNemar test to compare sensitivity and specificity of the Diamond-Forrester score with the combination of hs-cTnI and Diamond-Forrester score.

	P value
Sensitivity	1.000
Specificity	0.016

8.5. Sensitivity analyses: Missing data

By using the imputed dataset, the findings were as below:

- Diamond Forrester risk score: the sensitivity and NPV were slightly increased to 97.1% and 93.1% respectively (Table 10.29). However, this increase did not affect the conclusions, and NPVs were 96.8% and 92.6%. From these findings we can conclude that the missing data does not affect the overall results.
- CAD-C model: As for Diamond Forrester the sensitivity and NPV were also slightly increased by 1.7% and 2% respectively, again this increase did not affect the overall interpretation of the results.
- Estimated probability: the results were the same as the two previous risk scores, there were slight increase in the results, and however, this increase would not affect interpretation of the main findings (Table 10.29).

For Likert probability there was only one missing value and there were only two missing values for Chest pain typicality. Therefore, multiple imputation was not used for these variables. The 2*2 tables and sensitivity and NPV values can be found in the appendix (Table 10.26, Table 10.27, Table 10.28 and Table 10.29)

8.6. Discussion

Previously, in Chapter 6, we have demonstrated that risk scores alone have only moderate diagnostic accuracy for sCAD. In this Chapter, I analysed the diagnostic accuracy of hs-cTnI in combination with the risk scores in patients with suspected sCAD. The results showed significant disparity in sensitivity between each of the risk scores. Chest pain typicality had a sensitivity of 72.3% with NPV of 82.0%, whereas the Diamond-Forrester score had a sensitivity of 96.8% and NPV of 92.6%. These findings demonstrate that chest pain typicality cannot rule-out sCAD as it has low sensitivity and NPV. However, the Diamond-Forrester score had good sensitivity and NPV and may be sufficient to rule-out sCAD.

The rest of the risk scores showed a moderate sensitivity between 86.9, 86.1% and 85.2% with low NPVs of 88.4%, 86.6% and 87.3 receptively. This means that when we combined hs-cTnI with selected risk scores we had insufficient sensitivity to rule-out sCAD.

On logistic regression including both hs-cTnI and risk scores as independent variables, each risk score was found to be a significant predictor of sCAD. The p value of troponin I was low in comparison with Diamond-Forrester score, which did not add new value or identify new patients with sCAD than the Diamond-Forrester score already did.

Previous chapters showed that risk scores or troponin could not rule-out sCAD when they were used alone, however, when they combined together, we had a moderate sensitivity though it was also insufficient to rule-out sCAD. Therefore, this strategy could not be used to reduce the use of imaging in patients with suspected sCAD.

In the systematic review presented in Chapter 2, some studies used hs-cTn in combination with the risk scores. The study by Adamson et al used a combination of hs-cTnI with the CAD consortium model (CAD-C). The CAD-C model includes age, sex and chest pain characteristics. The authors found that when high-sensitivity troponin I was combined with the CAD-C model, overall diagnostic accuracy improved. We can see that the NPV was increased from 94.4 (95% CI 91.6 – 97.1%) to 94.9% (95% CI 92.4 – 97.3%). Moreover, the study showed that chest pain typicality further affected the probability of sCAD, such that patients with values in the highest quintile for CAD-C/hs-cTnI and typical anginal symptoms had very high (>90%) probability of sCAD, potentially 'ruling in' the diagnosis. On the contrary, patients with atypical symptoms with values in the lowest quintile for CAD-C/hs-cTnI had a very low probability of sCAD, which could potentially 'rule-out' the diagnosis (40).

Returning to the finding above we used the same concept of the combination, but we took an additional stride, by looking at the sensitivity and specificity at different cut-offs using the LoD of the troponin assay, which was different from systematic review studies. For example, we used three different cut-offs for troponin I at 2ng/L, 5ng/L and 10ng/L, and then we looked at the risk scores alone and whether the combination is statistically better than just the risk scores alone. However, we showed no added value with hs-cTnI in this study. Indeed, the risk scores alone (Diamond-Forrester and CAD-C model) had good overall accuracy, but this was not improved by adding hs-cTnI.

8.7. Limitations

One key limitation of this work is that there were some missing data for risk scores and hs-cTnI. This is a common limitation of real-world clinical research, where data are collected in busy clinical settings. However, the proportion of missing data was relatively small. Further, I explored the potential impact of missing data on my findings by using multiple imputation. Using the imputed datasets, I could see that the findings did not change in a meaningful way. Therefore, the missing data were unlikely to have affected my conclusions.

8.8. Conclusions

The analysis above showed that when we combined hs-cTnI with selected risk scores, the sensitivity and NPV were marginally improved compared to the use of risk scores alone. However, and showed that it could diagnose patients with sCAD especially with Diamond-Forrester score with a sensitivity of 96.8% and NPV 92.6%.

Chapter 9 : Discussion.

My thesis has focused on the diagnosis of cardiovascular disease (CVD), one of the most common causes of mortality and morbidity around the globe. Specifically, I focused on one of its most important types: stable angina. This happens when the myocardium does not receive enough oxygen due to the narrowing or blockage of the coronary arteries. This type of angina typically causes chest pain or chest discomfort upon physical exertion or emotional stress. The diagnosis of stable angina is suspected based on the patient medical history and confirmed or refuted using imaging investigations, such CT coronary angiography (CTCA).

In the presence of myocardial injury, cardiac troponin is released to the blood stream. This can be detected with high precision using high-sensitivity cardiac troponin (hs-cTn) assays. HS-cTn is used to diagnose acute myocardial infarction (AMI). However, I questioned whether low concentrations of hs-cTn could be used to reduce unnecessary imaging for patients with suspected stable angina.

