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NOVEL APPROACHES TO THE DIAGNOSTIC AND PROGNOSTIC ASSESSMENT OF CORONARY HEART DISEASE

Philip Douglas Adamson

BHB, MB ChB, FRACP

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But there is a disorder of the breast marked with strong and peculiar symptoms, considerable for the kind of danger belonging to it, and not extremely rare, which deserves to be mentioned more at length. The seat of it, and sense of strangling, and anxiety with which it is attended, may make it not improperly be called angina pectoris. They who are afflicted with it, are seized while they are walking, (more especially if it be up hill, and soon after eating) with a painful and most disagreeable sensation in the breast, which seems as if it would extinguish life if it were to increase or to continue; but the moment they stand still, all this uneasiness vanishes.

William Heberden, 1772.
Novel approaches to the diagnostic and prognostic assessment of coronary heart disease

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ABBREVIATIONS

ACC  American College of Cardiology
AHA  American Heart Association
CAD  Coronary artery disease
CADC Coronary Artery Disease Consortium
CI   Confidence interval
CK   Creatine kinase
COPD Chronic obstructive pulmonary disease
CTCA Computed tomography coronary angiography
CV   Coefficient of variation
ESC  European Society of Cardiology
FDG  Fluorodeoxyglucose
HL   Hosmer-Lemeshow
HR   Hazard ratio
IQR  Interquartile range
NICE National institute of clinical excellence
NPV  Negative predictive value
OR   Odds ratio
PET  Positron emission tomography
PROMISE PROspective Multicenter Imaging Study for Evaluation of chest pain
RR   Relative risk
SCOT-HEART Scottish COmputed Tomography of the HEART
SUV  Standardised uptake value
TBR  Target-to-background ratio
TnC  Troponin C
TnI  Troponin I
TnT  Troponin T
URL  Upper reference limit
DECLARATION

This thesis represents research undertaken in the Centre for Cardiovascular Sciences, University of Edinburgh, the Department of Cardiology, Royal Infirmary of Edinburgh.

The SCOT-HEART trial was funded by The Chief Scientist Office of the Scottish Government Health and Social Care Directorates (CZH/4/588), with supplementary awards from Edinburgh and Lothian’s Health Foundation Trust and the Heart Diseases Research Fund. Measurement of high-sensitivity cardiac troponin I concentrations for the studies presented in chapters 5 and 6 was provided free of charge by Singulex and Abbott Diagnostics. The DIAMOND study, from which the data for chapter 7 is derived was undertaken as an investigator sponsored study with funding and provision of ticagrelor from AstraZeneca. These companies were not involved in the study designs or analysis.

I was the primary investigator for the DIAMOND study and was directly responsible for study design, patient recruitment and follow-up, cardiac PET-CT supervision and reporting and analysis of study findings. Documentation regarding trial protocol, patient consent, ethics and regulatory approvals is supplied in the electronic appendix to this thesis. Regarding the clinical trial data utilised for the remainder of the results chapters of this thesis, I was personally involved in the conception, initiation, conduct and data analysis of the sub-studies described within this thesis. In particular, I was responsible for planning and undertaking all statistical analysis described herein. In keeping with the nature of collaborative research, imaging data for the SCOT-HEART
analyses was provided by Professor David Newby, Dr Michelle Williams and Dr Amanda Hunter. Imaging data for the Danish external validation cohort in chapter 5 was provided by Professor Hans Mickley from the University of Southern Denmark. Clinical trial data from the SUMMIT trial presented in chapter 6 was provided by Professor David Newby.

Chapters 1, 3 and 4 are based on work accepted for publication in peer-reviewed journals. The thesis has not been accepted in any previous applications for a degree and all sources of information have been acknowledged. All studies were undertaken in accordance with the regulations of the local Ethics Board within NHS Lothian and with the Declaration of Helsinki of the World Medical Association.

PHILIP D ADAMSON 17th August 2017
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ABSTRACT

BACKGROUND
Cardiovascular disease, principally manifest as myocardial infarction or stroke, is the dominant cause of death worldwide and despite therapeutic advances, the global burden of these conditions continues to increase. In order to address this ongoing disease burden, there is a clear need to more effectively target the use of existing and novel diagnostic investigations and medical therapies. Emerging cardiovascular biomarkers include the biochemical, such as high-sensitivity cardiac troponin, and the radiological, such as computed tomography coronary angiography (CTCA) and 18F-fluoride positron emission tomography (PET). Cardiac troponins can now be reliably quantified in clinically stable or asymptomatic populations and provide information about myocardial pathophysiology, whilst CTCA can non-invasively quantify atherosclerotic burden and 18F-fluoride PET imaging offers insight into plaque vulnerability. Improved targeting of diagnostic investigations requires more reliable estimation of pre-test probability of coronary disease whilst optimizing the use of pharmacological or interventional treatments requires more accurate prognostic stratification. Achieving both objectives in an equitable manner across all population groups will depend upon updated clinical guidelines containing improved risk models and enhanced management pathways.

The objective of this thesis was to investigate the potential clinical benefit of novel approaches to the diagnostic and prognostic assessment of coronary heart disease.
EVALUATION OF THE 2016 NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE (NICE) GUIDANCE ON THE ASSESSMENT OF SUSPECTED STABLE ANGINA.

A post-hoc analysis was undertaken of the Scottish COnputed Tomography of the HEART (SCOT-HEART) trial of 4,146 participants with suspected angina randomised to assessment with computed tomography coronary angiography or standard care. Patients were dichotomised according to guideline definitions into groups representing possible angina and non-anginal presentations. The primary (diagnostic) endpoint was diagnostic certainty of angina at 6 weeks and the prognostic endpoint comprised fatal and non-fatal myocardial infarction.

In 3,770 eligible participants, CTCA increased diagnostic certainty more in those with possible angina (relative risk [RR] 2.22 (95% CI 1.91-2.60), p<0.001) than those with non-anginal symptoms (RR 1.30 (1.11-1.53), p=0.002; pinteraction<0.001). In the possible angina cohort, CTCA did not change rates of invasive angiography (p=0.481) but markedly reduced rates of normal coronary angiography (hazard ratio [HR] 0.32 (0.19-0.52), p<0.001). In the non-anginal cohort, rates of invasive angiography increased (HR 1.82 (1.13-2.92), p=0.014) without reducing rates of normal coronary angiography (HR 0.78 (0.30-2.05), p=0.622). At 3.2 years of follow-up, fatal or non-fatal MI was reduced in patients with possible angina (3.2% to 1.9%; HR 0.58 (0.34-0.99), p=0.045) but not in those with non-anginal symptoms (HR 0.65 (0.25-1.69), p=0.379).

Overall the updated NICE guidance on patient assessment maximises the benefits of CTCA with respect to diagnostic certainty, the use of invasive coronary angiography, and reductions in fatal and non-fatal myocardial infarction. Patients with non-anginal
chest pain derive minimal benefit from CTCA, which instead increases rates of invasive investigation.

**EXTERNAL VALIDATION OF THE PROspective MULTICENTER IMAGING STUDY FOR EVALUATION OF CHEST PAIN (PROMISE) TOOL FOR DETERMINING MINIMAL-RISK OF CORONARY ARTERY DISEASE.**

The PROspective Multicenter Imaging Study for Evaluation of chest pain (PROMISE) minimal-risk tool was recently developed to identify patients with suspected stable angina at very low risk of coronary artery disease and clinical events. The external validity of this tool was investigated within the context of the Scottish Computed Tomography of the HEART multicenter randomised controlled trial of patients with suspected stable angina due to coronary artery disease. Model discrimination and calibration was determined amongst 1,764 patients in whom complete CCTA data were available and compared with the European Society of Cardiology guideline-endorsed Coronary Artery Disease Consortium (CADC) risk score.

The PROMISE minimal-risk tool improved discrimination compared with the CADC model (c-statistic 0.785 vs 0.730, p<0.001) and was improved further following re-estimation of covariate coefficients (c-statistic 0.805, p<0.001). Model calibration was initially poor ($\chi^2$ 197.6, Hosmer-Lemeshow [HL] p<0.001), with significant overestimation of probability of minimal risk, but improved significantly following revision of the PROMISE minimal-risk intercept and covariate coefficients ($\chi^2$ 5.6, HL p=0.692).
**HIGH-SENSITIVITY CARDIAC TROPONIN I IN THE DIAGNOSIS OF STABLE CORONARY ARTERY DISEASE**

In a pre-specified sub-study of the Scottish COMputed Tomography of the Heart trial, plasma cardiac troponin was measured using a high-sensitivity single molecule counting assay in 943 adults with suspected stable angina who had undergone coronary computed tomography angiography. Rates of obstructive coronary artery disease were compared with the pre-test probability determined by the European Society of Cardiology Coronary Artery Disease Consortium risk model with and without cardiac troponin concentrations. External validation was undertaken in an independent study population from Denmark comprising 487 patients with suspected stable angina.

Higher cardiac troponin concentrations were associated with obstructive coronary artery disease with a 5-fold increase across quintiles (9 to 48%, p<0.001) independent of known cardiovascular risk factors (odds ratio [OR] 1.35 [95% confidence interval (CI) 1.25-1.46] per doubling of troponin). Cardiac troponin concentrations improved the discrimination of the ESC model for identifying obstructive coronary artery disease (c-statistic 0.785 to 0.800, p=0.003) and improved classification into ESC-recommended categories of clinical risk (net reclassification improvement 0.143 [95% CI, 0.093-0.193]). The revised model achieved similar improvements in discrimination and net reclassification when applied in the external validation cohort.

**HIGH-SENSITIVITY CARDIAC TROPONIN I IN CARDIOVASCULAR RISK STRATIFICATION OF PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND HEIGHTENED CARDIOVASCULAR RISK.**

The association between plasma high-sensitivity cardiac troponin I concentration and cardiovascular events in patients with chronic obstructive pulmonary disease and heightened cardiovascular risk was examined within the context of a double-blind
randomised controlled trial of inhaled corticosteroids and bronchodilators (1 placebo arm and 3 different treatment arms). Plasma cardiac troponin I concentrations were measured with a high-sensitivity assay in a subgroup of 1,599 patients. The cardiovascular endpoint was a composite of cardiovascular death, myocardial infarction, stroke, unstable angina and transient ischaemic attack during follow-up of 1.5 years.

Baseline plasma cardiac troponin I concentrations were above the lower limit of detection (1.0 ng/L) in 1,559 (97.5%) patients and were unaffected by inhaled therapies at 3 months (p>0.05 for all). Compared with the lowest tertile (cardiac troponin I ≤3.0 ng/L), patients in the highest tertile (≥ 5.5 ng/L) were at greater risk of cardiovascular events (hazard ratio 3.0, 95% confidence interval 1.5 to 6.2, p=0.002) and cardiovascular death (hazard ratio 9.6, 95% confidence interval 2.6 to 35.6, p<0.001) after adjustment for cardiovascular risk factors. There were no differences in COPD exacerbations between tertiles even after adjustment (p>0.05).

**Reproducibility of coronary 18F-fluoride PET-CT imaging**

The inter-observer and scan-rescan reproducibility of coronary 18F-fluoride PET-CT imaging was investigated in 20 patients with clinically stable but high risk multi-vessel coronary artery disease who underwent repeated 18F-fluoride PET-CT scans 11.5±4.5 days apart. Scan analysis using the currently accepted approach of normalisation to a referent coronary segment (TBR\textsubscript{REFERENT}) identified 10 (50%) patients with evidence of focal coronary 18F-fluoride uptake and demonstrated moderate agreement across observers on a per-patient level (κ = 0.56). This was similar to the level of agreement achieved with visual assessment alone (κ = 0.64). Reproducibility was improved by semi-quantitative reporting combining visual assessment with a threshold uptake value
for determining the presence of tracer uptake ($\kappa = 0.84$). Using the optimised approach achieved excellent agreement on overall segmental uptake counts (intra-class correlation = 0.97).

**Conclusion**

Cardiovascular diagnostic and prognostic assessments represent a complex endeavour and established tools for risk prediction can demonstrate suboptimal predictive accuracy when evaluated in patient cohorts that are independent of the population used for model derivation. The process of risk stratification has important potential for improvement through the integration of existing approaches with novel biochemical and non-invasive diagnostic imaging technologies that provide unique insights into structural and pathophysiological processes. The promise such developments hold requires rigorous assessment in well-designed trials involving patients who closely reflect the population most likely to receive treatment. Such trials are difficult and costly to conduct with traditional methods and careful consideration should be given to more pragmatic clinical trial designs.
LAY SUMMARY OF THESIS

Cardiovascular disease, is the dominant cause of death worldwide and despite therapeutic advances, the global burden of this condition continues to increase. In order to address this ongoing disease burden, there is a clear need to more effectively target the use of existing and emerging tests and treatments. The objective of this thesis was to investigate the potential clinical benefit of novel approaches to the diagnostic and prognostic assessment of coronary heart disease. This objective can be divided into three component parts.

The first was to investigate, using the SCOT-HEART study as an exemplar, how pragmatically designed trials, with broad inclusion criteria, can be used to evaluate clinical care processes and robustly validate the findings of traditional randomised trials that typically recruit a highly-selected patient population. In doing so, I tested the diagnostic and prognostic value of the recently updated guidelines from the UK National Institute for Health and Care Excellence when applied within the SCOT-HEART trial population. This analysis identified a number of important benefits arising from the new guidance including enhanced diagnostic certainty, a reduction in inappropriate testing and improved clinical outcomes. I also undertook an external validation study of a recently published risk calculator that was developed in the context of the US-based PROMISE clinical trial population and identified sub-optimal predictive accuracy, highlighting the need to confirm the reliability of such models within the context of the specific population in which they will be applied.
The second objective relates to the potential role of the high-sensitivity cardiac troponin I protein measured on blood tests as a biomarker of coronary disease in clinically stable outpatients. To do this I measured troponin concentrations on blood samples collected from patients within the SCOT-HEART trial and used this information to update and extend an established risk model for determining the pre-test probability of coronary disease in patients being assessed for suspected stable angina. This revised risk calculator, which I also validated in an external study population from Denmark, demonstrated diagnostic benefits over the established risk model which is currently endorsed by clinical guidelines. I also analysed troponin concentrations in a large international clinical trial of chronic obstructive pulmonary disease therapies (SUMMIT) and identified a strong association between troponin concentrations and future cardiovascular risk.

The final aim of this thesis was to determine the reproducibility of coronary imaging with 18F-fluoride PET. To do this I performed repeated PET scans on 20 patients with established coronary artery disease and compared the qualitative and quantitative results obtained when these scans were reported by 3 independent observers. This study demonstrated good overall reproducibility and will provide important information to assist in the ongoing conduct of trials using this imaging technique to stratify cardiovascular risk.
CHAPTER 1

INTRODUCTION

Including adaptations from:


CHAPTER 1  DIAGNOSTIC AND PROGNOSTIC ASSESSMENT OF SUSPECTED STABLE ANGINA INCLUDING THE CURRENT STATUS OF TROPONIN AND IMAGING BIOMARKERS

1.1 SUMMARY

Despite recent advances in testing and treatment, coronary heart disease remains one of the largest causes of morbidity and mortality worldwide. In order to address this ongoing disease burden, there is a clear need to more effectively target the use of existing and novel diagnostic investigations and medical therapies. Improved targeting of diagnostic investigations requires more reliable estimation of pre-test probability of coronary disease whilst optimizing the use of pharmacological or interventional treatments requires more accurate prognostic stratification. Achieving both objectives in an equitable manner across all population groups will depend upon updated clinical guidelines containing improved risk models and enhanced management pathways.

This thesis endeavours to bridge these diverse fields of research by investigating the potential diagnostic and prognostic applications of high-sensitivity cardiac troponin as a circulating biomarker along with 18F-fluoride positron emission tomography as an advanced imaging biomarker of coronary heart disease risk. However, the true promise of such investigations can only be realised if such technologies are demonstrated to work in real-world patient populations and are incorporated into safe and efficient clinical guidelines.
1.2 CLINICAL GUIDELINES IN THE DIAGNOSIS OF SUSPECTED STABLE ANGINA

1.2.1 HISTORICAL ASPECTS IN THE DIAGNOSIS OF ANGINA PECTORIS

In 1768, William Heberden coined the term ‘angina pectoris’ during a seminal presentation to the Royal College of Physicians. Afflicted individuals described being seized whilst walking ‘with a painful and most disagreeable sensation in the breast’, however, ‘the moment they stand still, all this uneasiness vanishes’ (Heberden 1772). It took another 20 years before Edward Jenner first reported an association between these symptoms and the pathological features of coronary artery disease (Bruce Fye 1994). One hundred years later, angina pectoris remained ‘a most inconstant symptom of heart disease’, precluding reliable in vivo diagnosis (Osler 1897). The introduction of the electrocardiogram and reports of exercise associated deviation of the ST segment offered the first objective evidence of inducible myocardial ischaemia (Chaitman 1986), whilst invasive coronary angiography soon became the gold-standard diagnostic investigation for coronary stenosis. However, by the latter half of the 20th century it was widely appreciated that these investigations were not suited to broad application in very low, or high-risk populations due to imperfect sensitivity and specificity. There was a clear need for more reliable methods for identifying intermediate risk patients for whom diagnostic testing is most likely to be informative (Enthoven 1978).

1.2.2 RISK ESTIMATION FOR DETERMINING PRE-TEST PROBABILITY OF CORONARY DISEASE

The first rigorous attempt to create accurate and reproducible estimates of likelihood of obstructive coronary disease was published by Diamond and Forrester in 1979
(Diamond and Forrester 1979). Their report included a risk table incorporating age, sex and typicality of symptoms to determine the pre-test probability of disease (*Table 1.1*) and described how Bayes’ theorem could be applied to refine this estimate once the results of non-invasive testing was available. They subsequently published a clinical classification of chest pain symptoms (Diamond 1983) that remains in use today (*Table 1.2*). The risk models for determining pre-test probability of coronary artery disease have been updated on a number of occasions across the intervening years but remain similar in nature (Chaitman, Bourassa et al. 1981, Genders, Steyerberg et al. 2011, Genders, Steyerberg et al. 2012).

<table>
<thead>
<tr>
<th>AGE, y</th>
<th>NON-ANGINAL CHEST PAIN</th>
<th>ATYPICAL ANGINA</th>
<th>TYPICAL ANGINA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MALE</td>
<td>FEMALE</td>
<td>MALE</td>
</tr>
<tr>
<td>30-39</td>
<td>5.2±0.8</td>
<td>0.8±0.3</td>
<td>21.8±2.4</td>
</tr>
<tr>
<td>40-49</td>
<td>14.1±1.3</td>
<td>2.8±0.7</td>
<td>46.1±1.8</td>
</tr>
<tr>
<td>50-59</td>
<td>21.5±1.7</td>
<td>8.4±1.2</td>
<td>58.9±1.5</td>
</tr>
<tr>
<td>60-69</td>
<td>28.1±1.9</td>
<td>18.6±1.9</td>
<td>67.1±1.3</td>
</tr>
</tbody>
</table>

*Table 1.1 Pre-test likelihood of coronary artery disease in symptomatic patients according to age and sex*

Adapted from (Diamond and Forrester 1979)

Values expressed as % ±1 standard error.

y, years.

<table>
<thead>
<tr>
<th>Typical angina</th>
<th>Meets all three of the following characteristics:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• substernal chest discomfort of characteristic quality and duration;</td>
</tr>
<tr>
<td></td>
<td>• provoked by exertion or emotional stress;</td>
</tr>
<tr>
<td></td>
<td>• relieved by rest and/or nitrates within minutes.</td>
</tr>
</tbody>
</table>

| Atypical angina | Meets two of these characteristics. |

| Non-anginal chest pain | Meets only one or none of these characteristics. |

*Table 1.2 Clinical classification of chest pain*

Adapted from (Diamond 1983)
1.2.3 CURRENT GUIDELINES FOR THE DIAGNOSIS OF STABLE ANGINA

Three of the most widely applied clinical guidelines for the assessment of suspected stable angina are those produced by the American College of Cardiology/American Heart Association (ACC/AHA) (Fihn, Gardin et al. 2012), the European Society of Cardiology (ESC) (European Society of Cardiology Task Force 2013), and the UK National Institute for Health and Care Excellence (NICE) (National Institute for Health and Care Excellence 2016). The published documents have much in common and prior to 2016, all endorsed a risk-based approach, centred on using demographics and clinical features to estimate pre-test probability of coronary disease and defining risk thresholds that determine the appropriateness of further investigations for individual patients. Despite ongoing uncertainty regarding the accuracy of risk estimates and the potential for over or under-investigation of some patient groups, recent studies have demonstrated an association between increasing guideline compliance, reduced diagnostic testing and lower overall expenditure (Ashrafi, Raga et al. 2013, Lee, Michail et al. 2015, Ormerod, Wretham et al. 2015).

In November 2016, the NICE guideline was updated with two important changes made to the recommendations (National Institute for Health and Care Excellence 2016). First, the abolition of the explicit approach to the estimation of pre-test probability with patients now selected for further testing based simply on the description of chest pain or the presence of an abnormal resting electrocardiogram (ECG). Second, driven by technological developments and cost reductions, non-invasive testing for myocardial ischaemia has been replaced with broad indications for computed tomography coronary angiography (CTCA). The consequences arising from these changes have not previously been determined and concerns have already been raised.
that the new strategy will increase use of diagnostic investigations and subsequent coronary revascularisation without improving clinical outcomes (Cremer and Nissen 2017).

1.3 CURRENT STATUS OF HIGH-SENSITIVITY CARDIAC TROPONIN I

1.3.1 INTRODUCTION

Suspected acute coronary syndrome (ACS) represents one of the most common reasons for emergency department visits, accounting for 6-10% of all acute hospital presentations, with over 5 million cases per year in the US alone (Goodacre, Cross et al. 2005, Bhuiya F 2010). Chest pain, however, is a non-specific symptom, with possible aetiologies ranging from the trivial to the life threatening. Acute myocardial infarction (AMI) typically accounts for less than a third of final diagnoses but remains the focus of the initial assessment. In many cases, with the exception of presentations with ST segment elevation on the electrocardiogram (ECG), a firm diagnosis cannot be made reliably at the time of the first patient contact. Consequently, a period of close observation and serial testing is recommended with the principal aim of safely excluding an acute coronary event.

The inherent uncertainty involved in ruling out AMI prompted early recognition of the need for reliable biochemical tests to detect evidence of myocardial injury. Initial candidates, including lactate dehydrogenase (LDH) and aspartate aminotransferase (AST), were non-specific markers of cellular necrosis with a corresponding susceptibility for false positive results (Aldous 2013). Specificity gradually improved with the development of myocyte specific assays, such as myoglobin, creatinine kinase (CK), and its myocardial specific isoenzyme, CKMB. Such tests were soon adopted
into diagnostic guidelines (The Joint International Society and Federation of Cardiology/World Health Organization task force 1979). Unfortunately, it readily became apparent that these biomarkers had limited sensitivity to detect small infarcts, with potentially negative consequences. Beginning in the 1980s, two important developments ushered in a substantial improvement in the biochemical assessment of ACS. The first was the introduction of immunoassays that employed monoclonal antibodies to enable protein detection in low concentrations. The second was the arrival of assays targeting structural cardiomyocyte proteins, in particular, cardiac troponins (Figure 1.1).

1.3.2 Cardiac Troponin

Troponin is the central regulatory complex within striated muscle that modulates myocyte contractile force in response to the intracellular calcium concentration. It comprises three distinct sub-units including a calcium-binding troponin (TnC), a tropomyosin-binding protein (TnT), and an inhibitory troponin (TnI) (Gomes, Potter et al. 2002). Structural differences distinguish the cardiac and skeletal muscle forms of these proteins and have facilitated the development of diagnostic assays with near perfect myocardial specificity (Sharma, Jackson et al. 2004). Rising plasma cardiac troponin concentrations can be detected within hours of the onset of symptoms and typically peak after 12 to 24 hours before gradually falling over 7-10 days. Upon trialling these assays, it was discovered that elevated levels could be detected in as many as a third of suspected ACS cases where the CKMB level was within the normal range and such cases had significant risks for major adverse cardiac events (MACE) (Hamm, Ravkilde et al. 1992, Galvani, Ottani et al. 1997). In recognition of this, the
first Joint European Society of Cardiology/American College of Cardiology consensus document redefining the diagnosis of myocardial infarction recommended that any increase in cardiac troponin above the upper reference limit be reported as an AMI and emphasised the need for serial sampling to ensure a peak level was recorded (The Joint European Society of Cardiology/American College of Cardiology Committee 2000).

The upper reference limit was intended to represent the 99th centile of an apparently healthy population with an acceptable level of imprecision, or coefficient of variation of ≤10%. In reality, the early cardiac troponin assays, had limited sensitivity and fell well short of these analytical requirements. Over the subsequent decade successive generations of assay achieved incremental gains, enabling detection of correspondingly smaller infarcts. Contemporary sensitive assays, as currently marketed in the US, have improved analytical characteristics but continue to fail to meet the guideline recommended standards, with a typical coefficient of variation of 15-20% at the 99th centile (Apple and Collinson 2012).

**Figure 1.1 Timeline of circulating biomarkers in assessment of cardiovascular risk and diagnosis of acute myocardial infarction**

HDL, high-density lipoprotein; Apo, apolipoprotein; WBC, white blood cell; CRP, C-reactive protein; BNP, brain-natriuretic protein; AST, aspartate aminotransferase; CK, creatinine kinase; CK-MB, creatinine kinase – MB isoenzyme.
1.3.3 **HIGH-SENSITIVITY CARDIAC TROPOGIN ASSAYS**

The theoretical benefits of further improving the assay limits of detection (LoD) have recently prompted the emergence of a class of true high-sensitivity troponin assays. These assays have been defined as those able to measure circulating cardiac troponin above the LoD in at least 50% (often approaching 100%) of the healthy population and demonstrate a coefficient of variation at the 99th centile upper reference limit well below 10% (Apple, Ler et al. 2012). Implementation of high-sensitivity cardiac troponin is likely to address a number of unmet needs in the current assessment of suspected ACS. First, it is hoped that a more accurate reference range can be determined, potentially accounting for variations in age, gender or ethnicity. Second, improved accuracy at low concentrations will likely result in lower diagnostic thresholds than those employed by sensitive cardiac troponin assays, allowing more sensitive detection of myocardial injury, which may in turn may enable more appropriate therapeutic decision making. Third, this improved sensitivity may allow the use of differential thresholds, whereby acute myocardial infarction could effectively be ruled-out on the basis of a very low initial troponin concentration, and alternatively ruled-in when the concentration falls above the 99th centile. This would allow safe, early discharge in many patients, requiring only a smaller subset to remain for serial sampling. Fourth, the ability to detect subtle concentration changes over short time intervals may help distinguish an acute cardiac event, from a chronic process, thereby improving diagnostic specificity. Finally, the ability to reliably quantify troponin concentrations within apparently healthy individuals may facilitate the use of troponin as a biomarker of cardiovascular risk in primary or secondary prevention populations.
It is important to note that many high-sensitivity cardiac troponin assays remain in developmental stages and not all have yet received regulatory approval. The two tests employed in the following results chapters include the clinically approved Abbott ARCHITECT\textsuperscript{STAT} high-sensitive cardiac troponin I (hs-cTnI) assay, and the research-specific Singulex Erenna high-sensitivity cardiac troponin I assay.

1.3.4 **Defining a ‘healthy’ reference population**

One of the major advantages of high-sensitivity assays over contemporary-sensitive assays is that troponin concentrations can be quantified in the majority of healthy people thereby enabling more accurate identification of the 99\textsuperscript{th} centile upper reference limit. It has since become apparent that the upper reference limit exhibits substantial variation when different populations are studied and is highly dependent upon the criteria used to determine a disease-free population. Troponin elevation indicates the presence rather than the mechanism of myocardial injury. Established myocardial disease, cardiovascular risk factors and the presence of undiagnosed conditions including atherosclerosis may all increase plasma hs-cTnI concentrations in the absence of acute myocardial infarction (*Figure 1.2*). Even presumably non-pathological factors, such as gender and ethnicity, have been shown to be important contributors. Furthermore, even when troponin concentrations are elevated above the upper reference limit, this can be related to a number of acute or chronic conditions and the third universal definition of myocardial infarction (Thygesen, Alpert et al. 2012) has now created distinct sub-types of myocardial infarction and myocardial injury in recognition of this (*Figure 1.3*).
Figure 1.2 Important considerations when defining a healthy population upper reference limit

MI, myocardial infarction; CHF, congestive heart failure; LVH, left ventricular hypertrophy; DM, diabetes mellitus; HTN, hypertension.
Figure 1.3 Classification of myocardial infarction according to the Third Universal Definition with selected causes of high-sensitivity cardiac troponin concentrations above the 99th centile upper reference limit

Hs-cTnI, high-sensitivity cardiac troponin I; URL, upper reference limit; MI, myocardial infarction.
1.3.5 **HIGH-SENSITIVITY TROPINON I BEYOND MYOCARDIAL INFARCTION**

There is growing interest in the potential role for high-sensitivity troponin testing outside the ACS setting. There is a substantial right-skew to the distribution of troponin I concentrations in the general population and it is increasingly recognized that higher troponin concentrations are related to an array of chronic conditions. Regardless of the cause, higher concentrations of troponin correspond with a worse prognosis (Apple, Steffen et al. 2012, Omland, Pfeffer et al. 2013, Zeller, Tunstall-Pedoe et al. 2014, Everett, Zeller et al. 2015) and could support early implementation of intensive management strategies. Troponin may have a role in the primary or secondary prevention of cardiovascular events in a manner akin to traditional biomarkers of risk such as low-density lipoprotein. Already, high-sensitivity troponin concentrations have been shown to be modifiable with pharmacological agents such as statins (Ford, Shah et al. 2016) or glucose lowering treatments (Januzzi, Butler et al. 2017) and could offer a novel surrogate indicator of treatment efficacy.

It should be emphasized that such uses have yet to gain guideline endorsement and additional research is required before their clinical use is considered. Nevertheless, the future of high-sensitivity troponin testing looks likely to include these non-traditional applications.
1.4 THE VULNERABLE ATHEROSCLEROTIC PLAQUE: IN VIVO IDENTIFICATION AND POTENTIAL THERAPEUTIC AVENUES

1.4.1 INTRODUCTION

Worldwide more than 17 million people die every year from cardiovascular disease (CVD) with this number projected to increase to over 23 million by 2030 (World Health Organisation 2014). Within Europe, CVD results in four million deaths annually, accounting for 47% of all-cause mortality. Between them, heart attacks and strokes are responsible for around 80% of this mortality (Nichols M, Townsend N et al. 2012). The vast majority of acute ischaemic vascular events occur in relation to an underlying atherosclerotic plaque. Plaque rupture is the dominant initiating event, responsible for 60-70% of acute coronary syndromes, whilst plaque erosion is responsible for most of the remainder (Davies 2000, Falk, Nakano et al. 2013). Irrespective of the mechanism, the consequence is exposure of a thrombogenic substrate to circulating blood. This in turn triggers platelet aggregation and the coagulation cascade which compromises vascular blood flow resulting in downstream end-organ ischaemia and infarction. These events occur abruptly and often without warning. Despite intensive therapies, they recur in as many as 25% of patients.

Until recently, the high-risk plaque has only been identifiable retrospectively, predominantly from pathological examination of autopsy specimens. This has limited our ability to appreciate the dynamic nature of plaque vulnerability and rupture, and has placed a heavy reliance on invasive angiography to describe the anatomical luminal stenosis severity rather than plaque biology. Prospective identification of plaque rupture events has suggested that the majority of culprit lesions are non-flow limiting, and often overlooked by angiographic and traditional functional
investigations. Novel imaging techniques now have the potential to identify the pathological structures and processes associated with plaque rupture. This in turn has raised hopes for strategies targeted at modifying the natural history of these lesions and a new era of stratified, or ultimately personalised, medicine may be dawning.

This article aims to provide readers with an overview of the current status of the identification of high-risk atherosclerotic plaque, how emerging investigative technologies have furthered our understanding of the natural history of vulnerability and the implications that this knowledge may pose for established or novel therapeutic strategies. Here, we will principally focus on the process of plaque rupture resulting in coronary and cerebrovascular events.

1.4.2 PLAQUE BIOLOGY

Atherosclerosis describes the process of lipid accumulation and modification within the vascular wall that underlies the development of arterial plaque. It arises from a complex interplay of local factors, such as vascular shear stress patterns and endothelial injury: in addition to systemic mediators, including circulating lipoproteins, hyperglycaemia, environmental exposures and genetic predispositions. Fundamentally, it is a chronic inflammatory disorder governed by cellular and humoral components of the immune system (Packard, Lichtman et al. 2009). Its process can be subdivided into overlapping stages, which may remain clinically silent, or progress to the development of acute or chronic symptoms.

By early adulthood, there is near universal development of adaptive intimal thickening whereby vascular smooth muscle cells (VSMCs) accumulate within the superficial layers of the vessel wall at sites of low endothelial shear stress (Virmani, Kolodgie et
Concomitantly these regions retain low density lipoproteins (LDL) which bind to subendothelial proteoglycans (Tabas, Williams et al. 2007). Enzymatic reactions induce oxidisation of LDL and drive endothelial and VSMC expression of cellular adhesion molecules that promote migration and differentiation of circulating monocytes. The resultant scavenger macrophages in turn, phagocyte lipids and become foam cells within the developing "fatty streak" or xanthoma (Bentzon, Otsuka et al. 2014). Many of these lesions remain dormant or regress whilst others develop an acellular lipid pool (pathological intimal thickening). In some cases a fibroatheroma forms as persistent apoptosis and necrosis of macrophages and VSMCs generates a necrotic core overlain with a collagen-rich surface layer. The direction of atherosclerotic progression reflects the relative balance of certain cellular sub-types of the innate and adaptive immune systems, predominantly macrophages and T helper (T_h) cells respectively. Acting via secreted cytokines, M1-type macrophages and T_h1 lymphocytes promote chronic inflammation, in contrast to mediators released from M2-type macrophages and T_h2 lymphocytes that attempt to pacify this process (Pasterkamp, Schoneveld et al. 2000, Dutta, Courties et al. 2012, Scholtes, Johnson et al. 2012, Moore, Sheedy et al. 2013). Fortunately, in the majority of cases a stable lesion phenotype emerges comprising abundant fibrotic and calcific tissue. Alternatively, on going expansion of the necrotic core and degradation of surface collagen may result in the archetypal high-risk plaque: the thin-cap fibroatheroma (TCFA). The hallmarks associated with vulnerability can be loosely categorised as those either related to the macroscopic structure of the plaque or to the biological processes occurring within it. Indeed, histological and imaging data have consistently demonstrated that culprit plaques responsible for myocardial
Novel approaches to the diagnostic and prognostic assessment of coronary heart disease

Infarction have the following characteristics: a large plaque volume and lipid necrotic core, positive remodelling, peripheral neovascularisation, a thin fibrous cap, microcalcification, intra-plaque haemorrhage and chronic inflammation. Each of these represent a potential imaging target for in vivo identification of high-risk plaques and for guiding subsequent therapeutic modification (Figure 1.4).
Figure 1.4 Imaging targets of plaque vulnerability

Circulating monocytes migrate into early intimal thickening where they phagocytose lipid becoming foam cells and activated macrophages detectable on PET (18F-FDG) and MRI (USPIO). Vascular remodelling can be detected on CT and IVUS imaging prior to luminal stenosis developing. As the lipid core develops this can be detected as low-density signal on CT and IVUS and quantified with NIRS. The resulting hypoxic environment prompts neovascularisation with friable vessels prone to IPH, both of which can be detected on MRI. A necrotic core develops with microvesicles arising from apoptotic macrophages and vascular smooth muscle cells (VSMCs) giving rise to microcalcifications detectable on PET (18F-fluoride) before coalescing into more stable calcific nodules detectable on CT and IVUS. The fibrous cap can be accurately measured with OCT which can also detect plaque rupture and early intraluminal thrombosis. PET, positron emission tomography; FDG, fluorodeoxyglucose; USPIO, ultrasmall superparamagnetic iron oxide; IVUS, intravascular ultrasound; NIRS, near-infrared spectroscopy; IPH, intraplaque haemorrhage; NaF, sodium fluoride; OCT, optical coherence tomography.
1.4.3 **Why the Vulnerable Plaque?**