First, I performed a systematic review of the literature for the available evidence for the use of hs-cTn to diagnose patients with stable angina. I identified 570 papers, of which 7 were identified as relevant to my research question. Three separate hs-cTn assays were identified in these studies, the first was the Abbott Architect hs-cTnI assay. The study conducted by Walter et al used the lowest possible cut-off value of 1.5 ng/L. They found a sensitivity of 97% with 76% NPV. This showed very good sensitivity at this cut-off, however as the post-test probability of sCAD was 24%, the NPV was not high enough. These numbers suggested that at this sensitivity it is possible to detect almost all cases of the condition being investigated, however, with low NPV there will be chance of false negative results. Moreover, in the study by Mueller et al, they implemented a cut-off value of 1.3 ng/L for their test, and they found a sensitivity of 95% with 89% NPV. Again, the sensitivity was high but in return the NPV was not high enough with an 11% chance of patients having sCAD despite a negative test.

The second troponin assay used was Roche hs-cTnT Elecsys. The study by Muller et al used a cut-off of 3ng/L and reported a sensitivity of 95% and a NPV of 88%. On the other hand, the study by Ciwikal et al used a higher cut-off of 6ng/L, which resulted in a lower sensitivity of 71% and a lower NPV of 63%. This implies that patients with negative troponin test results may still have sCAD, and further diagnostic tests should be conducted to rule-out the possibility of sCAD. Orisini et al and Moridson et al also used the Roche hs-cTnT Elecsys assay with cut-off values of 14ng/L and 3ng/L, respectively. However, the sensitivity of both studies was low at 80% and 71%, respectively, and the NPV was also low, at 87.5% for Moridson. The studies showed varying levels of sensitivity and NPV for the Roche hs-cTnT Elecsys troponin assay, depending on the specific cut-off used. Muller et al reported a high sensitivity and NPV at a cut-off of 3ng/L, while Ciwikal et al had lower values for both parameters at a higher cut-off of 6ng/L. Orisini et al and Moridson et al had lower sensitivities and NPVs, suggesting that this troponin type may not be the most reliable option for ruling-out CAD at these specific cut-offs.

The third troponin assay evaluated was Singulex hs-cTnI, and was used by Adamson et al. unfortunately, in this study the sensitivity and specificity were not reported as it was not being used alone, but alongside a risk score.

These troponin assays evaluated in these studies had different levels of sensitivity and NPVs depending on their cut-offs. For all troponin types the sensitivity was improved when they used the lowest possible cut-offs values. However, because the prevalence of sCAD was relatively high in these cohorts, the NPV was still relatively low. This means that there is a relatively high probability of false negative results. On the other hand, when a higher cut-off was used that lead to low sensitivity values. In some instances, NPV increased because more patients were ‘ruled out’, but the number of false negative diagnoses was likely still unacceptable. All these results had demonstrated that hs-cTn

alone cannot rule-out CAD sufficiently, due to low sensitivity and NPV. However, I then questioned whether we could combine hs-cTn with risk scores that are widely used in clinical practice to risk stratify patients with suspected stable angina to achieve higher sensitivity and NPV. In the study by Adamson, they used hs-cTnI in combination with CAD-C risk score, and it showed that the NPV was increased after the combination from 94.4% (95% CI 91.6 – 97.1%) to 94.9% (92.4 – 97.3%). Using both hs-cTn and the CAD-C risk score improved the ability to diagnose CAD and decrease the numbers of false negative results. Similarly, the study by Mouridison et al evaluated the use of hs-cTnT in combination with Exercise stress test (EST). The results of sensitivity and NPV were improved, the results showed a sensitivity at 70.7% and 87.5% NPV of hs-cTnT when used alone. These results were improved when combined with EST, the sensitivity was increased at 85.4% with increased in NPV at 91.3%. Overall, these studies highlighted the benefits of use the available risk scores in combination with hs-cTn assays to improve the diagnosis of CAD.

In the first phase of my work, I added to this body of evidence by evaluating the diagnostic accuracy of hs-cTnT to diagnose patients with stable CAD. The lowest possible cut-off was used in this chapter, which is 3ng/L, the limit of the blank of this assay. For an adjudicated diagnosis of CAD, we found that hs-cTnT at this cutoff had a sensitivity of 82.6% with NPV at 81.0%. Specificity was low at 24.1%. The area under the curve was 0.588, showing poor overall diagnostic accuracy. Thus, the main finding was that hs-cTnT alone at the lowest possible cut-offs could not rule-out CAD. As we increased the cut-off, sensitivity fell further. On the contrary, specificity increased as the cut-off was raised. We had a specificity of 100% at the highest cut-off of 35ng/L, which could enable us to rule-in CAD. This study showed that hs-cTnT was not sufficient to rule-out CAD. Therefore, this suggested that, if hs-cTnT was to be a useful diagnostic marker in this situation, we would need to combine it with other information. For example, hs-cTnT could be used alongside a risk score that has been developed to risk stratify patients with suspected sCAD. If hs-cTnT was used in a low risk patient group, it may still be possible to achieve a satisfactory sensitivity and NPV to rule-out CAD.