Before considering how to identify vulnerability, it is important to discuss the benefits of attempting to do so. Indeed, given that the underlying paradigm has been recognised for many decades and has only had modest impact on clinical care, it could be argued that we should abandon this objective in favour of the broader concept of managing the vulnerable patient. In reality, both of these strategies are worthy of pursuit if we are to reduce the morbidity associated with cardiovascular disease. It is important to note that the process of vulnerability is not localised to a single plaque but reflects a high-risk internal milieu. Both autopsy and *in vivo* imaging studies describe two distinct patient cohorts exhibiting contrasting natural histories. Patients with *unstable coronary artery disease* have an underlying pro-inflammatory state, with evidence of pancoronary vulnerability, high rates of coincident vulnerable plaques at baseline and the tendency to develop more over time. This translates into high rates of plaque rupture, more rapid disease progression and a predisposition to myocardial infarction. Meanwhile the opposite appears true for lower-risk individuals with *stable coronary artery disease*, who have little in the way of disease activity, few vulnerable plaques and low event rates (Hong, Mintz et al. 2008, Kubo, Maehara et al. 2010, Zhao, Witzenbichler et al. 2013, Tian, Ren et al. 2014, Vergallo, Ren et al. 2014). In this way, whilst not all vulnerable plaques will go on to cause cardiac events, identification of their widespread presence might more objectively identify vulnerable patients at increased cardiovascular risk.
1.4.4 Imaging the Vulnerable Plaque

On-going delineation of the essential elements of vulnerability has paralleled a corresponding expansion in diagnostic approaches (*Table 1.3, Figure 1.5*). The scope of technologies includes both invasive and non-invasive imaging techniques that can be combined with the use of novel targeted probes to identify structural features or physiological processes of interest. These molecular markers remain largely preclinical but promise further insights into *in vivo* plaque biology. Coupling these tools with additional advances in systemic biomarkers raises the future potential for a stratified population-based approach to risk assessment and management (*Figure 1.6*).
<table>
<thead>
<tr>
<th>Imaging Technique</th>
<th>Clinical feasibility (cost, availability, ease of use etc)</th>
<th>Key imaging targets</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-invasive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasound</td>
<td>+++</td>
<td>cIMT</td>
<td>Carotid imaging only. Conflicting evidence for use as surrogate end-point in drug trials</td>
</tr>
<tr>
<td>CT</td>
<td>+++</td>
<td>Calcification (macroscopic) Plaque volume/composition Remodelling</td>
<td>Widely available</td>
</tr>
<tr>
<td>MRI</td>
<td>++</td>
<td>Neovascularisation and IPH Inflammation (USPIO)</td>
<td>Technically challenging to image coronary plaque</td>
</tr>
<tr>
<td>PET</td>
<td>+</td>
<td>Inflammation (18F-FDG) Microcalcification (18F-fluoride)</td>
<td>Limited availability Short T₁/₂ of tracers necessitates close proximity of cyclotron</td>
</tr>
<tr>
<td>Invasive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary angiography</td>
<td>+++</td>
<td>Stenosis severity</td>
<td>Essential pre-requisite to other invasive imaging tools Can be combined with FFR for assessing coronary physiology</td>
</tr>
<tr>
<td>IVUS</td>
<td>++</td>
<td>Plaque volume/composition Positive remodelling Calcification (macroscopic) Fibrous cap thickness</td>
<td>Prospective studies have demonstrated predictive potential</td>
</tr>
<tr>
<td>OCT</td>
<td>++</td>
<td>Plaque rupture Thrombus</td>
<td>Can differentiate plaque rupture from erosion</td>
</tr>
<tr>
<td>NIRS</td>
<td>+</td>
<td>Lipid core</td>
<td>Predictive of peri-procedural MI Prospective validation study underway</td>
</tr>
</tbody>
</table>

**Table 1.3 Clinically available techniques for imaging features of plaque vulnerability**

CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomography; IVUS, intravascular ultrasound; OCT, optical coherence tomography; NIRS, near-infrared spectroscopy; cIMT, carotid intima-media thickness; USPIO, ultra-small superparamagnetic iron oxide; FDG, fluorodeoxyglucose; FFR, fractional flow reserve; MI, myocardial infarction; T₁/₂, half-life.
Figure 1.5 Features of vulnerability as detected on multiple imaging modalities

Carotid US of a low density plaque with ulcerated surface (arrow) (A). CTCA of a positively remodelled arterial segment with low density plaque and spotty calcification (arrow) (B). MRI of a carotid plaque using IR TFE sequencing showing intraplaque haemorrhage as a hyperintense signal (arrow) with a dark appearance in regions of dense calcification (*) (C). Co-registered PET-CT imaging using 18F-fluoride of a venous bypass graft demonstrating avid binding to microcalcification (arrow) (D). Invasive coronary angiography demonstrating visible intraluminal thrombus (arrow) (E). VH-IVUS of a coronary plaque with large necrotic core (red), spotty calcification (white regions), and sparse fibrous tissue (green regions) (F). Coronary OCT showing a ruptured fibrous cap (*) with adherent luminal thrombus (arrow) (G). NIRS chemogram from a coronary plaque showing a region with high lipid core burden (yellow) (H). US, ultrasound; CTCA, CT coronary angiography; IR TFE, inversion recovery turbo field echo; PET-CT, positron emission tomography-CT; VH-IVUS, virtual histology-intravascular ultrasound; OCT, optical coherence tomography; NIRS, near-infrared spectroscopy.
Figure 1.6 Hierarchical approach to identifying risk

The future of cardiovascular medicine lies in a personalised approach to risk stratification and modification. On a population basis this concept is already well established with widespread uptake of screening using demographics such as age, gender and family history; clinical features including blood pressure (A); and biochemical or metabolic profiling. The potential is now arising to augment this process with higher-risk individuals offered non-invasive imaging (B) to identify subclinical plaque vulnerability that may benefit from further intensification of treatment. Invasive plaque characterisation (C) may similarly, enable tailored therapeutic decision-making in a subgroup of patients who are scheduled for coronary angiography.
1.4.5 **Non-invasive Plaque Imaging**

**Ultrasound**

B-mode ultrasonography is a readily available tool for assessing carotid plaque. Its key role to date has been in the quantification of stenosis severity, which correlates modestly with cerebrovascular clinical events and can help determine the relative merits of revascularisation (North American Symptomatic Carotid Endarterectomy Trial Collaborators 1991, Executive Committee for the Asymptomatic Carotid Atherosclerosis Study 1995, European Carotid Surgery Trialists’ Collaborative Group 1998). Measurement of the carotid plaque thickness (carotid intima-media thickness [cIMT]) is also possible, providing a measure of plaque burden that has been frequently employed as a surrogate end-point in clinical studies. Beyond stenosis and wall thickness, it is possible to use plaque echogenicity to assess tissue composition with echolucent lesions demonstrating features of vulnerability including high lipid content, intraplaque haemorrhage and inflammation (European Carotid Plaque Study Group 1995, Gronholdt, Wiebe et al. 1997, Sannino, Brevetti et al. 2014).

**Computed Tomography**

Computed tomography (CT) angiography provides high spatial and temporal resolution imaging. In addition to describing luminal stenosis, its key strength lies in the capacity to image the vascular wall enabling quantification of atherosclerotic burden and detailed plaque characterisation that compares favourably with intravascular imaging and histology (Leber, Knez et al. 2005, Pundziute, Schuijf et al. 2008, Kashiwagi, Tanaka et al. 2009, Voros, Rinehart et al. 2011, Voros, Rinehart et al. 2011, Boogers, Broersen et al. 2012). CT calcium scoring quantifies the macroscopic calcification in the coronary arteries. Calcification is believed to occur as
a healing response to intense plaque inflammation, reflecting an attempt at sequestration akin to chronic granulomatous disorders such as tuberculosis (Ånestad, Hoel et al. 2001, Johnson and Newby 2009, New and Aikawa 2013). The macroscopic calcium visible on CT (>200 µm) occurs in the latter stages of this process and has been associated with increased plaque stability (Lin, Tintut et al. 2006). Although CT calcium scoring does not identify high-risk plaques directly it does provide a surrogate marker of plaque burden and powerful prediction of cardiovascular events, presumably on the basis that the more plaques a patient has the more likely one is to rupture and cause an event (Detrano, Guerci et al. 2008, Thilo, Gebregziabher et al. 2010). Detecting calcification earlier in its development may improve risk prediction by identifying inflamed plaques that are still in the process of healing. Indeed recent CT-derived evidence supports a much more nuanced role for calcium in plaque rupture with risk inversely related to mineral density (Hutcheson, Maldonado et al. 2014). Microcalcification (5-50 µm in diameter) represents the very earliest stages of this healing process (New and Aikawa 2011, Roijers, Debernardi et al. 2011) and has been consistently associated with high-risk and culprit atherosclerotic plaques. In part this may reflect the residual inflammation in the plaque and in part because finite element analysis has demonstrated that these deposits dramatically amplify tensile stresses within the cap (Kelly-Arnold, Maldonado et al. 2013). A critical window of microcalcification size (5-65 µm) can concentrate sufficient stress to overcome the structural integrity of the fibrous cap (Bluestein, Alemu et al. 2008, Vengrenyuk, Cardoso et al. 2008, Rambhia, Liang et al. 2012, Maldonado, Kelly-Arnold et al. 2013) and therefore directly predispose to rupture.
CT imaging has additionally reinforced our recognition that arterial expansion begins early in plaque development and that substantial plaque growth can be accommodated by this positive remodelling without luminal compromise. Mechanistically it appears that macrophages are central in this process by releasing proteases into the necrotic core that have a similar degradative effect on the arterial wall as they do on the fibrous cap (Pasterkamp, Schoneveld et al. 2000). Experimental arterial models derived from in vivo imaging data have revealed how positive remodelling promotes low endothelial shear stress and high plaque wall stress - both key contributors to vulnerability (Stone, Coskun et al. 2007, Phinikaridou, Hua et al. 2013, Chatzizisis, Antoniadis et al. 2014).

From a clinical perspective, the CT detection of low attenuation plaque within a positively remodelled coronary segment shows clear association with additional features of plaque vulnerability and may act as a surrogate marker for the presence of TCFA (Pundziute, Schuijf et al. 2008, Raffel, Merchant et al. 2008, Voros, Rinehart et al. 2011). In one prospective study the presence of these two features in combination resulted in a 40-fold increase in clinical events (Motoyama, Sarai et al. 2009).

**Magnetic Resonance Imaging**

Magnetic resonance imaging (MRI) avoids the use of ionising radiation, provides excellent soft tissue contrast, and, within the carotid artery can distinguish stable fibroatheromas from those with thin or ruptured caps (Yuan, Zhang et al. 2002). One particularly important attribute is the ability to detect neovascularisation and resultant intra-plaque haemorrhage (IPH). This process occurs within the expanding and increasingly hypoxic necrotic core that causes immature micro-vessels to develop from vaso vasorum. This vascular network is fragile and prone to IPH that in turn provokes further inflammation and plaque growth (Michel, Virmani et al. 2011).
Autopsy studies have suggested a relationship between IPH and clinical events (Kolodgie, Gold et al. 2003) but it has been unclear whether the plaque haemorrhage triggered plaque rupture or vice versa (Davies and Thomas 1985). Prospective serial imaging studies of the carotid circulation with MRI support the former hypothesis and have associated the presence of IPH with an annualised cerebrovascular event rate approaching 20% (Saam, Hetterich et al. 2013, Teng, Sadat et al. 2014).

Assessment of coronary atherosclerosis has previously proven challenging as a result of modest spatial resolution, long scan times and cardiac motion. Attempts to address these issues are on going and, under optimal conditions, it now compares favourably with CT for anatomic detection of coronary stenosis (Hamdan, Asbach et al. 2011, Makowski, Hennigsson et al. 2013). The presence of high-intensity coronary plaque on T1-weighted images corresponds with high-risk plaque features as determined by intra-vascular ultrasound and is similarly predictive of future cardiac events (Kawasaki, Koga et al. 2009, Noguchi, Kawasaki et al. 2014).

Tissue characterisation can be further improved through the use of gadolinium-based contrast media that accumulate in regions of plaque inflammation and a variety of targeted contrast agents are now also under investigation (Yeon, Sabir et al. 2007, Ibrahim, Makowski et al. 2009, Hur, Park et al. 2010). The majority remain preclinical although ferumoxytol, an ultra-small superparamagnetic particle of iron oxide (USPIO), has approval for clinical use and may allow non-invasive detection of tissue macrophages (Weissleder, Nahrendorf et al. 2014).
**Nuclear Imaging (PET and SPECT)**

Single-photon emission computed tomography (SPECT) and positron emission tomography (PET) allow *in vivo* assessment of both structural and physiological features of plaque that may predict vulnerability. Both report the distribution of a chosen tracer with remarkable sensitivity, detecting concentrations in the pico-molar range, creating the ability to develop specific agents with unique targets of interest (Knuuti and Bengel 2008). Until recently both PET and SPECT were hampered by poor spatial resolution (~4 mm for PET, ~10 mm for SPECT). This limitation is being addressed with the introduction of hybrid imaging whereby PET scans can be co-registered with high resolution CT or MRI.

The scope of targeted radiopharmaceuticals is near limitless and is comprehensively described elsewhere (Jaffer and Verjans 2014, Press and Jaffer 2014). With regards to PET tracers, most remain in early development stages except $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) and $^{18}$F-fluoride which have been used for several decades in the oncology setting and more recently adapted to vascular imaging. $^{18}$F-FDG is a radiolabeled glucose analogue that is taken up by metabolically active tissues and indicates increased macrophage activity within plaque (Tarkin, Joshi et al. 2014). Uptake in carotid atherosclerosis is associated with recent cerebrovascular events (Rudd, Warburton et al. 2002), corresponds with high-risk plaque features such as positive remodelling and ulceration (Figueroa, Subramanian et al. 2012), and predicts recurrent ipsilateral events (Marnane, Merwick et al. 2012). Similar uptake can be seen in a wide variety of vascular territories including the coronary arteries (Rudd, Myers et al. 2008). Unfortunately, as a non-specific metabolic marker, coronary imaging is made difficult
by intense uptake in the closely adjacent myocardium that represents a significant challenge to the future coronary application of this tracer (Rogers, Nasir et al. 2010).

\(^{18}\)F- fluoride demonstrates avid binding to hydroxyapatite with minimal myocardial uptake. It was recently discovered to accumulate in a variety of vascular wall locations and represents a unique tool for \textit{in vivo} detection of early active microcalcification below the limits of detection by CT (Derlin, Richter et al. 2010, Irkle, Vesey et al. 2015). In support of this hypothesis are several intriguing studies demonstrating tracer accumulation is not always concordant with CT determined calcification but instead predicts the subsequent development of advanced cardiovascular calcification (Dweck, Chow et al. 2012, Li, Berenji et al. 2012, Dweck, Jenkins et al. 2014). Its potential for identification of vulnerable plaques has been assessed in both peripheral and coronary vessels, and histologically validated with carotid endarterectomy specimens (Derlin, Richter et al. 2010, Dweck, Chow et al. 2012, Joshi, Vesey et al. 2014). In patients with clinically stable coronary disease it predicts the presence of high-risk features on intravascular imaging. Finally, in patients with recent myocardial infarction, it appears to identify accurately the culprit plaque (Joshi, Vesey et al. 2014). Further studies seeking to replicate these findings prospectively in larger, multi-centre cohorts are now underway (NCT02110303, NCT02278211).

1.4.6 \textbf{INTRAVASCULAR IMAGING}

\textbf{CORONARY FLOW DYNAMICS}
Routine revascularisation of stenoses assessed solely by coronary angiography has not been shown to reduce the risk of future cardiovascular events when compared with optimal medical therapy (Boden, O'Rourke et al. 2007). However, augmenting visual
assessment with the use of fractional flow reserve (FFR) determined myocardial ischaemia, does reduce rates of urgent revascularisation, indeed on this basis, the FAME-II trial was terminated early. Yet this trial was similarly unable to demonstrate a reduction in the rate of myocardial infarction (De Bruyne, Fearon et al. 2014), leading many to question whether obstruction to flow in the coronary arteries contributes importantly to the risk of plaque rupture. From a mechanistic perspective it seems plausible that disturbed coronary flow and the consequent changes in shear and mechanical stresses, induced by these FFR positive lesions, would contribute to atherosclerotic progression and plaque rupture. The PREDICTION study, using 3-dimensional vascular profiling, identified the presence of low endothelial shear stress (ESS) distal to the site of maximal stenosis to predict progressive loss of luminal area and increase in plaque burden (Stone, Saito et al. 2012). Furthermore, it appears that plaque-mediated perturbations in shear stress may promote platelet sensitisation, lowering their threshold for activation and potentiating the risk of subsequent thrombosis (Sheriff, Bluestein et al. 2010, Yin, Rouf et al. 2014). Whether such assessments of haemodynamic obstruction can help predict myocardial infarction in isolation or when incorporated into clinical risk prediction models remains unclear and a topic of great clinical relevance.

**INTRAVASCULAR ULTRASOUND**
Perhaps the most comprehensively investigated diagnostic tool for plaque vulnerability is intravascular ultrasound (IVUS). Using an intracoronary catheter, detailed grey-scale images of the vessel wall can be obtained and analysed in real time. More recently, software utilizing spectral analysis of radiofrequency backscatter has been developed to allow crude tissue characterisation. This technique was validated
with *ex vivo* histology and correlates with 4 plaque tissue types: fibrotic, fibro-fatty, calcific and necrotic (Diethrich, Pauliina Margolis et al. 2007, Nair, Margolis et al. 2007, Brugaletta, Cola et al. 2014). Three important prospective studies have sought to determine whether features detected on IVUS might predict plaque-related recurrent events. The strongest predictors were virtual-histology-IVUS determined TCFA, large plaque burden and reduced luminal area but unfortunately these characteristics were too ubiquitous and had insufficient predictive accuracy to be of significant clinical use (Calvert, Obaid et al. 2011, Stone, Maehara et al. 2011, Cheng, Garcia-Garcia et al. 2014).

**Optical Coherence Tomography**

Frequency-domain optical coherence tomography (OCT) makes use of the shorter wavelength of light and this increases spatial resolution by an order of magnitude compared to IVUS. However, this increased resolution is at the cost of reduced tissue penetration (1-3 mm) and the requirement for a blood-free field, usually generated by contrast flushing during imaging (Bezerra, Costa et al. 2009). With an axial resolution of 10-20 µm, OCT has greater sensitivity than IVUS for detecting plaque rupture (Kubo, Imanishi et al. 2007), and, importantly allows *in vivo* diagnosis of plaque erosion as a cause of acute coronary syndromes (Jang, Tearney et al. 2005, Jia, Abtahian et al. 2013, Prati, Uemura et al. 2013, Otsuka, Joner et al. 2014, Ozaki 2014). Plaque characterization is feasible with cholesterol and calcific deposits producing low backscattering signal whilst fibrous tissue produces high backscatter (Otsuka, Joner et al. 2014). It may even be possible to detect macrophage accumulation (Tearney, Yabushita et al. 2003, Tearney, Regar et al. 2012). The greatest advantage of OCT, however, is the ability to determine directly the integrity of the fibrous cap and make
accurate measurements of cap thickness: a vital contributor to plaque vulnerability and potential target in future therapeutic trials (Hattori, Ozaki et al. 2012).

**Near-infrared Spectroscopy**

Spectroscopy is a well-developed tool within analytical chemistry for identifying organic molecules. The underlying principle relates to different molecular bonds absorbing light at specific wavelengths. An intra-coronary imaging catheter is now available that determines coronary plaque composition based on its characteristic spectroscopic signature (Waxman, Ishibashi et al. 2007). It employs the ability of near-infrared light (780-2500 nm wavelength) to ‘see’ through blood and uses an automated algorithm to generate a longitudinal image of the scanned artery known as a ‘chemogram’. A numerical score, known as the lipid core burden index (LCBI), can be calculated. Recently a coronary catheter that combines NIRS and IVUS imaging components has become available allowing both techniques to be performed simultaneously with accurate co-registration of images. Validation studies have been performed *ex vivo* and compared with histology in explanted hearts (Gardner, Tan et al. 2008), and *in vivo* in the Spectroscopic Assessment of Coronary Lipid (SPECTACL) trial (Waxman, Dixon et al. 2009). The ability of NIRS-IVUS hybrid imaging to detect vulnerable plaques is currently being assessed prospectively in the Lipid Rich Plaque (LRP) study (NCT02033694).

Raman spectroscopy is a related technique being investigated for plaque characterisation based on light scatter between molecules creating a unique shift in reflected frequency. It has the potential to determine individual chemicals with greater specificity than NIRS but has been more difficult to implement *in vivo* and it remains a research tool (Puri, Worthley et al. 2011).
1.4.7 **Emerging Techniques**

Two important and inter-related pathways in imaging the vulnerable plaque are in progress. The first of these relates to the development of a broad array of molecular markers targeted at specific characteristics present in high-risk lesions. These probes can be conjugated with tracers that can be detected by existing imaging tools such as PET (Jaffer and Verjans 2014). Secondly there are a number of additional intracoronary imaging modalities under investigation. One of the most interesting of these is near infrared fluorescence (NIRF) that detects, with high sensitivity, fluorochromes coupled with probes that bind to molecular indicators of plaque biology, identifying features such as protease activity or fibrin deposition (Jaffer, Calfon et al. 2011, Hara, Bhayana et al. 2012). NIRF, when combined with either IVUS or OCT, holds the potential to allow simultaneous hybrid structural and functional imaging (Thukkani and Jaffer 2013, Lee, Lee et al. 2014). Such developments, however, remain preclinical to date and are described in more detail elsewhere (Wilensky, Song et al. 2006, Brugaletta, Garcia-Garcia et al. 2012, Zaman, Kosuge et al. 2014).

1.4.8 **What are the clinical implications of identifying the vulnerable plaque?**

Whilst the ability for serial, non-destructive plaque imaging creates the opportunity to better understand plaque biology and the processes by which atherosclerotic lesions develop over time, the ultimate goal of identifying high-risk plaques is to provide accurate event prediction and enable targeted interventions. This could allow more intensive pharmacological strategies in high-risk individuals or potentially procedures that target focal plaque stabilisation. Alternatively, it may obviate the need to use expensive novel therapies in those unlikely to derive benefit thereby optimising use of
limited resources. Although this ideal remains an elusive long-term goal, we believe there are important additional opportunities for detailed plaque characterisation that justify continued research.

**Therapeutic Targeting in Primary and Secondary Prevention**

In addition to encouraging healthy lifestyle modification, consensus guidelines for the management of asymptomatic individuals identified to be at elevated cardiovascular risk frequently recommend pharmacological interventions including anti-platelet agents, as well as blood pressure and cholesterol lowering medications. Such a strategy results in both substantial over and under treatment given most patients would remain asymptomatic in the absence of intervention, and, an important minority continue to have clinical events despite it. A similar dilemma exists in individuals with known, clinically stable vascular disease, or high-risk patients undergoing non-cardiac surgery. These groups are typically already receiving aspirin and statin therapy, and the evidence for further treatment escalation in the absence of ongoing symptoms is currently unclear. It seems plausible that determining the widespread presence or absence of vulnerable plaques in these cohorts may allow incremental gains in patient risk stratification and more rational drug prescribing.

**Improved Efficiency of Drug Development**

Novel cardiovascular medications are continually in development but large scale, long term trials to determine their clinical benefits are costly to perform and create unfortunate delays in their use. Evidence is now emerging that techniques to monitor plaque vulnerability may provide useful surrogate end-points that can inform and focus the findings of later phase III studies. Proof of principle for this concept was seen in two statin studies using $^{18}$F-FDG PET imaging of vascular inflammation that have
shown reductions independent of their effect on LDL (*Figure 1.7*): lending support to the much discussed pleiotropic effects of this drug class and perhaps explaining the benefit of rosvastatin in patients with normal cholesterol concentrations but elevated C-reactive protein (Tahara, Kai et al. 2006, Ridker, Danielson et al. 2008, Ishii, Nishio et al. 2010); although the proposed anti-inflammatory mechanism underpinning this association remains disputed by some (Lodi, Evans et al. 2010, Ferri and Corsini 2014, Oesterle, Laufs et al. 2017). In contrast, inhibitors of pro-inflammatory lipoprotein-associated phospholipase A2, failed to modify PET determined vascular inflammation, which predicted the subsequent failure to improve hard clinical endpoints in a larger clinical trial (O'Donoghue, Braunwald et al. 2014, Tawakol, Singh et al. 2014, White, Held et al. 2014). A similar relationship was seen in a study of dalcetrapib - a cholesteryl ester transfer protein (CETP) inhibitor - where no impact on vascular inflammation was detected pre-empting the neutral findings of the subsequent trial assessing clinical endpoints (Fayad, Mani et al. 2011, Schwartz, Olsson et al. 2012).

The impact that other novel lipid lowering agents may have on plaque stability in humans is unknown, but promisingly, the PCSK9 antibody alirocumab reduced plaque macrophage and necrotic core content whilst increasing collagen in a mouse model of atherosclerosis (Kuhnast, van der Hoorn et al. 2014). It is easy to see how a similar approach using features of coronary plaque vulnerability as surrogate markers of treatment efficacy could be applied to a broad spectrum of pharmacological interventions. Meanwhile it is also worthwhile considering the role of coronary stenting in plaque passivation. The pilot SECRITT trial performed serial OCT imaging of non-obstructive TCFA before and 6 months after stenting and demonstrated an increase in fibrous cap thickness. A similar study using IVUS determined vulnerability
in a larger cohort to guide implantation of an absorbable scaffold is on going (NCT02171065).

Figure 1.7 Effects of simvastatin on 18F-FDG uptake in atherosclerotic plaque inflammation

Representative 18F-FDG PET images at baseline and after 3 months of treatment (post-treatment) with dietary management alone (diet) or simvastatin. (Top) Dietary management alone had no effect on 18F-FDG uptakes (arrows) in the aortic arch and the carotid arteries. (Middle) 18F-FDG uptakes were attenuated by simvastatin treatment. (Bottom) The co-registered images of 18F-FDG PET and CT clearly show that the plaque 18F-FDG uptakes (arrowheads) disappeared after 3-month treatment with simvastatin. PET, positron emission tomography; FDG, fluorodeoxyglucose. Adapted with permission from (Tahara, Kai et al. 2006).
**Optimised Management of Patients with Acute Coronary Syndromes**

The current invasive approach to the management of ACS is to stent the culprit lesion with the intention of relieving luminal obstruction and stabilising the plaque surface. In practice, this strategy is typically driven by the severity of angiographic stenosis, which, particularly in cases of multi-focal lesions, may not always correlate with the true location of culprit plaque rupture. Accurate identification of ruptured or vulnerable plaques however, could be employed to reliably determine the culprit site and additionally guide decisions concerning the optimal interventional strategy for non-culprit lesions. This has particular importance in light of recent trials suggesting patients may benefit from early and complete revascularisation of high-risk bystander disease (Kelly, McCann et al. 2013, Wald, Morris et al. 2013).

Questions also remain around how to manage the 10% of patients with ACS with no obstructive disease identified on angiography (De Ferrari, Fox et al. 2014). The ability to determine if plaque rupture is to blame or an alternative diagnosis, coronary or non-coronary, should be considered has major therapeutic and prognostic implications. The potential benefits of this approach were raised by one report suggesting OCT diagnosed plaque erosion may be safely managed conservatively without coronary stenting (Prati, Uemura et al. 2013).

1.4.9 **Limitations of the Vulnerable Plaque Paradigm**

It is important to recognise that despite the years of research undertaken, our ability to predict with certainty which plaque will result in a future cardiac event remains suboptimal. Three points in particular are worth noting.
PLAQUE VULNERABILITY IS MULTIFACTORIAL
Most investigative tools provide information on a limited number of features of plaque vulnerability. For example, CT angiography can detect the presence of spotty calcification and positive remodelling but cannot measure fibrous cap thickness or describe the inflammatory processes occurring within the necrotic core. Correspondingly, OCT can accurately quantify cap thickness but has limited capacity to describe tissue characteristics deep within the plaque. This in part may explain the ubiquitous nature of these high risk imaging features and the observed low predictive value. Accurately predicting which plaques will rupture or erode is a major challenge that is likely to require hybrid or multi-modal imaging approaches so that multiple high-risk features can be identified simultaneously and the predictive capability improved.

VULNERABLE PLAQUES ARE COMMON, CLINICAL EVENTS (COMPARATIVELY) RARE
In many cases, potentially vulnerable plaques may heal without surface disruption. Additionally, plaque erosion or rupture can occur in the absence of clinical symptoms. Multiple such events may occur concurrently, either in the coronary circulation or elsewhere in the vascular system and the resultant, non-obstructive thrombus is organised and becomes incorporated into the underlying atherosclerotic lesion (Mann and Davies 1999, Davies 2000, Tian, Ren et al. 2014, Vergallo, Ren et al. 2014). An individual plaque focused approach may therefore not be feasible (Arbab-Zadeh and Fuster 2015). However, at the patient level it may be that individuals with the greatest number of vulnerable plaques are statistically more likely to experience a clinical event. Again, we believe an integrative approach is likely to be most successful, perhaps combining such measures of vulnerability/disease activity with assessments
of plaque burden, and haemodynamic obstruction to provide complementary prognostic information. However it should be noted that other non-plaque related factors may also need to be considered including the coagulations status of the blood, environmental exposures and myocardial sensitivity to ischaemia (Newby 2010).

**Predicting Plaque Erosion Remains Elusive**

Plaque erosion events can be determined on OCT as the presence of luminal thrombosis with an intact fibrous cap. Eroded culprit plaques tend to show more advanced stenosis and greater inflammation than non-eroded non-culprit plaques but are less stenotic with fewer macrophages and T lymphocytes, less calcification and thicker caps when compared with ruptured culprit plaques (Farb, Burke et al. 1996, Tian, Vergallo et al. 2014). The underlying lesion morphologies are heterogenous including pathological intimal thickening and fibroatheroma making prospective identification challenging (Yahagi, Otsuka et al. 2015). Given around one third of all ACS are related to plaque erosion, this represents a substantial cohort unaddressed by current approaches to detecting vulnerability although novel molecular techniques targeting myeloperoxidase, an important feature in eroded plaques, are under investigation (Te Boekhorst, van Tilborg et al. 2012).
1.4.10 Conclusion

The vulnerable plaque is the focal manifestation of a systemic process. It directly contributes to the majority of acute cardiovascular events and is consequently responsible for substantial morbidity and mortality. Recent advances in imaging have improved our ability to detect this lesion in vivo and may offer the possibility for prospective identification. Unfortunately, vulnerable plaques are common in relation to clinical events and it remains to be clarified which are the critical determinants of the divergent natural history of these apparently high-risk lesions. Nevertheless, progress is being made and already we have seen tantalising hints of the impact such knowledge may have on patient care. Ultimately it is to be hoped that these advances will enable truly personalised approaches to prognostication and implementation of therapeutic strategies.

1.5 Thesis aims and hypotheses

Although the overarching aim of this thesis is to explore novel approaches to the diagnostic and prognostic assessment of coronary heart disease, this objective can be divided into three component parts. The first is to investigate, using the SCOT-HEART study as an exemplar, how pragmatically designed trials can be used to evaluate clinical care processes and robustly validate the findings of traditional randomised trials. The second relates to the potential role of high-sensitivity cardiac troponin I as a circulating biomarker of coronary atherosclerosis outwith the acute coronary syndrome setting. The final aim is to determine the reproducibility of coronary imaging with 18F-fluoride positron emission tomography and thereby assist
in the ongoing conduct of trials using this imaging technique to stratify cardiovascular risk.

The following hypotheses will be tested:

i. Application of computed tomography coronary angiography in accordance with the symptom-based approach endorsed in the 2016 NICE guideline will improve diagnostic efficiency and clinical outcomes in patients referred for assessment of suspected stable angina (Chapter 3).

ii. The PROMISE minimal-risk tool for assessment of suspected stable angina will provide less accurate risk estimates when applied to the broader range of patients included in the SCOT-HEART trial compared with the derivation cohort in the PROMISE trial (Chapter 4).

iii. High-sensitivity cardiac troponin I, when added to the existing CADC risk model, will improve the estimation of pre-test probability of coronary artery disease and allow better targeting of non-invasive testing in patients with suspected stable angina (Chapter 5).

iv. High-sensitivity cardiac troponin I, when applied to patients with COPD, is of prognostic significance and will predict risk in a manner that is specific to cardiovascular events (Chapter 6).

v. Coronary imaging with 18F-fluoride positron emission tomography is a reproducible imaging technique for the assessment of atherosclerotic microcalcification (Chapter 7).
CHAPTER 2

METHODOLOGY
CHAPTER 2  METHODOLOGY

2.1 SUMMARY

Data for chapters 3, 4, 5 and 6 have been derived from the multicentre, Scottish COmputed Tomography of the HEART (SCOT-HEART) randomised controlled trial involving patients recruited from 12 chest pain clinics across Scotland. Data for the external validation cohort described in chapter 5 is derived from a previously reported cohort study from the Odense University Hospital, Denmark (Hosbond, Diederichsen et al. 2014). Data for chapter 6 were derived from a pre-specified biomarker sub-study of the previously published Study to Understand Mortality and Morbidity (SUMMIT) (Vestbo, Anderson et al. 2013, Vestbo, Anderson et al. 2016), an international, randomised clinical trial. Data for chapter 7 were produced as a pre-specified reproducibility sub-study of the ongoing Dual antiplatelet therapy to Inhibit coronary Atherosclerosis and Myocardial injury in patients with Necrotic high-risk coronary plaque Disease (DIAMOND) prospective randomised controlled trial (ClinicalTrials.gov identifier: NCT02110303) for which I am the principal investigator. Specific study design and methodology have been explained fully in each separate chapter.
2.2 Patient cohorts

2.2.1 SCOT-HEART trial population

The study population of the SCOT-HEART trial has previously been described (The SCOT-HEART investigators 2015). The trial adopted a pragmatic design, aiming for broad inclusion criteria and maximising outcome ascertainment through routinely collected national registry data. Patients who had been referred for assessment of suspected stable angina were recruited from 12 rapid access chest pain clinics across Scotland. Between November 18th 2010 and September 24th 2014, 4,146 participants were enrolled of whom 2,073 were randomly assigned to standard care plus CCTA and 1,778 of these underwent CCTA at one of three sites. The chief investigator for this trial was Professor David Newby.

For the purposes of the analysis in chapter 3, in accordance with the National Institute for Health and Care Excellence (NICE) 2016 guideline concerning the assessment of suspected stable angina, 376 patients were excluded due to an existing history of documented coronary heart disease resulting in a final analysis population of 3,770.

For the purposes of the pre-specified biomarker sub-study, patients who were randomised to the CT intervention arm and were scheduled to undergo this scan at the Clinical Research Imaging Centre at the Royal Infirmary of Edinburgh were approached to consent to the collection of blood samples at the time of their CT scan. Blood samples were obtained from 987 participants at the time of CCTA imaging at a single centre and 943 had plasma cardiac troponin I concentrations measured. CCTA image quality was non-diagnostic in 6 cases resulting in an analysis set comprising 937 participants.
2.2.2 DANISH COHORT WITH SUSPECTED STABLE ANGINA

This previously reported study population (Hosbond, Diederichsen et al. 2014) comprised 487 patients with suspected stable angina who underwent biomarker sampling in addition to coronary imaging at the Odense University Hospital, Denmark. The chief investigator for the original study was Professor Hans Mickley.

2.2.3 SUMMIT TRIAL POPULATION

This previously reported international, multi-centre trial (Vestbo, Anderson et al. 2016) assessed the efficacy and safety of inhaled corticosteroids and long-acting beta agonists in 16,485 patients with COPD and heightened cardiovascular risk. Blood samples were taken prior to randomization from 1,673 patients based in the United States (SUMMIT biomarker population), of which baseline cardiac troponin I concentrations were assessed in 1,599 patients and 1,259 had a second troponin measurement performed 3 months after randomisation. The chief investigator for the original study was Professor Jurgen Vestbo.

2.2.4 DIAMOND TRIAL POPULATION

The study population comprised patients ≥ 40 years of age with established, multi-vessel coronary artery disease that were recruited at least 12 months after any previous acute coronary syndrome or at least 3 months after any coronary revascularisation. Patients were recruited from a coronary angiography database at the Royal Infirmary of Edinburgh, UK and underwent PET-CT imaging in the Clinical Research Imaging Centre at the Royal Infirmary of Edinburgh. From a total recruited population of 220, 20 patients were enrolled in the imaging reproducibility sub-study and underwent a
second PET-CT scan within 2 weeks of the baseline scan. The chief investigator for this study was Professor David Newby.

2.3 NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE GUIDELINES

As investigated in chapter 3, the 2010 and 2016 editions of the National Institute for Health and Care Excellence (NICE) guidelines for the assessment of suspected stable angina (National Institute for Health and Clinical Excellence 2010, National Institute for Health and Care Excellence 2016) were accessed from the NICE website (www.nice.org.uk).

2.4 PROMISE MINIMAL-RISK SCORE

As investigated in chapter 4, the risk score for determination of minimal-risk of coronary artery disease was applied according to the model published by the PROMISE trial group (Fordyce, Douglas et al. 2017).