Following this Bayesian principle, I moved on to evaluate the accuracy of hs-cTnT with the available risk scores (Diamond-Forester, CAD-C model, Percentage probability and Likert probability). Firstly, I evaluated the accuracy of the risk scores alone to rule-out sCAD. This analysis gave us a moderate sensitivity and NPV. We got a sensitivity of 95.5% for the Diamond-Forrester score with 93.0% NPV, which was the highest between selected risk scores. However, the sensitivity decreased with the other risk scores at 90.4%, 67.1% and 77.3% with 87.5%, 83.7% and 87.7% NPV for CAD-C model, Likert probability and estimated probability respectively. Then, I evaluated the combination of the risk scores plus hs-cTnT for sCAD. By adding in hs-cTnT, the sensitivity for sCAD increased. However, the increase was relatively modest, whereas specificity fell. The combination of hs-cTnT and the Diamond-Forrester risk score gave a sensitivity of 98.6% with 95.8% NPV, again this was the highest between the selected risk scores. This tells us that adding risk scores can increase the value of clinical information and the diagnostic performance of hs-cTnT, although the increase in sensitivity was relatively marginal compared to the use of risk scores alone. Arguably, however, this sensitivity and NPV may be accepted as sufficient to avoid the use of imaging in patients with suspected sCAD.

Though we did not find that hs-cTnT could rule-out sCAD, we know that there are other troponin assays. The hs-cTnI assays detect troponin in more apparently healthy individuals, which may enhance their ability to rule-out sCAD. Therefore, we evaluated a high-sensitivity hs-cTnI assay (Abbott ARCHITECT hs-cTnI). Hs-cTnI assays can detect a very small amount of troponin after a cardiac injury, depending on this principle our expectations was we will have a high sensitivity and NPV to rule-out CAD. I used the lowest possible cut-off of this assay which is 2ng/L LOD, however, the sensitivity and NPV was low at 66.1% and 83.0% respectively. One possible explanation for the lower sensitivity found with hs-cTnI is that those assays do not report results down to the LoB (only the LoD), due to manufacturer's guidelines. With low specificity at 53.2%, we could not rule-in nor rule-out CAD.

Again, as the use of hs-cTnI alone was not sufficient to rule-out CAD, I evaluated the use of hs-cTnI with the available risk scores to rule-out CAD. As for hs-cTnT, I also evaluated the accuracy of the risk scores alone and the accuracy of risk scores plus hs-cTnI. Similar to the findings with hs-TnT, the highest sensitivity and NPV we had were for the Diamond-Forrester risk score at 97.9% and 95.8% respectively. However, the sensitivity decreased for other risk scores as for hs-cTnT. And when I combined the risk scores and hs-cTnI, the sensitivity increased only for the Diamond-Forrester risk score at 98.1% which was highest of the risk scores studied. Moreover, I evaluated whether hs-cTnI and the risk scores gave independent diagnostic information using multivariable analysis and calculating odds ratios, with hs-cTnI and risk scores as independent variables. The logistic regression analysis for the Diamond-Forrester risk score and hs-cTnI showed that, while the Diamond-Forrester risk score was predictive of sCAD (with an odds ratio of 1.013, 95% CI 1.002 – 1.025, p=0.024), hs-cTnI was not (odds ratio 0.993, 95% CI 0.937 – 1.052, p=0.809). This suggests that hs-cTnI does not add new diagnostic information once the Diamond-Forrester risk score has been taken into account. Hs-cTnI alone showed a low sensitivity and NPV, however, when we added the risk scores, we had a marginal increase in sensitivity and NPV. However, I used McNemar's test to compare the sensitivity of the Diamond-Forrester risk score alone versus Diamond-Forrester plus hs-cTnI. This analysis showed that the increase in sensitivity by adding in hs-cTnI was not statistically significant (p=1.000). Again, this suggested that there was little value in adding hs-cTnI once the Diamond-Forrester risk score had been accounted for.

From the all results above hs-cTnT and hs-cTnI alone could not rule-out sCAD alone, as well as when combined with the risk scores. We had a low sensitivity for the troponin alone, and when combined with the risk scores the sensitivity and NPV was moderately increased. This can tell us that using these biomarkers can be not sufficient to rule-out sCAD as definitive diagnosis. Moreover, these biomarkers

also have limitations for ruling in sCAD and could not provide the definitive diagnosis. CAD can be considered a complicated disease that can occur due to multiple factors, and the patients with CAD can present with different symptoms and patterns. They have varying risk factor profiles and comorbidities. This could explain why troponin by itself is not effective to rule-out sCAD.

The combination of hs-cTn with risk scores gave marginal increases in sensitivity, but I found no suggestion that hs-cTn added value once risk scores (particularly the Diamond-Forrester risk score) had been accounted for. From this information the clinicians can upgrade the inclusion and exclusion criteria for the test. These analyses also demonstrated the limitations of currently available risk scores. Only the Diamond-Forrester risk score was able to achieve a sensitivity that came close to ruling out sCAD. Taken together, the findings emphasise that CAD can be a complex disease and there remains an urgent need for further research into novel approaches to rule-out sCAD without requiring the use of imaging.

An important issue that I did not study in this work is that hs-cTn may be used to predict future cardiac events. The study by Rubin et al, looked at the relation between hs-cTnT and multiple risk factors for cardiovascular disease CVD. This study focused on weather hs-cTnT can predict patients with myocardial ischemia and the risk of systolic hypertension between populations. In this study cardiovascular risks were measured such as blood pressure, HbA1C and silent ischemia using the electrocardiogram ECG. The findings showed that hypertension, HbA1C and silent myocardial ischemia were increased, and indicates that hs-cTnT can be a diagnostic marker especially for silent myocardial ischemia. In this study they used a lower cut-off of hs-cTnT at 4.2 ng/L, at this cut-off hs-cTnT was able to predict systolic hypertension which can identify patients at risk of systolic hypertension. In conclusion this study showed that hs-cTnT can be linked with CVD risk factors mentioned in the study (hypertension and silent myocardial ischemia). As the findings indicates that

hs-cTnT can be a biomarker for silent myocardial ischemia and can give a good value in risk stratification for cardiovascular events (79). In my research I focused only on the diagnostic accuracy of hs-cTn as a biomarker for sCAD. The research did not focus on whether hs-cTn can play a role as a predictor of adverse events like MI or death in the future. Potentially, hs-cTn could be used to identify patients who are at very low risk of future adverse events, even if they have anatomical CAD.

Hs-cTnT and hs-cTnI are very sensitive assays and detect cardiac troponin even in the majority of healthy individuals without cardiovascular disease. From that concept the latest evidence suggests that hs-cTnT and hscTnI can estimate cardiovascular events to assist with primary prevention and can add a high value when added to risk stratification modules. This may enable early detection of CVD, using hs-cTn as a marker for risk stratification in healthy individuals without any signs of CVD. The Multi-Ethnic study of atherosclerosis (MESA) illustrates the relationship between hs-cTnT concentrations and cardiovascular events in primary prevention. The study showed the relation between the increase in hs-cTnT and cardiovascular risk events such as stroke. Moreover, in this study they used the combination of hs-cTnT and coronary artery calcium score. This combination showed good value in risk stratification for atherosclerotic cardiovascular disease. This showed that patients with high detectable hs-cTnT concentration and nil coronary artery calcium score had a higher risk of atherosclerotic cardiovascular disease in compared with patients with undetectable hs-cTnT. Overall, the study demonstrated that hs-cTn can be a valuable biomarker for cardiovascular risk events (80). In my thesis I did not look at the risk of future adverse events, however, there is promising research showing that hs-cTn at the baseline could predict patients at very low risk of future adverse events and at that point it is possible only to focus on symptom control in those patients.

9.1. Limitations

As with every study I faced several limitations that affected the progress and the results of my study, these limitations can be illustrated in three points:

1. Missing data: one of the most important limitations I faced was missing data. Despite the best efforts of everyone who participated in the study including me, research nurses and my supervisor, we still experienced missing data, due to different reasons. These reasons included the incomplete data collection during consultations.
2. Incomplete sample size: another important limitation was the inability to complete the originally intended sample size of the study. The study sample size that supposed to be collected was 450 patients. But, unfortunately due to the bankruptcy of the funding company, the recruitment was halted and we were not able to complete the full sample size. We collected only 350 samples between two sites. The incomplete sample size may affected the sensitivity, specificity and the final results of the study.
3. Bankruptcy of the funding company: the most significant limitation that happened to my study was the unanticipated bankruptcy of the funding company. This event made deferent negative impacts to my study. Firstly, it affected the planed timeline of my study resulted in delay of my thesis submission. Secondly, lack of finance, which affected the recruitment of the patients and analysis of the blood samples. Finally, incomplete sample size of the study due to this bankruptcy. However, despite this limitation and the substantial impact of the COVID-19 pandemic (which precluded further recruitment), we were able to complete analyses in stored samples using an alternative hs-cTnI assay.
4.

9.2. Future work

Further research should be conducted evaluating the accuracy of hs-cTn at lower cut-offs in combination with the most promising risk scores (especially the Diamond-Forrester risk score) to rule-out sCAD without imaging. In future, we should also focus on other biomarkers. For example, C-

reactive protein (CRP) is produced by the liver. It has been used as a marker of inflammation and has been shown to predict future cardiovascular events (81).

While this research has not found a role for hs-cTn as a lone biomarker for ruling out sCAD, this future research could give big advantages to patients if they can safely reduce the need for cardiac imaging, unnecessary radiation and high-cost tests.

9.3. Conclusions

In conclusion in this research, I evaluated the accuracy of hs-cTn to diagnose patients with sCAD. Hs-cTn was unable to rule-out patients with sCAD in a single blood test. Even when combined with available patient risk scores, hs-cTn added little value to the risk scores and could not rule-out sCAD.

Chapter 10 : Appendices

Appendix 1

Table 10.1: List of variables collected for the MCAD study

Variable	Type of data
Patient Study number	Numeric
Site of study	Location
Date of recruitment	Date
Age	Numeric Categorical (yes/no)
Gender	Categorical (Male/Female)
Pain (Substernal - Location)	Categorical (yes/no)
Pain (Worse on exertion)	Categorical (yes/no)
Pain (Relived by rest)	Categorical (yes/no)
Presenting complaint	String
Past history of hyperlipidaemia	Categorical (yes/no)
Past history of hypertension	Categorical (yes/no)
Past history of diabetes type 1	Categorical (yes/no)
Past history of diabetes type 2	Categorical (yes/no)
Past history of CVA or TIA	Categorical (yes/no)
Past history of PVD	Categorical (yes/no)
Prior MI	Categorical (yes/no)
Prior PCI/CABG	Categorical (yes/no)
Smoker	Categorical (yes/no)
Past history of heart failure	Categorical (yes/no)
Family history of heart disease	Categorical (yes/no)
Systolic blood pressure	Numeric

Diastolic blood pressure	Numeric
Heart rate	Numeric
Normal resting ECG	Categorical (yes/no)
ST elevation	Categorical (yes/no)
LBBB	Categorical (yes/no)
ST depression	Categorical (yes/no)
Abnormal T inversion	Categorical (yes/no)
Date and time of blood collection	Numeric/date
Stress echo	Categorical (yes/no)
Date of stress echo	Date
Outcome of stress echo	Categorical (Normal-abnormal)
Myocardial perfusion scan	Categorical (yes/no)
Date of MPS	Date
Outcome of MPS	Categorical (Normal-abnormal)
CT angiogram	Categorical (yes/no)
Date of CT	Date
Outcome of CT	Categorical (Normal-abnormal)
Exercise test	Categorical (yes/no)
Date of ETT	Date
Outcome of ETT	Categorical (Normal-abnormal)
Diagnosis	General
Date of diagnosis	Date
Discharge back to GP	Categorical (yes/no)
Discharge to GP date	Date
Discharge to GP outcome	General