2.5 EUROPEAN SOCIETY OF CARDIOLOGY GUIDELINE

As described in chapter 5, the 2013 European Society of Cardiology (ESC) guideline for the assessment of suspected stable angina (European Society of Cardiology Task Force 2013) was accessed from the ESC website (www.escardio.org). The details of the Coronary Artery Disease Consortium risk model for the estimation of pre-test probability of obstructive coronary artery disease have been previously published (CAD Consortium 2011).
2.6 **COMPUTED TOMOGRAPHY CORONARY ANGIOGRAPHY**

2.6.1 **SCAN ACQUISITION**

The details of the respective computed tomography (CT) scanning platforms employed are specified in the respective results chapters. All scans were performed using a minimum 64-slice scanner with simultaneous recording of the electrocardiogram (ECG). Oral and intravenous beta-blocking agents were administered to achieve a target heart rate of <60 beats per minute. In most cases, scans were reconstructed using prospective ECG-gated image sets. Retrospective gating was employed where the resting heart rate remained ≥60 beats per minute.

2.6.2 **SCAN ANALYSIS**

Computed tomography images were analysed by 2 trained observers. We have previously assessed the intra-observer and inter-observer reproducibility of 210 randomly selected CT scans from the SCOT-HEART trial and demonstrated excellent agreement for the presence of obstructive disease, non-obstructive disease, or normal coronary arteries (κ = 0.807 [intra-observer] and 0.721 [inter-observer] respectively) (Williams, Golay et al. 2015). Differences in categorisation were resolved by consensus. Coronary artery calcium scoring was performed using dedicated software (VScore, Vital Images, Minnetonka, USA). Agatston score was calculated using a threshold of 130 HU (Hounsfield units) for each vessel and summed to give a total score. The coronary arteries were assessed using a 15-segment model with each segment classified into one of five categories dependent on the degree of luminal cross-sectional area stenosis: normal (<10% stenosis), mild non-obstructive (10-49% stenosis), moderate non-obstructive (50-69% stenosis), obstructive (70-99% stenosis)
or total/sub-total occlusion (100% stenosis). Obstructive coronary artery disease was defined as a luminal cross-sectional area stenosis of ≥70% (approximating to a 50% diameter stenosis) in at least one major epicardial vessel. Using previously described methods, the segment stenosis score (SSS) (Min, Shaw et al. 2007) and CT-adapted Leaman score (de Araujo Goncalves, Garcia-Garcia et al. 2013) were quantified as measures of overall atherosclerotic burden. All image analysis was performed blinded to the biomarker results.

2.7 **HIGH-SENSITIVITY CARDIAC TROPONIN I ASSAYS**

2.7.1 **ABBOTT ARCHITECT STAT HIGH-SENSITIVE TROPONIN I ASSAY**

The ARCHITECT\textsuperscript{Stat} high-sensitive troponin I assay (Abbott Diagnostics, Illinois, USA) is a clinically approved assay able to reliably quantify troponin concentrations in the majority of healthy persons. It has a limit of detection of 1.2 ng/L, a coefficient of variation <10% at 4.7 ng/L and gender-specific 99\textsuperscript{th} centile upper reference limits of 16 and 34 ng/L in women and men respectively (Apple and Collinson 2012, Shah, Griffiths et al. 2015).

The Abbott Diagnostics ARCHITECT\textsuperscript{Stat} high-sensitivity cardiac troponin I assay makes use of the existing ARCHITECT i2000SR and i1000SR analysers, thereby enabling straightforward adoption by clinical laboratories already using these platforms (National Institute for Health and Care Excellence 2014). The specific technique is a proprietary non-competitive, chemiluminescent microparticle immmunoassay (CMIA) known as ChemiFlex\textsuperscript{®}. By employing a 2-step process, with distinct 'capture' and 'detection' antibodies, it reduces the false-positives related to non-specific binding and the potential impact of sample interferents such as human anti-
mouse antibodies (Wild, Sheehan et al. 2013). In the first step the sample is incubated in a buffer solution containing paramagnetic microparticles coated with anti-troponin I antibodies that bind any cardiac troponin I present. The microparticles (and bound cardiac troponin I) can then be magnetically attracted to the wall of the reaction vessel and the sample washed to remove all unbound material. The second step consists of adding a solution of anti-troponin I antibodies conjugated with acridinium. Following a second wash, a pretrigger reagent (hydrogen peroxide) is added causing the label to be released from the solid state. Finally, a trigger agent (sodium hydroxide) causes the acridinium to initiate a light-generating reaction. The resultant luminescence is measured by a photomultiplier tube as reactive light units (RLUs) and the signal generated is directly proportional to the sample cardiac troponin I concentration facilitating for accurate quantification (Figure 2.1). The assay can be performed on plasma or serum samples including those with gel separators. Specimens can be stored at room temperature for up to 8 hours, at 2-8°C for up to 72 hours, and beyond this must be frozen. Any frozen specimens must be thoroughly mixed after thawing to produce a homogenous sample. Although the assay itself can return results in as little as 16 minutes (National Institute for Health and Care Excellence 2014), there are additional sample processing delays to be accounted for. In particular, serum samples must be allowed sufficient time for complete clot formation prior to centrifugation and all specimens should be free of particulate matter.
An appropriately prepared plasma or serum sample is incubated in a solution of magnetic microparticles with surface bound cTnI-antibodies (1). Following an initial wash to remove unbound troponin, soluble cTnI-binding antibodies conjugated with a patented derivative acridinium are added to the test sample (2). A second wash is performed to remove unbound antibodies. An acridinium light-emitting reaction is then initiated by the addition of a pre-trigger and trigger agent (3). The resultant luminescence is directly proportional to the initial cTnI concentration within the initial sample (4).
2.7.2 Singulex Erenna High-Sensitivity Troponin I Assay

The Singulex Erenna Single Molecule Counting™ high-sensitivity troponin I immunoassay has a limit of detection (LoD) of 0.1 ng/L, a limit of quantification (LOQ, co-efficient of variation <10%) of 0.4 ng/L and a 99th centile upper reference limit of 10.9 ng/L (Apple and Collinson 2012, Wu, Estis et al. 2015). The assay process has many similarities with the Abbott ARCHITECT STAT (Figure 2.2). The process begins with incubation of the prepared sample in a solution of microparticles with surface bound cardiac troponin I antibodies and secondary incubation with soluble antibodies. In this case, the soluble antibodies are conjugated with a fluorophore consisting of a chemical that emits light in response to excitation with a specific light frequency. Following an elution phase, the soluble antibody is drawn into a capillary tube and passes in front of a narrow frequency laser that causes the fluorophore to emit intense light flashes. Detected signals with peak intensity above the threshold of background fluorescence are counted as events.
Figure 2.2 Singulex Erenna Single Molecule Counting™ immunoassay

An appropriately prepared plasma or serum sample is incubated in a solution of microparticles with surface bound cTnI-antibodies (1). Following an initial wash to remove unbound troponin, soluble cTnI-binding antibodies conjugated with a fluorophore are added to the test sample (2). A second wash is performed to remove unbound antibodies. During the modified elution step an elution agent is added causing the fluorescent dye-labeled detection antibodies to be released from the immune complexes (3). The eluate is then drawn into a capillary tube, and illuminated by a laser. Single fluorescently labeled molecules are detected as they generate intense flashes of light when passing through the interrogation space. Detected signals with peak intensity above the threshold of background fluorescence are counted as events (4).
2.8 **18F-FLUORIDE POSITRON EMISSION TOMOGRAPHY**

2.8.1 **SCAN ACQUISITION**

Participants were administered a target dose of 250 MBq 18F-fluoride intravenously and subsequently rested in a quiet environment for 60 min prior to scanning. An attenuation correction computed tomography (CT) scan was performed before electrocardiographic (ECG)-gated positron emission tomography of the thorax in list-mode for 30 min within a hybrid scanner (64-multidetector Biograph mCT, Siemens Medical Systems, Erlangen, Germany). This was immediately followed by ECG-gated coronary CT calcium scanning and contrast-enhanced CT coronary angiography (CTCA) during held expiration. To correct for cardiac motion, both positron emission tomography (PET) and CT scans were reconstructed in multiple phases of the cardiac cycle, with the mid-diastolic phase used for analysis.

2.8.2 **SCAN ANALYSIS**

Analysis of the CT images was performed as described previously in this chapter.

Qualitative and quantitative analysis of the PET images from all 40 scans was performed independently by 3 trained observers using an OsiriX workstation (OsiriX version 3.5.1 64-bit; OsiriX Imaging Software, Geneva, Switzerland). Images were anonymised and presented to the observers randomly with separate blinded study codes used for initial and repeat scans. Careful co-registration of PET and CT images was undertaken prior to image analysis using residual blood pool 18F-fluoride activity on the PET scan to align with contrast enhanced CT images of the cardiac chambers. Scans were reported on both a per-patient and per-segment basis. Coronary assessment
began with visual inspection of tracer localisation to confirm its origin from within the coronary artery, that uptake followed the course of the coronary artery in 3 dimensions and to exclude signal arising from nearby structures such as the aortic valve or mitral valve annulus. Quantitative PET analysis was undertaken of all proximal coronary segments in addition to any atherosclerotic segments with suspected focal 18F-fluoride tracer uptake. Measurements were performed by drawing a volume of interest encompassing the site of maximal tracer uptake and the maximum standardised uptake value (SUV\(_{\text{MAX}}\)) recorded. The peak SUV (SUV\(_{\text{PEAK}}\)), defined as the mean SUV for all voxels within 5mm of the hottest voxel, was also recorded. Finally, as previously recommended (Dweck, Chow et al. 2012, Joshi, Vesey et al. 2014), a referent plaque without visual tracer uptake was also measured to enable semi-quantitative categorisation of plaques as 18F-fluoride positive lesions (those with visual tracer localisation and SUV\(_{\text{MAX}}\) at least 25% greater than that measured within a proximal reference lesion). Measurement of blood pool activity was undertaken as previously described (Pawade, Cartlidge et al. 2016) with elliptical regions of interest drawn within the brachiocephalic vein, superior vena cava, the interventricular septum, and all 4 cardiac chambers with mean and maximum SUV recorded for each region. Coronary SUV\(_{\text{MAX}}\) and SUV\(_{\text{PEAK}}\) were divided by the blood pool SUV\(_{\text{MEAN}}\) to calculate coronary target to background ratios (TBR\(_{\text{MAX}}\), TBR\(_{\text{PEAK}}\) respectively), or the coronary SUV\(_{\text{MAX}}\) was divided by the referent plaque SUV\(_{\text{MAX}}\) (TBR\(_{\text{REFERENT}}\)).
2.9 Data linkage and electronic patient records

Chapters 3, 4 and 5 comprise patient cohorts within Scotland. As such, all patients have an existing, unique Community Health Index (CHI) number. The Community Health Index number is a population register containing details of all Scottish residents registered with a General Practitioner and was used for linkage to regional and national registries provided by the Information and Statistics Division of the National Health Service (NHS) Scotland. Within Scotland, this has previously been demonstrated as a robust approach to clinical trial endpoint identification (Barry, Dinnett et al. 2013), including outcomes such as recurrent hospitalisation, cardiovascular procedures, myocardial infarction and death.

2.10 Ethical considerations

All patients for whom data has been included in the following results chapters provided written informed consent prior to involvement in the respective studies. The details of the research governance and ethical oversight are outlined in the relevant results chapters.

2.11 Statistical analysis

The details of the statistical methods, including statistical modelling, are outlined in the respective results chapters. Due to significant positive skew, troponin concentrations were log transformed prior to incorporation in models. Unpaired categorical data were assessed by chi-square test and paired data with the McNemar test. Continuous data were examined using parametric and non-parametric data. Statistical significance was assigned at p-value <0.05.
CHAPTER 3

EVALUATION OF THE 2016 NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE GUIDANCE ON THE ASSESSMENT OF SUSPECTED STABLE ANGINA

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CHAPTER 3  EVALUATION OF THE 2016 NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE GUIDANCE ON THE ASSESSMENT OF SUSPECTED STABLE ANGINA

3.1  SUMMARY

OBJECTIVES:
To evaluate the diagnostic and prognostic benefits of computed tomography coronary angiography (CTCA) using the 2016 National Institute for Health and Care Excellence (NICE) guidelines for the assessment of suspected stable angina.

METHODS
Post-hoc analysis of the Scottish COmputed Tomography of the HEART (SCOT-HEART) trial of 4,146 participants with suspected angina randomised to CTCA. Patients were dichotomised into NICE guideline-defined possible angina and non-anginal presentations. Primary (diagnostic) endpoint was diagnostic certainty of angina at 6 weeks and prognostic endpoint comprised fatal and non-fatal myocardial infarction (MI).

RESULTS
In 3,770 eligible participants, CTCA increased diagnostic certainty more in those with possible angina (relative risk [RR] 2.22 (95% CI 1.91-2.60), p<0.001) than those with non-anginal symptoms (RR 1.30 (1.11-1.53), p=0.002; \( p_{interaction} < 0.001 \)). In the possible angina cohort, CTCA did not change rates of invasive angiography (p=0.481) but markedly reduced rates of normal coronary angiography (hazard ratio [HR] 0.32 (0.19-0.52), p<0.001). In the non-anginal cohort, rates of invasive angiography increased (HR 1.82 (1.13-2.92), p=0.014) without reducing rates of normal coronary
angiography (HR 0.78 (0.30-2.05), p=0.622). At 3.2 years of follow-up, fatal or non-fatal MI was reduced in patients with possible angina (3.2% to 1.9%; HR 0.58 (0.34-0.99), p=0.045) but not in those with non-anginal symptoms (HR 0.65 (0.25-1.69), p=0.379).

CONCLUSION
NICE-guided patient selection maximises the benefits of CTCA on diagnostic certainty, use of invasive coronary angiography, and reductions in fatal and non-fatal myocardial infarction. Patients with non-anginal chest pain derive minimal benefit from CTCA and increase the rates of invasive investigation.
3.2 INTRODUCTION

Chest pain is a common symptom within the community and is responsible for at least 1% of all presentations to general practitioners (Ruigomez, Rodriguez et al. 2006, Frese, Mahlmeister et al. 2016). It is frequently a cause of concern for patients and clinicians alike, with both eager to identify or exclude potentially serious underlying conditions. Although stable coronary heart disease is responsible for only 10% of such presentations (Bosner, Becker et al. 2009), the resources required to exclude this diagnosis have important public health implications. Clearly it is in the interests of all parties to develop an efficient and effective strategy for the assessment and management of these symptoms.

In response to this clinical need, the National Institute of Health and Care Excellence (NICE) first published an innovative guideline (CG95) on the assessment of chest pain of recent onset in 2010 (National Institute for Health and Clinical Excellence 2010). This publication encouraged a systematic approach to determining the pre-test probability of coronary heart disease using routinely available clinical features. Furthermore, it established explicit thresholds of risk, below which additional investigation for coronary disease was regarded as unnecessary and unhelpful. These changes were met with initial scepticism related to the potential for increased costs (Ghosh, Qasim et al. 2012), underestimation of disease prevalence (Patterson, Nicol et al. 2011) and frequent pathway non-adherence arising from the unacceptability of discharging low risk patients without further investigation (Hoey 2011). Fortunately, the ensuing years have allayed many of these concerns with more recent studies demonstrating an association between increasing guideline compliance, reduced
diagnostic testing and lower overall expenditure (Ashrafi, Raga et al. 2013, Lee, Michail et al. 2015, Ormerod, Wretham et al. 2015).

In November 2016, the NICE guideline was updated with two important changes made to the recommendations (National Institute for Health and Care Excellence 2016). First, the abolition of the explicit approach to the estimation of pre-test probability with patients now selected for further testing based simply on the description of chest pain or the presence of an abnormal resting electrocardiogram (ECG). Second, driven by technological developments and cost reductions, non-invasive testing for myocardial ischaemia has been replaced with broad indications for computed tomography coronary angiography (CTCA). However, concerns have already been raised that this new strategy has not been adequately assessed and should be tested in a clinical trial (Cremer and Nissen 2017). We therefore aimed to determine the diagnostic and prognostic implications of these changes to the assessment of patients presenting with stable chest pain of recent onset using the Scottish COmputed Tomography of the HEART (SCOT-HEART) trial dataset.
3.3 METHODS

3.3.1 STUDY DESIGN AND POPULATION

The SCOT-HEART study is a prospective, multi-centre, randomised controlled trial investigating the role of CTCA in patients referred to a specialist clinic with suspected angina due to coronary heart disease. The study design (The SCOT-HEART investigators 2012) and principal findings (The SCOT-HEART investigators 2015) have previously been reported. The study population comprised individuals without a documented history of prior coronary heart disease referred for assessment of suspected stable angina of recent onset who were randomised 1:1 to CTCA plus standard care or standard care alone. Participants were recruited from 12 cardiology chest pain clinics across Scotland and those randomised to the intervention arm underwent CTCA imaging at one of 3 sites in addition to routine clinical assessment. The main exclusion criteria related inability to undergo CTCA due to renal failure (estimated glomerular filtration rate <30 mL/min), major allergy to iodinated contrast media, or known pregnancy. Recruitment began November 18, 2010 and follow-up of clinical events is ongoing with planned reporting of five-year outcomes in early 2018. The study was performed in accordance with the Declaration of Helsinki and with research ethics committee approval.

Chest pain symptoms were defined as typical angina, atypical angina or non-anginal according to established criteria (Table 3.1) (National Institute for Health and Care Excellence 2016). An abnormal resting electrocardiogram was determined by the presence of any of the following: pathological Q waves; left bundle branch block; or either ST segment or T wave abnormalities. As per the 2016 NICE guideline
recommendations, participants were categorised into two groups: those with non-
anginal chest pain and a normal ECG (non-anginal cohort), and those with either
 typical or atypical chest pain, or non-anginal chest pain and an abnormal ECG
(possible angina cohort).

Pre-test probability of coronary disease was estimated according to the 2010 NICE
guidelines (National Institute for Health and Clinical Excellence 2010) and 10-year
cardiovascular risk was calculated with the ASSIGN score, a validated Scottish
cardiovascular risk score that incorporates social deprivation (Woodward, Brindle et
al. 2007).