Medical management	Categorical (yes/no)
Medical management date	Date
Medical management outcome	General
Revascularisation	Categorical (yes/no)
Revascularisation date	Date
Revascularisation outcome	General
PCI	Categorical (yes/no)
PCI date	Date
PCI outcome	General
CABG	Categorical (yes/no)
CABG date	Date
CABG outcome	General
Date of CRF completion	Date
HS-cTnT_Roche	Numeric

Appendix 2

Table 10.2: EQ-5D-3L list collected for the MCAD study

Mobility	Categorical
Self-care	Categorical
Usual activities	Categorical
Pain/discomfort	Categorical
Anxiety/depression	Categorical
Vas	Numeric

Appendix 3 for high-sensitivity cardiac troponin T (hs-cTnT) with risk scores

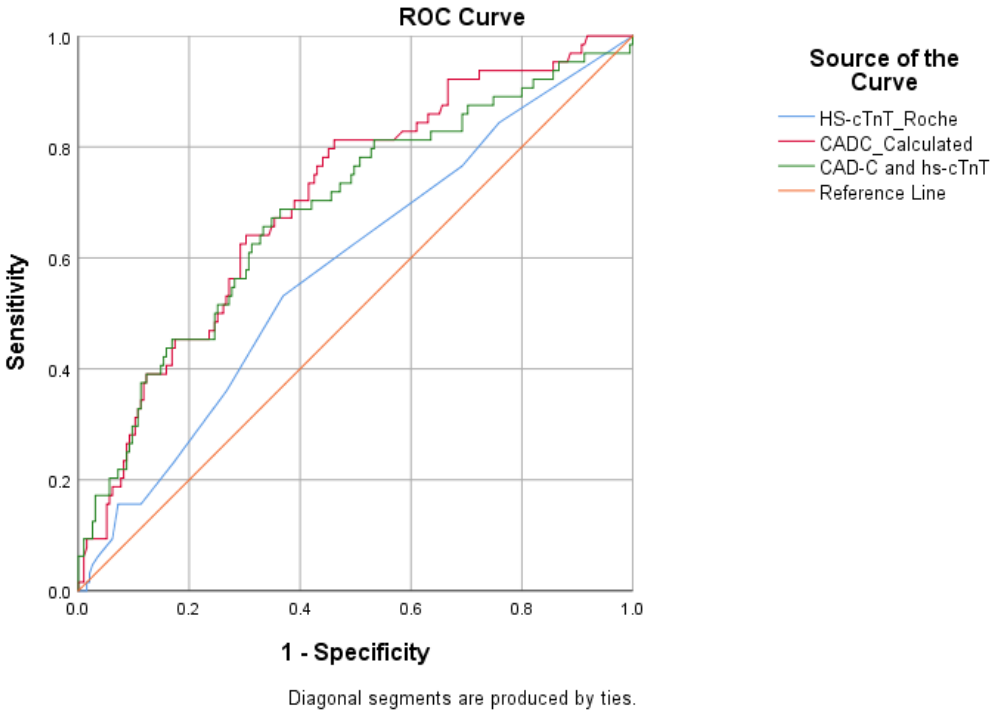

Figure 10.1: ROC curve for the risk scores (CAD-C module) when combined with hs-cTnT.

Table 10.3: AUC for CAD-C module of CAD when combined with hs-cTnT.

Risk score	AUC	95% CI	P value (AUC >0.5)
CAD-C module	0.705	0.633 – 0.777	<0.001
Hs-cTnT Roche	0.581	0.501 – 0.662	0.052
CAD-C and hs-cTnT	0.685	0.607 – 0.763	<0.001

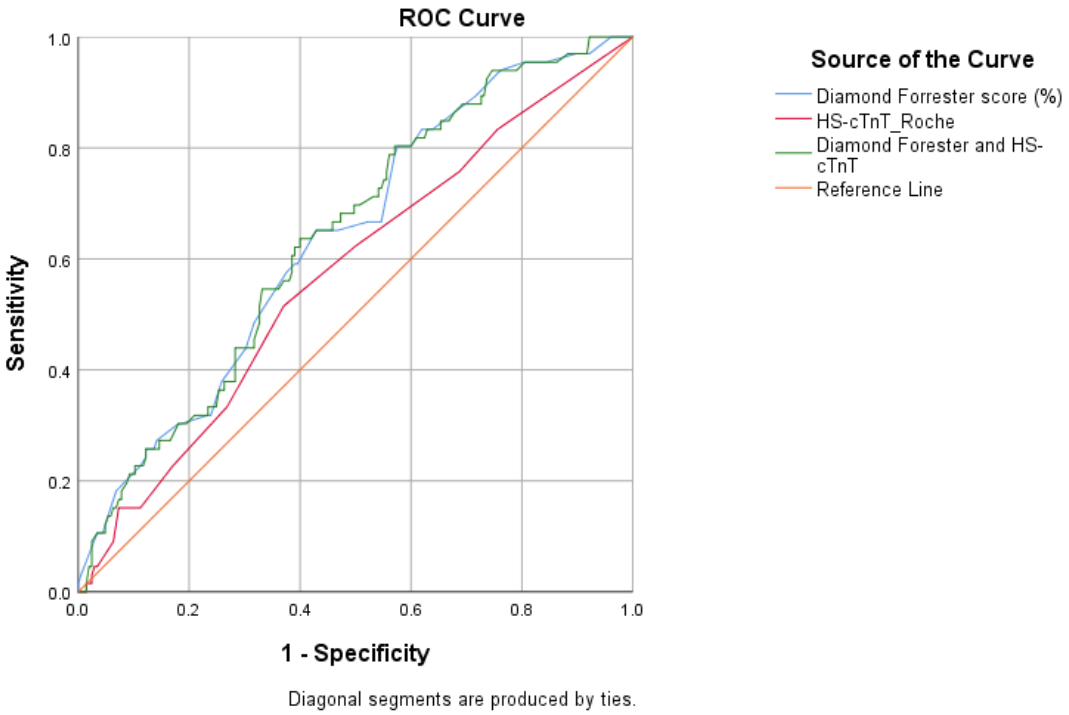

Figure 10.2: ROC curve for the risk scores (Diamond Forrester risk score) when combined with hs-cTnT.

Table 10.4: AUC for Diamond Forrester risk score of CAD when combined with hs-cTnT.

Risk score	AUC	95% CI	P value (AUC >0.5)
Diamond-Forrester risk score	0.637	0.563 – 0.711	0.001
Hs-cTnT Roche	0.573	0.494 – 0.652	0.076
Diamond-Forrester and hs-cTnT	0.640	0.567 – 0.713	0.001

Cross tabulation: Risk scores versus sCAD:

Table 10.5: Risk scores (chest pain) versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
Typical chest pain	20	48
Atypical chest pain	40	116
Non-anginal chest pain	10	51
Missing data	0	1

Table 10.6: Risk scores (Diamond-Forrester risk score) versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
Diamond-Forrester score at least 10% probability	63	166
Diamond-Forrester score less than 10% probability	3	39

Table 10.7: Risk scores (CAD-C module) versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
CAD-C score at least 10% probability	85	113
CAD-C score less than 10% probability	17	48

Table 10.8: Risk scores (Estimated probability) versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
Estimated probability of sCAD at least 10%	51	102
Estimated probability of sCAD less than 10%	15	107

Table 10.9: Risk scores (Likert probability) versus sCAD.

Likert scale	CAD present, n (%)	CAD not present, n (%)
Definite sCAD	3	2
Probable sCAD	12	12
Could be sCAD	32	81
Probably not sCAD	23	115
Definitely not sCAD	0	3

Cross-tabulation: Risk scores + troponin T to 'rule-out' sCAD

Table 10.10: Chest pain plus hs-cTnT versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
Chest pain 'typical' OR hs-cTnT at least 3ng/L	62	177
Chest pain 'atypical' or 'non-anginal' AND hs-cTnT <3ng/L	8	39

Table 10.11: Diamond Forrester risk score plus hs-cTnT versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
Diamond-Forrester score at least 10% probability OR hs-cTnT at least 3ng/L	69	193
Diamond-Forrester score <10% AND hs-cTnT <3ng/L	1	23

Table 10.12: CAD-C module plus hs-cTnT versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
CAD-C score at least 10% probability OR hs-cTnT at least 3ng/L	64	178
CAD-C score <10% AND hs-cTnT <3ng/L	6	37

Table 10.13: Estimated probability plus hs-cTnT versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
Estimated probability of sCAD at least 10% OR hs-cTnT at least 3ng/L	64	176
Estimated probability of sCAD <10% AND hs-cTnT <3ng/L	6	40

Table 10.14: Likert probability plus hs-cTnT versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
Likert scale 'definite', 'probable' or 'could be' sCAD OR hs-cTnT at least 3ng/L	66	184
Likert scale 'probably not' of 'definitely not' sCAD AND hs-cTnT <3ng/L	4	32

Appendix 4 for high-sensitivity cardiac troponin I in combination with risk scores

Table 10.15: The odds ratios for CAD-C module and hs-cTnI.

Variable	Beta coefficient	Odds ratio (95% CI)
CAD-C	3.530	34.138 (5.751 – 202.642)
HS-cTnI	0.013	1.013 (0.953 – 1.077)
Constant	-1.778	0.169

Table 10.16: The odds ratios for Predicted probability and hs-cTnI.

Variable	Beta coefficient	Odds ratio (95% CI)
Estimated probability	0.048	1.049 (1.021 – 1.077)
HS-cTnI	0.003	1.003 (0.941 – 1.068)
Constant	-1.903	0.149

Table 10.17: The odds ratios for Likert Probability and hs-cTnI.

Variable	Beta coefficient	Odds ratio (95% CI)
Likert Probability.	0.717	2.048 (1.729 – 2.425)
Hs-cTnI.	0.006	1.006 (0.983 – 1.030)
Constant.	-3.071	0.046