<table>
<thead>
<tr>
<th>(1) Clinical classification of chest pain symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical angina</td>
</tr>
<tr>
<td>Meets all three of the following characteristics:</td>
</tr>
<tr>
<td>• constricting discomfort in the front of the chest,</td>
</tr>
<tr>
<td>or in the neck, shoulders, jaw, or arms</td>
</tr>
<tr>
<td>• precipitated by physical exertion</td>
</tr>
<tr>
<td>• relieved by rest or GTN within about 5 minutes</td>
</tr>
<tr>
<td>Atypical angina</td>
</tr>
<tr>
<td>Meets two of these characteristics</td>
</tr>
<tr>
<td>Non-anginal chest pain</td>
</tr>
<tr>
<td>Meets only one or none of these characteristics</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(2) Resting ECG interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal ECG</td>
</tr>
<tr>
<td>Any of the following changes:</td>
</tr>
<tr>
<td>• pathological Q waves</td>
</tr>
<tr>
<td>• LBBB</td>
</tr>
<tr>
<td>• ST-segment and T wave abnormalities</td>
</tr>
<tr>
<td>Normal ECG</td>
</tr>
<tr>
<td>None of the above changes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(3) NICE 2016 diagnostic cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possible angina</td>
</tr>
<tr>
<td>Meets any of the following criteria:</td>
</tr>
<tr>
<td>• Typical or atypical angina</td>
</tr>
<tr>
<td>• Non-anginal chest pain and an ABNORMAL</td>
</tr>
<tr>
<td>resting ECG</td>
</tr>
<tr>
<td>Non-anginal</td>
</tr>
<tr>
<td>Non-anginal chest pain and a NORMAL resting ECG</td>
</tr>
</tbody>
</table>

Table 3.1 Classification of diagnostic cohorts according to the revised NICE
guideline

GTN, glyceryl trinitrate; ECG, electrocardiogram; NICE, national institute for health and care
excellence.
3.3.2 **Computed Tomography Coronary Angiography**

Participants underwent coronary artery calcium scoring and CTCA using either a 64-detector row scanner (Brilliance 64, Philips Medical Systems, Netherlands, and Biograph mCT, Siemens, Germany) or a 320-detector row scanner (Aquilion One, Toshiba Medical Systems, Nasushiobara, Japan). Computed tomography images were analysed by 2 trained observers with excellent reproducibility (Williams, Golay et al. 2015) and differences in categorisation were resolved by consensus. A complete description of the image analysis procedure has been previously published (The SCOT-HEART investigators 2012, The SCOT-HEART investigators 2015). For the purposes of this study, prognostically important coronary heart disease was defined as either of the following: ≥50% stenosis in the left main stem, or ≥70% stenosis in all three major epicardial vessels (Yusuf, Zucker et al. 1994).

3.3.3 **Outcomes**

The primary (diagnostic) endpoint was clinician certainty (yes/no versus unlikely/probable) in the diagnosis of angina secondary to coronary heart disease at 6 weeks. The prognostic end-point for this study was a composite of fatal and non-fatal myocardial infarction. Additional secondary endpoints included the requirement for invasive coronary angiography, changes in clinician prescribing of cardiovascular pharmacotherapy, coronary revascularisation, all-cause death and non-fatal stroke.

Outcome data were updated on 29th June 2016 and were identified via record linkage from regional and national registries provided by the Information and Statistics Division of the National Health Service (NHS) Scotland and when appropriate, confirmed by review of patient health records. Within Scotland, this has previously
been demonstrated as a robust approach to clinical trial endpoint identification (Barry, Dinnett et al. 2013). Categorisation for analysis was performed whilst masked to randomised allocation.

### 3.3.4 Statistical analysis

Statistical analysis was performed using R version 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria). All analyses were post-hoc and were performed stratified by study cohort and according to intention-to-treat, irrespective of compliance with scanning. Diagnostic endpoints were analysed using log-binomial regression (Pocock, Assmann et al. 2002, McNutt, Wu et al. 2003) or log-Poisson regression employing robust variance estimates for analysis of those secondary endpoints where log-binomial regression failed to converge (Yelland, Salter et al. 2011). For ease of interpretability, results are reported as the relative risk with 95% confidence intervals and p-value (Knol, Le Cessie et al. 2012, Grant 2014). The diagnosis of coronary heart disease, and angina due to coronary heart disease (diagnostic endpoints) were assessed for certainty (yes/no versus unlikely/probable) and change within these four categories. Clinical outcome endpoints were analysed with Cox regression, and reported as hazard ratios with cumulative incidence plots constructed. In addition to these stratum specific analyses, we modelled interaction terms for allocation and study cohort to provide hypothesis testing for interaction on the relative scale. All primary and secondary endpoints are reported after adjustment for the minimisation variables of age, sex, body-mass index, diabetes mellitus and atrial fibrillation. Data are presented as mean ± standard deviation or mean differences with 95% confidence intervals. Statistical significance was taken as two-sided p<0.05.
3.4 RESULTS

3.4.1 DATA COLLECTION AND STUDY POPULATION

The study population of the SCOT-HEART trial has previously been described (The SCOT-HEART investigators 2015). Between 18\textsuperscript{th} November 2010 and 24\textsuperscript{th} September 2014, 4,146 participants were recruited of whom 4 patients had incomplete description of their chest pain symptoms recorded and were excluded from the analysis. As recommended in the updated NICE guidelines, a further 372 participants were excluded from the primary analysis due to a documented history of prior coronary heart disease. The median duration of follow-up was 3.2 years (interquartile range [IQR], 2.5 to 4.1). In total 1,884 were randomly assigned to standard care and 1,886 to standard care plus CTCA. Of these, 3 participants allocated to standard care and 1,616 within the standard care plus CTCA arms underwent CTCA at one of three sites (Figure 3.1).

The mean age of the participants was 56.6±9.7 years and 1,721 (45.6\%) were women. Overall, 1,447 (38.3\%) of participants had non-anginal symptoms and a normal ECG whilst 2,323 (61.6\%) participants had symptoms or ECG changes consistent with possible angina. The non-anginal cohort were typically younger and had fewer cardiovascular risk factors than those with possible angina (Table 3.2).
Figure 3.1 Consort Diagram

CTCA, computed tomography coronary angiography
### Table 3.2 Baseline characteristics

Data are mean (SD) or value (%); BMI, body mass index; CVD, cerebrovascular disease; PVD, peripheral vascular disease; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; ECG, electrocardiogram; CHD, coronary heart disease; PTP, pre-test probability.

*ASSIGN Score (see http://assign-score.com/)

<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>Non-anginal</th>
<th>Possible angina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard care</td>
<td>CT Intervention</td>
<td>Standard care</td>
</tr>
<tr>
<td>n</td>
<td>3770</td>
<td>735</td>
<td>712</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.62 (9.73)</td>
<td>53.47 (9.68)</td>
<td>54.37 (9.67)</td>
</tr>
<tr>
<td>Male</td>
<td>2049 (54.4)</td>
<td>405 (55.1)</td>
<td>373 (52.4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.69 (5.97)</td>
<td>29.45 (6.38)</td>
<td>29.60 (6.28)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1211 (32.1)</td>
<td>172 (23.4)</td>
<td>190 (26.7)</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>2078 (55.1)</td>
<td>275 (37.4)</td>
<td>305 (42.8)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>370 (9.8)</td>
<td>52 (7.1)</td>
<td>63 (8.8)</td>
</tr>
<tr>
<td>Smoking habit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>1182 (31.4)</td>
<td>198 (26.9)</td>
<td>203 (28.5)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>772 (20.5)</td>
<td>176 (23.9)</td>
<td>159 (22.3)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>76 (2.0)</td>
<td>11 (1.5)</td>
<td>13 (1.8)</td>
</tr>
<tr>
<td>Previous CVD</td>
<td>123 (3.3)</td>
<td>12 (1.6)</td>
<td>25 (3.5)</td>
</tr>
<tr>
<td>Previous PVD</td>
<td>42 (1.1)</td>
<td>3 (0.4)</td>
<td>12 (1.7)</td>
</tr>
<tr>
<td>Family history</td>
<td>1558 (41.3)</td>
<td>285 (38.8)</td>
<td>295 (41.4)</td>
</tr>
<tr>
<td>Antiplatelet agent</td>
<td>1662 (44.1)</td>
<td>126 (17.1)</td>
<td>133 (18.5)</td>
</tr>
<tr>
<td>Statin</td>
<td>1459 (38.7)</td>
<td>121 (16.5)</td>
<td>132 (18.5)</td>
</tr>
<tr>
<td>Beta-blockade</td>
<td>786 (20.8)</td>
<td>46 (6.3)</td>
<td>65 (9.1)</td>
</tr>
<tr>
<td>ACE inhibitor or ARB</td>
<td>497 (13.2)</td>
<td>69 (9.4)</td>
<td>67 (9.4)</td>
</tr>
<tr>
<td>Chest pain symptoms</td>
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<td></td>
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<tr>
<td>Non-anginal</td>
<td>1616 (42.9)</td>
<td>735 (100.0)</td>
<td>712 (100.0)</td>
</tr>
<tr>
<td>Atypical angina</td>
<td>893 (23.7)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Typical angina</td>
<td>1261 (33.4)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Abnormal resting ECG</td>
<td>512 (13.6)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Exercise ECG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>2047 (54.3)</td>
<td>544 (74.0)</td>
<td>529 (74.3)</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>505 (13.4)</td>
<td>40 (5.4)</td>
<td>47 (6.6)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>460 (12.2)</td>
<td>14 (1.9)</td>
<td>8 (1.1)</td>
</tr>
<tr>
<td>Baseline diagnosis of CHD</td>
<td>1619 (42.9)</td>
<td>69 (9.4)</td>
<td>82 (11.5)</td>
</tr>
<tr>
<td>Baseline diagnosis of angina</td>
<td>1246 (33.1)</td>
<td>9 (1.2)</td>
<td>8 (1.1)</td>
</tr>
<tr>
<td>Predicted 10-year CHD risk*</td>
<td>17.08 (11.57)</td>
<td>13.60 (10.11)</td>
<td>14.99 (10.37)</td>
</tr>
<tr>
<td>Estimated PTP of CHD (NICE 2010)</td>
<td>17.08 (11.57)</td>
<td>13.60 (10.11)</td>
<td>14.99 (10.37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;10%</td>
<td>412 (10.9)</td>
<td>173 (23.5)</td>
</tr>
<tr>
<td></td>
<td>10-29%</td>
<td>717 (19.0)</td>
<td>255 (34.7)</td>
</tr>
<tr>
<td></td>
<td>30-59%</td>
<td>997 (26.4)</td>
<td>232 (31.6)</td>
</tr>
<tr>
<td></td>
<td>60-89%</td>
<td>942 (25.0)</td>
<td>75 (10.2)</td>
</tr>
<tr>
<td></td>
<td>&gt;90%</td>
<td>702 (18.6)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>
### 3.4.2 Computed Tomography Coronary Angiography

Compared with the non-anginal cohort, participants with possible angina were more likely to have obstructive coronary disease identified on CTCA (29.7% versus 9.5%; RR 2.81, 95% CI 2.15 to 3.68, p<0.001) and less likely to have normal coronary arteries (33.1% versus 50.1%; relative risk [RR] 0.74, 95% confidence interval [CI] 0.66 to 0.83, p<0.001) (Table 3.3). The pre-test probability assessment recommended in the 2010 NICE guidelines substantially overestimated the risk of obstructive coronary disease (Table 3.4) whilst the revised diagnostic cohorts demonstrated persistent heterogeneity in risk by age and sex (Table 3.5). The average rate of the primary diagnostic and prognostic endpoints for both men and women is presented in Table 3.6 and additional, non-coronary findings made on CTCA are reported in Table 3.7.

<table>
<thead>
<tr>
<th></th>
<th>Non-anginal</th>
<th>Possible angina</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>592</td>
<td>1027</td>
</tr>
<tr>
<td><strong>Coronary calcium score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (&lt;100 AU)</td>
<td>478 (80.7)</td>
<td>646 (63.0)</td>
</tr>
<tr>
<td>Medium (100-400 AU)</td>
<td>68 (11.5)</td>
<td>197 (19.2)</td>
</tr>
<tr>
<td>High (&gt;400 AU)</td>
<td>46 (7.8)</td>
<td>183 (17.8)</td>
</tr>
<tr>
<td><strong>CTCA findings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>295 (50.0)</td>
<td>339 (33.3)</td>
</tr>
<tr>
<td>Mild (&lt;50%)</td>
<td>158 (26.8)</td>
<td>195 (19.1)</td>
</tr>
<tr>
<td>Moderate (50-70%)</td>
<td>81 (13.7)</td>
<td>182 (17.9)</td>
</tr>
<tr>
<td>Obstructive</td>
<td>56 (9.5)</td>
<td>303 (29.7)</td>
</tr>
<tr>
<td>Prognostic CHD</td>
<td>8 (1.4)</td>
<td>86 (8.4)</td>
</tr>
</tbody>
</table>

**Table 3.3 Findings of CTCA**

AU, Agatston units; CTCA, computed tomography coronary angiography; CHD, coronary heart disease.
Table 3.4 CTCA findings according to previous or revised diagnostic classification cohorts

PTP, pre-test probability; CHD, coronary heart disease; NICE, National Institute of Health and Care Excellence; CTCA, computed tomography coronary angiography.

Table 3.5 Predicted risk of CHD within each diagnostic cohort according to age and gender

CHD, coronary heart disease.

Table 3.6 Average rate of the primary diagnostic and prognostic endpoints for the study population

MI, myocardial infarction.
## Table 3.7 Non-coronary findings on CTCA according to diagnostic cohort

Data are value (%). CTCA, computed tomography coronary angiography.

<table>
<thead>
<tr>
<th></th>
<th>Non-anginal</th>
<th>Possible Angina</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1447</td>
<td>2323</td>
</tr>
<tr>
<td>Non-coronary cardiac findings, %</td>
<td>133 (22.5)</td>
<td>312 (30.3)</td>
</tr>
<tr>
<td>Aortic valve calcification, %</td>
<td>29 (2.0)</td>
<td>99 (4.3)</td>
</tr>
<tr>
<td>Mitral valve calcification, %</td>
<td>5 (0.3)</td>
<td>22 (0.9)</td>
</tr>
<tr>
<td>Dilated left ventricle, %</td>
<td>11 (0.8)</td>
<td>17 (0.7)</td>
</tr>
<tr>
<td>Dilated right ventricle, %</td>
<td>8 (0.6)</td>
<td>4 (0.2)</td>
</tr>
<tr>
<td>Dilated left atrium, %</td>
<td>16 (1.1)</td>
<td>45 (1.9)</td>
</tr>
<tr>
<td>Dilated right atrium, %</td>
<td>4 (0.3)</td>
<td>7 (0.3)</td>
</tr>
<tr>
<td>Left ventricular wall thinning, %</td>
<td>2 (0.1)</td>
<td>12 (0.5)</td>
</tr>
<tr>
<td>Left ventricular hypertrophy, %</td>
<td>15 (1.0)</td>
<td>51 (2.2)</td>
</tr>
<tr>
<td>Hypertrophic obstructive cardiomyopathy, %</td>
<td>2 (0.1)</td>
<td>2 (0.1)</td>
</tr>
<tr>
<td>Pericardial disease, %</td>
<td>0 (0.0)</td>
<td>2 (0.1)</td>
</tr>
<tr>
<td>Pulmonary hypertension, %</td>
<td>0 (0.0)</td>
<td>1 (0.0)</td>
</tr>
<tr>
<td>Other, %</td>
<td>71 (4.9)</td>
<td>143 (6.2)</td>
</tr>
<tr>
<td>Non-cardiac findings, %</td>
<td>187 (31.5)</td>
<td>427 (41.5)</td>
</tr>
<tr>
<td>Pulmonary mass, %</td>
<td>45 (3.1)</td>
<td>122 (5.3)</td>
</tr>
<tr>
<td>Mediastinal mass, %</td>
<td>1 (0.1)</td>
<td>4 (0.2)</td>
</tr>
<tr>
<td>Lymphadenopathy, %</td>
<td>4 (0.3)</td>
<td>7 (0.3)</td>
</tr>
<tr>
<td>Pulmonary embolism, %</td>
<td>1 (0.1)</td>
<td>4 (0.2)</td>
</tr>
<tr>
<td>Parenchymal lung disease, %</td>
<td>70 (4.8)</td>
<td>184 (7.9)</td>
</tr>
<tr>
<td>Emphysema, %</td>
<td>45 (3.1)</td>
<td>95 (4.1)</td>
</tr>
<tr>
<td>Pulmonary fibrosis, %</td>
<td>1 (0.1)</td>
<td>6 (0.3)</td>
</tr>
<tr>
<td>Other parenchymal disease, %</td>
<td>27 (1.9)</td>
<td>90 (3.9)</td>
</tr>
<tr>
<td>Pleural disease, %</td>
<td>7 (0.5)</td>
<td>21 (0.9)</td>
</tr>
<tr>
<td>Hiatus hernia, %</td>
<td>43 (3.0)</td>
<td>79 (3.4)</td>
</tr>
<tr>
<td>Liver pathology, %</td>
<td>20 (1.4)</td>
<td>19 (0.8)</td>
</tr>
<tr>
<td>Other, %</td>
<td>36 (2.5)</td>
<td>88 (3.8)</td>
</tr>
</tbody>
</table>
3.4.3 Diagnostic certainty and additional investigations

The use of CTCA increased the certainty with which a diagnosis of angina was made (Figure 3.2A). This benefit was greatest (cohort interaction: p<0.001) in those with possible angina where the proportion of participants with a certain diagnosis of angina at 6 weeks was 34.9% with CTCA and 15.7% with standard care (RR 2.22, 95% CI 1.91 to 2.60, p<0.001). The improvement in diagnostic certainty remained, albeit attenuated, in the non-anginal cohort (CTCA 32%, standard care 25.2%; RR 1.30, 95% CI 1.11 to 1.53, p=0.002). The use of CTCA was associated with a change in diagnosis in 341 (29.0%) participants with possible angina and 120 (16.9%) participants with non-anginal symptoms. These improvements were associated with treatment changes in 26.8% of those with possible angina and 19.4% in those with non-anginal chest pain.

The use of CTCA was associated with an increase in new requests for invasive coronary angiography at 6 weeks in both the possible angina (71 [6.0%] versus 7 [0.6%]) and the non-anginal (12 [1.7%] versus 0 [0.0%]) groups. Overall, CTCA only increased the total number of angiograms performed during the complete follow-up period in the non-anginal cohort (6.6% versus 3.7%; HR 1.82, 95% CI 1.13 to 2.92, p=0.014), with no change in the possible angina cohort (30.2% versus 32.1%; HR 0.95, 95% CI 0.82 to 1.10, p=0.481) (Figure 3.2A). In participants with possible angina, CTCA was associated with a reduced likelihood of the invasive angiogram revealing normal coronary arteries (RR 0.32; 95% CI 0.19 to 0.52, p<0.001) and an increased likelihood of identifying obstructive disease (RR 1.18; 95% CI 1.07 to 1.32, p=0.002). In contrast, invasive angiography performed in the non-anginal cohort demonstrated similar rates of normal arteries (RR 0.78; 95% CI 0.30 to 2.05, p=0.622) and
obstructive coronary disease (RR 0.82; 95% CI 0.50 to 1.34, p=0.422) in both treatment arms (Figure 3.2B).