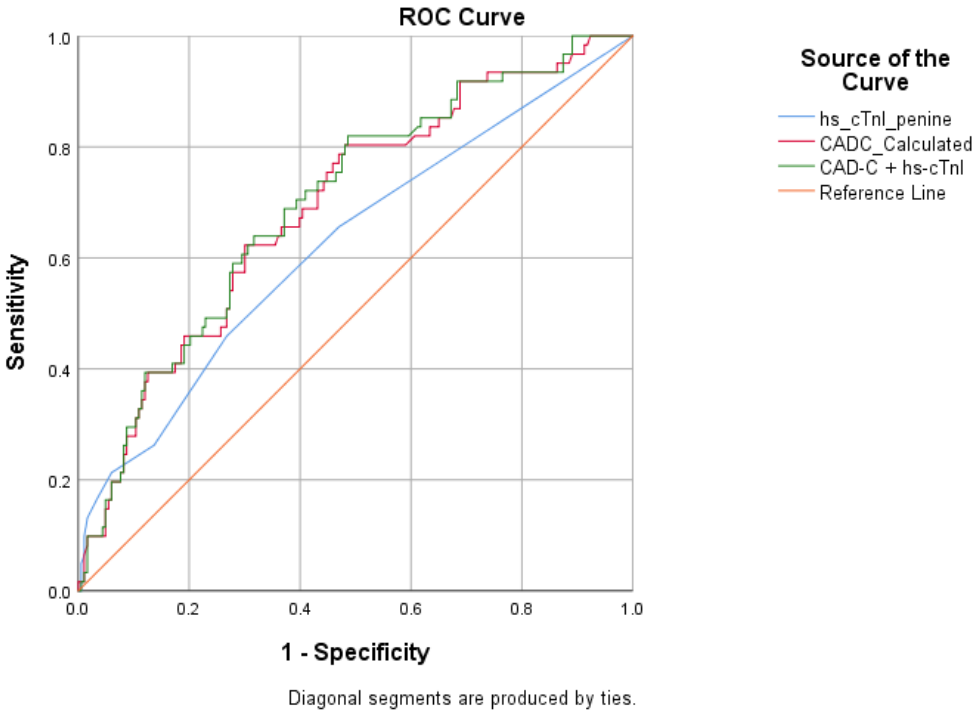

Figure 10.3: ROC curve for the risk scores (CAD-C module) when combined with hs-cTnI.

Table 10.18: AUC for CAD-C module of CAD when combined with hs-cTnI

Risk score	AUC	95% CI	P value (AUC >0.5)
CAD-C model	0.692	0.616 – 0.768	0.000
Hs-cTnI	0.626	0.542 – 0.709	0.003
CAD-C and hs-cTnI	0.700	0.625 – 0.775	0.000

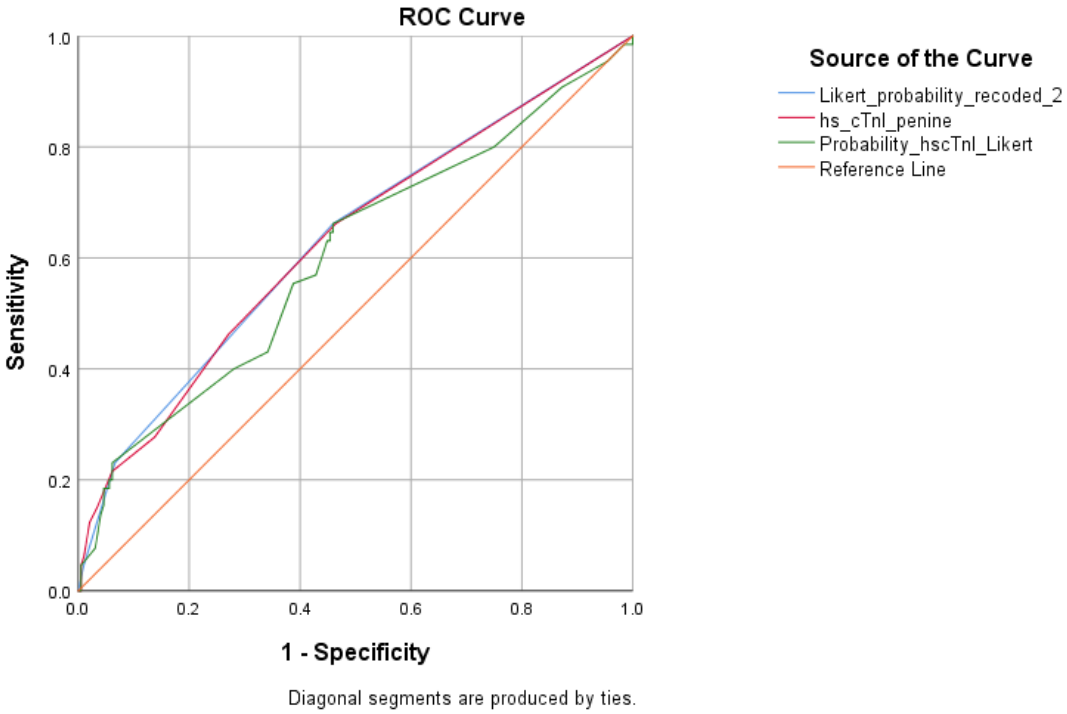

Figure 10.4: ROC curves for the risk scores (Likert Probability) when combined with hs-cTnI.

Table 10.19: AUC for Likert Probability of CAD when combined with hs-cTnI

Risk score	AUC	95% CI	P value (AUC >0.5)
Likert probability of CAD.	0.633	0.600 – 0.666	0.000
Hs-cTnI.	0.630	0.597 – 0.663	0.000
Likert probability and hs-cTnI.	0.603	0.569 – 0.637	0.000