Figure 3.2 Clinical impact of CTCA according to NICE diagnostic group
Diagnostic certainty, pharmacotherapeutic changes and effect on invasive angiography with standard care (blue) or standard care plus CTCA (red) according to diagnostic cohort. CHD, coronary heart disease; CTCA, computed tomography coronary angiography.
3.4.4 Clinical outcomes

During follow-up, 18 (1.2%) and 59 (2.5%) of participants experienced a fatal or non-fatal myocardial infarction in the non-anginal and possible angina groups respectively (Table 3.3). Allocation to standard care with CTCA reduced the likelihood of this endpoint in the cohort with possible angina from 3.2% to 1.9% (hazard ratio [HR] 0.58; 95% CI 0.34 to 0.99; p = 0.045, Figure 3.3). This was predominantly related to a reduction in non-fatal myocardial infarction from 3.0% to 1.6% (HR 0.55, 95% 0.31 to 0.96; p = 0.034). Although a similar effect size was seen in those allocated to CTCA in the non-anginal cohort, the confidence interval was wide, reflecting the lower event rate and thus did not achieve statistical significance (HR 0.65; 95% CI 0.25 to 1.69; p = 0.379, Figure 3.3). The treatment-group interaction p-value was 0.836.

The use of CTCA was not associated with an increase in coronary revascularisation in either the possible angina (18.7% versus 16.5%; HR 1.16, 95% CI 0.95 to 1.41, p=0.140) or non-anginal cohorts (2.2% versus 1.9%; HR 1.20, 95% CI 0.59 to 2.46, p=0.619).
Figure 3.3 Cumulative event curves for fatal and non-fatal myocardial infarction

Possible angina (solid lines) and non-anginal (dashed lines) cohorts in patients assigned to standard care (blue) and standard care plus CTCA (red).

HR, hazard ratio; CI, confidence interval.
3.5 **DISCUSSION**

We have applied the updated 2016 NICE guideline criteria to a prior large multicentre randomised controlled trial population. We have demonstrated that the selective investigation of patients with possible angina produced the greatest absolute benefits in terms of diagnostic certainty, use of invasive angiography, targeting of therapies and ultimately improving clinical outcome. In contrast, CTCA was not associated with a significant improvement in outcomes in patients with non-anginal symptoms and a normal resting ECG despite nearly doubling rates of invasive coronary angiography. These findings provide robust evidence to support the diagnostic strategy recommended within the new NICE guidelines.

This study has five notable strengths. First, the study participants were recruited from an unselected patient population referred to 12 chest pain clinics across Scotland and thereby accurately reflect the target cohort of the new guidelines. Second, as participants were allocated to CTCA in a randomised manner regardless of the typicality of chest pain symptoms, we minimised the potential for case ascertainment bias. Third, by not dictating the use of additional investigations in the standard care arm, we have focussed on the effect of CTCA on clinically significant outcomes rather than comparing the diagnostic accuracy of different imaging modalities. Fourth, all scans were performed on CT scanners meeting or exceeding the guideline technological requirements and were reported in accordance with the recommended definitions for obstructive CHD. Finally, the prospective nature of the SCOT-HEART trial enabled detailed and accurate phenotypic characterisation of patients at baseline and comprehensive clinical follow-up.
The use of CTCA in the assessment of patients with possible angina results in a 1.3% absolute risk reduction in the prognostic endpoint of fatal or non-fatal myocardial infarction over 3.2 years. This corresponds to 74 CTCA referrals (65 completed scans) to prevent a myocardial infarct. It should be noted that both cohorts had numerically similar hazard ratios and failed to demonstrate a statistically different treatment effect on formal interaction testing. Consequently, the non-significant risk reduction in the non-anginal cohort likely relates to the very low event rate observed within this group. Whilst this study was underpowered to reliably exclude a benefit in the non-anginal cohort, the results do suggest that the clinical significance of any benefits are likely to be small with an estimated number of 195 CTCA referrals to prevent a myocardial infarct.

An important innovation of the 2010 NICE guidelines was the recommendation to avoid further testing in patients with a low likelihood (<10%) of coronary heart disease. This has a sound theoretical basis in probability theory, reduces unnecessary investigations and is similar to the approach adopted by the European Society of Cardiology (European Society of Cardiology Task Force 2013). Despite this, the explicit calculation of risk has been removed from the updated recommendations due to the questionable applicability of the established scoring system – developed in 1979 within a US population – to the modern UK context, resulting in potential over-estimation of disease prevalence. Fortuitously, in this study, the cohort with non-anginal symptoms and a normal ECG had a prevalence of obstructive CHD of 9.5%, suggesting that the updated approach continues to provide an implicit method of pre-test probability estimation. It is important to note however, that within the non-anginal group, there is some underlying heterogeneity in risk by age and sex – the predicted
risk of obstructive CHD does vary from 3.4% in women aged less than 60 years of age to 20.2% in men aged over 60 – and it is likely that including routinely recorded clinical variables such as these could further optimise the assessment process (Fordyce, Douglas et al. 2017). Nonetheless, our study suggests that deferring the use of additional cardiac testing in these patients is safe, with an incidence of fatal or non-fatal myocardial infarction during the follow-up period of 1.5%, which was not reduced with the use of CTCA.

Within this study, CTCA increased the identification of both obstructive and non-obstructive coronary atherosclerosis which led to an increase in new requests for invasive coronary angiography in both patient cohorts. This increase in referrals has been raised as a potential drawback of adopting an anatomical approach to coronary assessment given the associated costs of unnecessary downstream testing (Cremer and Nissen 2017). Such concern is justified if CTCA is applied in an indiscriminate manner. Indeed, this study found no decrease in the likelihood of finding normal coronary arteries in those patients with non-anginal symptoms who underwent invasive evaluation, implying that CTCA did not improve appropriate test selection in this group. In contrast, when restricted to use in patients with possible angina, there was a reduction in the likelihood of normal coronary arteries and an increase in the rate of obstructive disease found on angiography suggesting that candidates for further testing had been appropriately selected. Furthermore, although both groups demonstrated higher rates of invasive angiography at 6 weeks, this increase only persisted in the non-anginal cohort by the conclusion of study follow-up. Interestingly, despite the increased detection of coronary obstruction on angiography, there was no increase in coronary revascularisation in patients with possible angina. This suggests
the adoption of a more nuanced approach to coronary intervention in the modern era. Our findings therefore refute previous commentators’ criticisms of the 2016 NICE guidance, and their assumptions regarding CTCA-guided use of both angiography and revascularisation (Cremer and Nissen 2017).

3.5.1 LIMITATIONS

Although this was a post-hoc analysis of the SCOT-HEART trial, it took place during the pre-specified period of follow-up of clinical events with systematic and robust collection of outcome data. Furthermore, the original trial was pragmatically designed in order to recruit patients with suspected stable angina of recent onset in a non-selective manner and the population enrolled is reflective of the heterogeneous group seen in chest pain clinics with an even spread of chest pain symptom typicality. In addition, participants had a broad range of estimated pre-test probability of coronary heart disease thereby ensuring direct applicability of the study outcomes to the proposed setting for implementation of the updated NICE guidelines.

It should be noted that, within this study, clinicians made use of additional ischaemia tests, particularly exercise ECG, that are no longer recommended by current guidelines. This does not necessarily detract from the overall findings. Indeed, it could be claimed that the high use of exercise ECG in both treatment arms would likely reduce the incremental benefit of CTCA compared with the recommended avoidance of this investigation.

Finally, it is uncommon for trials of diagnostic investigations to demonstrate improvements in clinical outcomes and this study cannot answer the question of how this reduction in event rates was achieved. It seems plausible that the identification of
coronary heart disease initiated a series of management changes including more personalised patient education, greater adherence to healthy lifestyle recommendations, and more appropriate use of risk modifying medications (The SCOT-HEART investigators 2016). Uncertainty persists concerning how to manage patients with no evidence of atherosclerosis on CTCA, specifically whether this warrants the cessation of preventative medications even in the presence of other cardiovascular risk factors. Furthermore, we have previously demonstrated a gradient of risk between the categories of normal, non-obstructive and obstructive coronary artery disease (The SCOT-HEART investigators 2016) and the ability to robustly quantify plaque burden is an important strength of CTCA. How this information is best used to inform treatment decisions however, remains an important unanswered question, particularly in light of recent effective but costly pharmacological interventions (Sabatine, Giugliano et al. 2017).

3.6 CONCLUSION

The clinical characterisation of symptoms is central to the 2016 updated NICE guidelines for the assessment of chest pain. When applied to a modern chest pain cohort, this revised approach appropriately selects patients requiring further investigation for coronary heart disease and minimises unnecessary testing in low risk individuals. Once patients with possible angina are identified, the use of computed tomography coronary angiography is associated with greater diagnostic certainty, more appropriate use of invasive angiography and a reduced risk of fatal and non-fatal myocardial infarction.
CHAPTER 4

IDENTIFICATION OF PATIENTS WITH STABLE CHEST PAIN DERIVING MINIMAL VALUE FROM CORONARY COMPUTED TOMOGRAPHY ANGIOGRAPHY: AN EXTERNAL VALIDATION OF THE PROMISE MINIMAL-RISK TOOL

Published in:

4.1 Summary

Background
The PROspective Multicenter Imaging Study for Evaluation of chest pain (PROMISE) minimal-risk tool was recently developed to identify patients with suspected stable angina at very low risk of coronary artery disease (CAD) and clinical events. We assessed the external validity of this tool within the context of the Scottish Computed Tomography of the HEART (SCOT-HEART) multicenter randomised controlled trial of patients with suspected stable angina due to coronary disease.

Methods
The minimal-risk tool was applied to 1,764 patients with complete imaging and follow-up data. External validity was compared with the guideline-endorsed CAD Consortium (CADC) risk score and determined through tests of model discrimination and calibration.

Results
A total of 531 (30.1%, mean age 52.4 years, female 62.0%) patients were classified as minimal-risk. Compared to the remainder of the validation cohort, this group had lower estimated pre-test probability of coronary disease according to the CADC model (30.0% vs 47.0%, p<0.001). The PROMISE minimal-risk tool improved discrimination compared with the CADC model (c-statistic 0.785 vs 0.730, p<0.001) and was improved further following re-estimation of covariate coefficients (c-statistic
0.805, p<0.001). Model calibration was initially poor ($\chi^2 197.6$, Hosmer-Lemeshow [HL] p<0.001), with significant overestimation of probability of minimal risk, but improved significantly following revision of the PROMISE minimal-risk intercept and covariate coefficients ($\chi^2 5.6$, HL p=0.692).

**CONCLUSION**
Despite overestimating the probability of minimal-risk, the PROMISE minimal-risk tool outperforms the CADC model with regards to prognostic discrimination in patients with suspected stable angina, and may assist clinicians in decisions regarding non-invasive testing.
4.2 **INTRODUCTION**

Chest pain is responsible for more than 1% of all presentations to family physicians, although stable coronary artery disease (CAD) is the underlying cause in only a minority (Bosner, Becker et al. 2009, Frese, Mahlmeister et al. 2016). Increased community awareness of CAD risk and improvements in primary prevention have led to progressively lower disease prevalence within this patient population and the frequency of abnormal results on ischaemia testing is now less than 10% (Rozanski, Gransar et al. 2013). There is a clear need to refine the assessment of suspected stable angina to optimise the efficient use of diagnostic resources and minimise unnecessary investigations. Recently, investigators from the North American PROspective Multicenter Imaging Study for Evaluation of chest pain (PROMISE) trial developed a risk model to identify individuals at very low risk of CAD (Fordyce, Douglas et al. 2017). To investigate the generalisability of this risk score, we undertook an external validation in a United Kingdom-based study of computed tomography in the diagnosis of CAD: the Scottish COmputed Tomography of the HEART (SCOT-HEART) trial.
4.3 METHODS

The SCOT-HEART study was a prospective multicenter randomised controlled trial investigating the role of coronary computed tomography angiography (CCTA) in patients referred to a specialist clinic with suspected angina due to CAD. The study design (The SCOT-HEART investigators 2012) and principal findings (The SCOT-HEART investigators 2015) have previously been reported. Briefly, participants were recruited from 12 cardiology chest pain clinics across Scotland and those randomised to the intervention arm underwent CCTA imaging in addition to routine clinical care. In contrast with PROMISE, there was a higher prevalence of obstructive coronary disease in the SCOT-HEART population (25.4% vs 10.7%) (Douglas, Hoffmann et al. 2015). For the purposes of this analysis, we limited the validation cohort to those individuals randomised to assessment with CCTA who had sufficient data to determine minimal-risk. The mean period of follow-up was 3.3±1.0 years.

Consistent with PROMISE (Fordyce, Douglas et al. 2017), minimal-risk was defined as requiring a coronary calcium score of 0, no CCTA evidence of coronary atherosclerosis, and the absence of any cardiovascular events (including all-cause death, non-fatal myocardial infarction or coronary revascularisation) during follow-up. All variables included in the published model were evaluated in this analysis with the exception of ethnicity because of the disparate ethnic composition of the study populations. The remaining variables include: age, sex, smoking history, diabetes mellitus, dyslipidemia, family history of premature coronary artery disease, hypertension, symptoms related to stress and high-density lipoprotein (HDL) concentration. In cases where HDL-cholesterol concentrations were not available,
multiple imputation using regression switching with predictive mean matching (MICE-PMM) was employed (Marshall, Altman et al. 2010).

Multivariable binomial logistic regression using the published model coefficients was used to estimate the probability of minimal-risk for each participant and the predicted risk was compared to the observed outcomes for these individuals. Model discrimination was determined from area under the receiver-operator curve (AUC), or c-statistic, and compared to the established CAD Consortium (CADC) risk score, which has recently been demonstrated to outperform the older Diamond-Forrester score (CAD Consortium 2011, Bittencourt, Hulten et al. 2016). The variables included in the CADC model include age, sex and typicality of presenting symptoms (typical, atypical or non-anginal). Discrimination reflects the ability of the model to correctly distinguish between minimal-risk (no plaque and no events) and other-risk individuals (i.e. place all subjects in the correct rank order of risk). Calibration describes the agreement between predicted and observed likelihood of minimal risk for an individual. Model calibration was assessed visually by plotting predicted versus observed risk in deciles and quantified with the Hosmer-Lemeshow (HL) statistic. Tests of discrimination and calibration were performed sequentially after step-wise updating of the model intercept (‘recalibration-in-the-large’) and slope to allow for differences in baseline risk between the derivation and validation populations. Finally, these tests were repeated following model revision, retaining the initial covariates but with coefficients re-estimated within the SCOT-HEART cohort. Statistical analysis was performed using R version 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria).
4.4 RESULTS

Within the 2,073 participants randomised to the intervention arm, 1,778 underwent CCTA scanning. In total, 1,764 patients (57.6, SD 9.5 years, female 44%) had complete imaging and outcome data available for analysis of whom 531 (30.1%; 52.4, SD 9.6 years, female 62.0%) fulfilled all criteria for minimal-risk (*Table 4.1*). High-density lipoprotein cholesterol (HDL-C) concentrations were unavailable in 506 patients (28.7%) and values were imputed.

In comparison with the remainder of the cohort, patients with minimal-risk were less likely to have symptoms of typical angina (24.1% vs 42.6%, \(p<0.001\)) and had lower pre-test probability of obstructive CAD as determined from the CADC risk score (30.0% vs 47.0%, \(p<0.001\)).

*Table 4.2* reports the observed probability of minimal risk, findings on CCTA, revascularisation and observed probability of all-cause death or non-fatal myocardial infarction within the SCOT-HEART population grouped by decile of predicted probability of no risk.

*Table 4.3* presents a comparison of the baseline characteristics for the PROMISE and SCOT-HEART trial populations.
### Table 4.1 Baseline characteristics

Data is presented as number (percentage) of patients unless otherwise stated.

- **BMI**, body mass index; **CVD**, cerebrovascular disease; **PVD**, peripheral vascular disease; **ACE**, angiotensin converting enzyme; **ARB**, angiotensin receptor blocker; **ECG**, electrocardiogram; **CHD**, coronary heart disease; **PTP**, pre-test probability.

- *ASSIGN Score (see http://assign-score.com/)
- ^Estimated according to the CAD Consortium risk model

<table>
<thead>
<tr>
<th></th>
<th>Minimal Risk</th>
<th>Other</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>531</td>
<td>1233</td>
<td></td>
</tr>
<tr>
<td><strong>Age, y (mean (SD))</strong></td>
<td>52.42 (9.59)</td>
<td>59.79 (8.50)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>329 (62.0)</td>
<td>439 (35.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Physician estimate of CAD</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>45 (8.5)</td>
<td>64 (5.2)</td>
<td></td>
</tr>
<tr>
<td>Unlikely</td>
<td>334 (62.9)</td>
<td>453 (36.7)</td>
<td></td>
</tr>
<tr>
<td>Probable</td>
<td>142 (26.7)</td>
<td>545 (44.2)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (1.9)</td>
<td>171 (13.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>124 (23.4)</td>
<td>484 (39.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>35 (6.6)</td>
<td>159 (12.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Dyslipidaemia</strong></td>
<td>222 (41.8)</td>
<td>853 (69.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Family history of premature CAD</strong></td>
<td>235 (44.3)</td>
<td>527 (42.7)</td>
<td>0.591</td>
</tr>
<tr>
<td><strong>Peripheral arterial disease</strong></td>
<td>4 (0.8)</td>
<td>28 (2.3)</td>
<td>0.046</td>
</tr>
<tr>
<td><strong>Prior stroke/TIA</strong></td>
<td>12 (2.3)</td>
<td>64 (5.2)</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>Smoking habit</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Never smoked</td>
<td>295 (55.6)</td>
<td>550 (44.6)</td>
<td></td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>136 (25.6)</td>
<td>453 (36.7)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>100 (18.8)</td>
<td>230 (18.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Predicted 10-year CVD risk</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>11.93 (8.45)</td>
<td>20.52 (11.11)</td>
<td></td>
</tr>
<tr>
<td><strong>Pre-test probability of obstructive CAD (mean (SD))^</strong></td>
<td>27.95 (18.96)</td>
<td>47.02 (24.53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Chest pain characterisation</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-anginal</td>
<td>261 (49.2)</td>
<td>423 (34.3)</td>
<td></td>
</tr>
<tr>
<td>Atypical angina</td>
<td>142 (26.7)</td>
<td>285 (23.1)</td>
<td></td>
</tr>
<tr>
<td>Typical angina</td>
<td>128 (24.1)</td>
<td>525 (42.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Symptoms precipitated by stress</strong></td>
<td>282 (53.1)</td>
<td>785 (63.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td>205 (38.6)</td>
<td>642 (52.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Abbreviations: CVD – cardiovascular disease; CHD – coronary heart disease; GTN – glyceryl trinitrate*
### Table 4.2 Test results and event rates by probability of minimal risk

Data is presented as number (percentage) of patients unless otherwise stated.