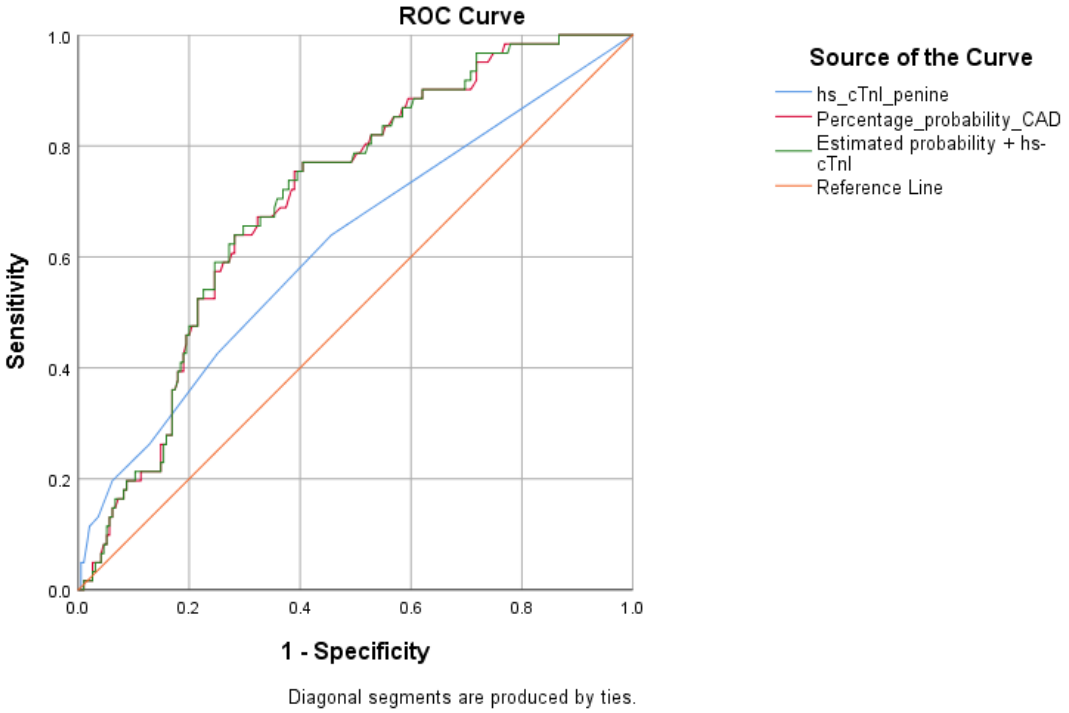

Figure 10.5: ROC curves for the risk scores (Percentage probability) when combined with hs-cTnI.

Table 10.20: AUC for percentage probability of CAD when combined with hs-cTnI

Risk score	AUC	95% CI	P value (AUC >0.5)
Percentage probability of CAD	0.707	0.638 – 0.776	0.000
Hs-cTnI	0.620	0.536 – 0.703	0.005
Percentage probability and hs-cTnI	0.709	0.640 – 0.778	0.000

Cross-tabulation: Risk scores + troponin I to 'rule-out' sCAD

Table 10.21: Chest pain plus hs-cTnI versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
Chest pain 'typical' OR hs-cTnI at least 2ng/L	47	118
Chest pain 'atypical' or 'non-anginal' AND hs-cTnI <2ng/L	18	82

Table 10.22: Diamond Forrester risk score plus hs-cTnI versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
Diamond-Forrester score at least 10% probability OR hs-cTnI at least 2ng/L	60	170
Diamond-Forrester score <10% AND hs-cTnI <2ng/L	2	25

Table 10.23: CAD-C model plus hs-cTnI versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
CAD-C model at least 10% probability OR hs-cTnI at least 2ng/L	53	122
CAD-C model <10% AND hs-cTnI <2ng/L	8	61

Table 10.24: Estimated probability plus hs-cTnI versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
Estimated probability of sCAD at least 10% OR hs-cTnI at least 2ng/L	52	133
Estimated probability of sCAD <10% AND hs-cTnI <2ng/L	9	62

Table 10.25: Likert probability plus hs-cTnI versus sCAD.

	CAD present, n (%)	CAD not present, n (%)
Likert scale 'definite', 'probable' or 'could be' sCAD OR hs-cTnI at least 2ng/L	56	141
Likert scale 'probably not' of 'definitely not' sCAD AND hs-cTnI <2ng/L	9	58

Appendix 5. Multiple imputation – the pooled dataset

Table 10.26: 2x2 table for the combination of Diamond-Forrester plus hs-cTnI versus a diagnosis of sCAD using the pooled imputed dataset (average values across 5 imputed datasets entered).

	CAD present, n (%)	CAD not present, n (%)
Diamond-Forrester >=10% or hs-cTnI >=2ng/L	68	189
Diamond-Forrester <10% and hs-cTnI <2ng/L	2	27

Table 10.27: 2x2 table for the combination of CAD-C model plus hs-cTnI versus a diagnosis of sCAD using the pooled imputed dataset (average values across 5 imputed datasets entered).

	CAD present, n (%)	CAD not present, n (%)
CAD-C model >=10% or hs-cTnI >=2ng/L	62	141
CAD-C model <10% and hs-cTnI <2ng/L	8	75

Table 10.28: 2x2 table for the combination of estimated probability plus hs-cTnI versus a diagnosis of sCAD using the pooled imputed dataset (average values across 5 imputed datasets entered).

	CAD present, n (%)	CAD not present, n (%)
Estimated probability >=10% or hs-cTnI >=2ng/L	61	147
Estimated probability <10% and hs-cTnI <2ng/L	9	69

Table 10.29: Test characteristics for different diagnostic strategies for imputation data.

Rule-out strategy	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Diamond-Forrester score <10% AND hs-cTnI <2ng/L	97.1 (90.1-99.6)	12.5 (8.4-17.7)	26.5 (25.2-27.7)	93.1 (76.7-98.2)	1.11 (1.04-1.18)	0.23 (0.06-0.94
CAD-C model <10% AND hs-TnI <2ng/L	88.6 (78.7-94.9)	34.7 (28.4-41.5)	30.5 (27.9-33.3)	90.4 (82.6-94.9)	1.36 (1.19-1.54)	0.33 (0.17-0.65)
Estimated probability of sCAD <10% AND hs-cTnI <2ng/L	87.1 (77.0-93.9)	31.9 (25.8-38.6)	29.3 (26.7-32.0)	88.5 (80.2-93.6)	1.28 (1.13-1.46)	0.40 (0.21-0.76)

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