SD, standard deviation; CCTA, coronary computed tomography angiography; MI, myocardial infarction.

<table>
<thead>
<tr>
<th>Predicted probability of no risk</th>
<th>N</th>
<th>Observed probability of no risk, SD</th>
<th>Findings on CCTA</th>
<th>Revascularisation</th>
<th>Death or MI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>Mild disease</td>
<td>Moderate disease</td>
</tr>
<tr>
<td>0.0-0.1</td>
<td>2 (0.1)</td>
<td>0.00 (0.00)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (100.0)</td>
</tr>
<tr>
<td>0.1-0.2</td>
<td>168 (9.5)</td>
<td>0.02 (0.15)</td>
<td>7 (4.2)</td>
<td>27 (16.1)</td>
<td>41 (24.4)</td>
</tr>
<tr>
<td>0.2-0.3</td>
<td>313 (17.7)</td>
<td>0.12 (0.33)</td>
<td>54 (17.3)</td>
<td>72 (23.0)</td>
<td>63 (20.1)</td>
</tr>
<tr>
<td>0.3-0.4</td>
<td>322 (18.3)</td>
<td>0.18 (0.38)</td>
<td>82 (25.5)</td>
<td>79 (24.5)</td>
<td>55 (17.1)</td>
</tr>
<tr>
<td>0.4-0.5</td>
<td>278 (15.8)</td>
<td>0.22 (0.41)</td>
<td>82 (29.5)</td>
<td>68 (24.5)</td>
<td>58 (20.9)</td>
</tr>
<tr>
<td>0.5-0.6</td>
<td>250 (14.2)</td>
<td>0.40 (0.49)</td>
<td>115 (46.0)</td>
<td>63 (25.2)</td>
<td>41 (16.4)</td>
</tr>
<tr>
<td>0.6-0.7</td>
<td>221 (12.5)</td>
<td>0.56 (0.50)</td>
<td>138 (62.4)</td>
<td>37 (16.7)</td>
<td>27 (12.2)</td>
</tr>
<tr>
<td>0.7-0.8</td>
<td>137 (7.8)</td>
<td>0.66 (0.47)</td>
<td>103 (75.2)</td>
<td>15 (10.9)</td>
<td>15 (10.9)</td>
</tr>
<tr>
<td>0.8-0.9</td>
<td>60 (3.4)</td>
<td>0.75 (0.44)</td>
<td>50 (83.3)</td>
<td>7 (11.7)</td>
<td>2 (3.3)</td>
</tr>
<tr>
<td>0.9-1.0</td>
<td>13 (0.7)</td>
<td>1.00 (0.00)</td>
<td>13 (100.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>
### Participant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>SCOT-HEART</th>
<th>PROMISE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57 ± 10</td>
<td>61 ± 8</td>
</tr>
<tr>
<td>Women</td>
<td>44%</td>
<td>53%</td>
</tr>
<tr>
<td>Body-mass index</td>
<td>30 ± 6</td>
<td>31 ± 6</td>
</tr>
<tr>
<td>Hypertension</td>
<td>34%</td>
<td>65%</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>11%</td>
<td>21%</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>53%</td>
<td>68%</td>
</tr>
<tr>
<td>Family history</td>
<td>41%</td>
<td>32%</td>
</tr>
<tr>
<td>Past or current smoking</td>
<td>53%</td>
<td>51%</td>
</tr>
<tr>
<td>Prior CHD</td>
<td>9%</td>
<td>0%</td>
</tr>
<tr>
<td>Prior CVD/PAD</td>
<td>4%</td>
<td>6%</td>
</tr>
</tbody>
</table>

### Baseline Treatments

<table>
<thead>
<tr>
<th></th>
<th>SCOT-HEART</th>
<th>PROMISE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>49%</td>
<td>45%</td>
</tr>
<tr>
<td>Statin</td>
<td>43%</td>
<td>45%</td>
</tr>
<tr>
<td>ACE/ARB</td>
<td>17%</td>
<td>44%</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>33%</td>
<td>25%</td>
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</tbody>
</table>

### Baseline Symptoms

<table>
<thead>
<tr>
<th></th>
<th>SCOT-HEART</th>
<th>PROMISE</th>
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</thead>
<tbody>
<tr>
<td>Typical</td>
<td>35%</td>
<td>12%</td>
</tr>
<tr>
<td>Atypical</td>
<td>24%</td>
<td>78%</td>
</tr>
<tr>
<td>Nonanginal</td>
<td>41%</td>
<td>11%</td>
</tr>
</tbody>
</table>

### Baseline Risk

<table>
<thead>
<tr>
<th></th>
<th>SCOT-HEART</th>
<th>PROMISE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Framingham 10-yr risk</td>
<td>17 ± 12%</td>
<td>22 ± 15%</td>
</tr>
<tr>
<td>Pretest probability of obstructive CHD</td>
<td>47%</td>
<td>53%</td>
</tr>
</tbody>
</table>

### Selection of Functional Testing

<table>
<thead>
<tr>
<th></th>
<th>SCOT-HEART</th>
<th>PROMISE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECG</td>
<td>85%</td>
<td>10%*</td>
</tr>
<tr>
<td>MPI</td>
<td>9%</td>
<td>68%*</td>
</tr>
<tr>
<td>Echo</td>
<td>1%</td>
<td>22%*</td>
</tr>
</tbody>
</table>

### Planned invasive coronary angiography at baseline

<table>
<thead>
<tr>
<th></th>
<th>SCOT-HEART</th>
<th>PROMISE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 4.3: Comparison of baseline characteristics for PROMISE and SCOT-HEART trial populations

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CHD, coronary heart disease; CVD, cardiovascular disease; ECG, electrocardiogram; Echo, echocardiography; MPI, myocardial perfusion imaging; PAD, peripheral arterial disease. Adapted from (Fordyce, Newby et al. 2016)
Model discrimination (**Figure 4.1**) was greater using the PROMISE minimal-risk score compared with the CADC model (c-statistic 0.785, 95% confidence interval [95% CI] 0.762-0.808, vs 0.730, 95% CI 0.706-0.755; p<0.001). Discrimination was unaffected by re-calibration of model intercept or slope but the c-statistic improved further following revision of the model coefficients (c-statistic 0.805, 95% CI 0.784-0.827; p<0.001).

Goodness-of-fit was not demonstrated for either established CADC model (χ² 1010.1, HL p<0.001) or the PROMISE minimal-risk risk tool (χ² 197.6, HL p<0.001). Goodness-of-fit for the PROMISE minimal-risk risk tool remained suboptimal following recalibration of the model intercept (χ² 32.2, HL p<0.001) and calibration slope (χ² 23.7, HL p=0.026) but was improved by the further addition of re-estimated model coefficients (χ² 5.6, HL p=0.692; **Table 4.4**) resulting in good calibration (**Figure 4.2**).
Figure 4.1 Receiver operating characteristic curve

The PROspective Multicenter Imaging Study for Evaluation of chest pain (PROMISE) minimal-risk score (blue) demonstrates significantly greater model discrimination compared to the existing Coronary Artery Disease Consortium (CADC) model (green). Discrimination improves further with updated model coefficients (red).
Table 4.4: Derived and updated minimal-risk model coefficients and baseline characteristics of these variables within the PROMISE (derivation) and SCOT-HEART (validation) cohorts.

Data is presented as number (percentage) of patients unless otherwise stated. CAD, coronary artery disease; NR, not reported.
Figure 4.2 Model calibration

Plot demonstrates poor calibration of predicted probability vs observed proportion of minimal risk using initial model coefficients and intercept (blue) in addition to the established Coronary Artery Disease Consortium (CADC) model (green). Calibration remains poor despite updating the model intercept (grey) and slope (purple). Good model calibration (HL, Hosmer-Lemeshow) is demonstrated once the model coefficients are re-estimated within the validation cohort (red). The dashed line represents perfect calibration (Steyerberg, Borsboom et al. 2004).
4.5 DISCUSSION

When applied to the SCOT-HEART population, the PROMISE minimal-risk tool improved model discrimination for excluding CAD when compared with the established CADC model. Indeed, the present c-statistic is greater than that reported in the original PROMISE model derivation (0.785 versus 0.725) (4). The improved discrimination of the PROMISE score compared with the CADC score likely reflects the different intended purpose of these scores. The CADC model was derived with the objective of accurately estimating the pre-test probability of obstructive coronary artery disease whilst the PROMISE model adopts a broader approach of excluding the presence of any coronary atherosclerosis or any clinical events throughout follow-up.

Model calibration – i.e. the ability to estimate accurately an individuals’ absolute risk – was poor when applied in the SCOT-HEART trial population. Such a finding is common when assessing model performance in clinically divergent settings (D'Agostino, Sr et al. 2001, Moons, Kengne et al. 2012, Rana, Tabada et al. 2016, Van Calster, Van Hoorde et al. 2017) and causes may be patient-related (including differences in case-mix, event rates, or predictor definitions), or model-related, such as over (or under-) fitting of coefficients or omission of important predictive variables.

Poor goodness-of-fit does not necessarily signify lack of model value, and the robust discrimination demonstrated in this report suggests appropriate and informative covariate selection. Therefore, we adopted the recommended stepwise approach to model updating (Moons, Kengne et al. 2012) that successfully improved model calibration within the SCOT-HEART population. It is plausible, that the PROMISE risk model remains correct in its initial form for application within the North American
context, whilst the updated coefficients may provide more accurate risk estimates for European populations. Regardless, to achieve accurate predictions of absolute risk, it is likely that such population-specific recalibration would be desirable for each setting in which the model was to be applied. However, even in the absence of this recalibration, our findings have important implications for determining appropriate diagnostic pathways and highlight that clinicians need to be aware of the imprecision of the estimates determined from the minimal-risk score. In recognition of this, the current guidelines for prevention of atherosclerotic cardiovascular disease already recommend incorporating explanation of the uncertainty of prognostic models in the clinician-patient risk discussion (Martin, Sperling et al. 2015).

In order to understand the reasons for risk estimate imprecision we need to consider carefully the differences in patient populations between the PROMISE and SCOT-HEART trials. Such differences have previously been reported (Fordyce, Newby et al. 2016), but specific areas of relevance to the current findings warrant mention. First, the improvement in discrimination seen with the SCOT-HEART population is likely to reflect the greater breadth of baseline risk, particularly regarding distribution of chest pain symptom typicality, which gives rise to a broader spread of the linear predictors within the validation cohort (Debray, Vergouwe et al. 2015). Relatedly, there is an apparent difference in the proportion of the trial populations fulfilling the criteria for minimal-risk between the studies, with slightly more low-risk patients identified in the SCOT-HEART cohort. This ‘miscalibration-in-the-large’ is a frequent challenge but can be straightforwardly addressed, if the average patient risk is known within the external clinical setting in which the model is being applied, by adjustment of the model intercept. Furthermore, although the exact nature of the effects of this
difference in case-mix or ‘spectrum bias’ is difficult to quantify, some insight can be gained from examination of the change in specific variable coefficients. With one exception, all the coefficients increased in magnitude when re-estimated. Those covariates where the increase was most substantial, for example female sex, appear to be more powerful predictors of minimal-risk in the SCOT-HEART population than was identified in PROMISE.

Ultimately, the clinical value of any prognostic score relies on a previously defined and broadly accepted threshold of risk that suitably assists clinicians in identifying those patients who can have further testing safely deferred. In this regard, the accuracy of absolute predicted risk may be of secondary importance to how reliably a model categorizes or reclassifies an individual into appropriate diagnostic pathways. In the absence of updated clinical guidelines incorporating the minimal-risk tool, it is unclear where this threshold should be set. However, it is reassuring to note that the probability of obstructive coronary artery disease remains less than 10% in the highest four deciles of predicted probability of minimal risk, whilst the corresponding risk of death or myocardial infarction remains below 2% in these groups. International guidelines have previously adopted low-risk thresholds in this range, below which further investigation is unhelpful (National Institute for Health and Clinical Excellence 2010, European Society of Cardiology Task Force 2013). Should such an approach be continued using the PROMISE model, it would identify 1 in 4 patients who could safely avoid further testing, enabling potentially important reductions in diagnostic resource use.

In the United Kingdom, the National Institute for Health and Care Excellence (NICE) has recently updated guidance for the assessment of suspected stable angina (National
Institute for Health and Care Excellence 2016) and has recommended a shift to a symptom-focused approach to chest pain management, moving away from the broader cardiovascular risk-based approach (National Institute for Health and Clinical Excellence 2010). The utility of this approach has yet to be established. However, given the more limited role of symptoms as a covariate, the minimal risk tool may have a role beyond the chest pain clinic setting and could be used to guide management in the primary or secondary prevention settings. This would need to be prospectively confirmed.

This study has some limitations. Failure to undergo CCTA as randomised, non-diagnostic images and the absence of coronary calcium scans, precluded the determination of minimal-risk in 309 (14.9%) patients. HDL-C concentrations were not available in a fifth of patients although the MICE-PMM technique for imputation achieves minimally biased estimates and satisfactory model performance with up to 50% missingness (Marshall, Altman et al. 2010). Finally, we excluded unstable angina not leading to revascularisation as a minimal risk exclusion criterion since unlike PROMISE, unstable angina events were not adjudicated in SCOT-HEART. However, with the widespread use of high-sensitivity troponin in Scotland, unstable angina accounts for less than 5% of all acute coronary syndromes.
4.6 CONCLUSION

When assessing patients with suspected stable angina, the PROMISE minimal-risk tool outperforms the CADC model and improves discrimination of the pre-test probability of normal coronary arteries and no clinical events. Suboptimal model calibration may overestimate probability of minimal risk in external populations. Nevertheless, the PROMISE minimal-risk tool may assist clinicians in decisions regarding non-invasive testing.
CHAPTER 5

HIGH-SENSITIVITY CARDIAC TROPONIN I IN THE DIAGNOSIS OF STABLE CORONARY ARTERY DISEASE
CHAPTER 5  HIGH-SENSITIVITY CARDIAC TROPONIN I IN THE DIAGNOSIS OF STABLE CORONARY ARTERY DISEASE

5.1 SUMMARY

OBJECTIVES
To determine whether high-sensitivity cardiac troponin I can improve the estimation of the pre-test probability for obstructive coronary artery disease in patients with suspected stable angina.

METHODS
In a pre-specified sub-study of the Scottish COmputed Tomography of the Heart (SCOT-HEART) trial, plasma cardiac troponin was measured using a high-sensitivity single molecule counting assay in 943 adults with suspected stable angina who had undergone coronary computed tomography angiography. Rates of obstructive coronary artery disease were compared with the pre-test probability determined by the European Society of Cardiology (ESC) Coronary Artery Disease Consortium risk model with and without cardiac troponin concentrations. External validation was undertaken in an independent study population from Denmark comprising 487 patients with suspected stable angina.

RESULTS
Higher cardiac troponin concentrations were associated with obstructive coronary artery disease with a 5-fold increase across quintiles (9 to 48%, p<0.001) independent of known cardiovascular risk factors (odds ratio [OR] 1.35 [95% confidence interval (CI) 1.25-1.46] per doubling of troponin). Cardiac troponin concentrations improved the discrimination of the ESC model for identifying obstructive coronary artery disease
(c-statistic 0.785 to 0.800, p=0.003) and improved classification into ESC-recommended categories of clinical risk (net reclassification improvement 0.143 [95% CI, 0.093-0.193]). The revised model achieved similar improvements in discrimination and net reclassification when applied in the external validation cohort.

**CONCLUSION**
High-sensitivity cardiac troponin I concentration is an independent predictor of obstructive coronary artery disease in patients with suspected stable angina. Use of this test may improve the selection of patients for further investigation and treatment.
5.2 INTRODUCTION

Presentations with suspected stable angina are common yet determining an accurate diagnosis is frequently challenging. Patients and clinicians alike are understandably keen to identify the cause of the symptoms in order that these can be treated and hopefully ameliorated. Of equal importance, is the concern that these symptoms may reflect prognostically significant atherosclerotic disease with the associated risk of future cardiovascular events. These concerns are appropriate given that 1 in 6 patients will suffer coronary death or non-fatal acute coronary syndrome in the 3 years following a diagnosis of stable angina (Sekhri, Feder et al. 2007). Importantly, this risk remains substantial even in those patients with symptoms deemed non-cardiac in origin (Sekhri, Feder et al. 2007). Consequently, despite the central role of the clinical history and cardiovascular risk factor ascertainment in the assessment process, supplementary investigations are frequently required to provide additional certainty related to the presence or absence of obstructive coronary artery disease (Euro Heart Survey Investigators 2005). A number of national and international bodies have proposed standardised pathways that employ risk models to estimate the pre-test probability (PTP) of obstructive coronary artery disease and guide decision-making with regards to appropriate use of investigations (National Institute for Health and Clinical Excellence 2010, Fihn, Gardin et al. 2012, European Society of Cardiology Task Force 2013). However, there is evidence both that these models may overestimate risk (Genders, Steyerberg et al. 2012, Kumamaru, Arai et al. 2014, Almeida, Fonseca et al. 2016) and that clinician use of stratification tools remains sub-optimal (Patel, Peterson et al. 2010, Mudrick, Cowper et al. 2012).
In light of these challenges, there is widespread interest in identifying suitable biomarkers that may improve diagnostic accuracy in patients with suspected stable coronary artery disease. As yet, no novel circulating biomarker has been shown to improve diagnostic classification (European Society of Cardiology Task Force 2013). It is in this context that a role may emerge for the most recent generation of high-sensitivity cardiac troponin assays. These tests offer the ability to reliably measure troponin in the majority of the healthy population and have already had a significant impact on the assessment of suspected acute coronary syndromes (Shah, McAllister et al. 2015). Meanwhile, evidence is emerging of potential roles in the context of stable cardiovascular diseases (Omland, Pfeffer et al. 2013, Everett, Brooks et al. 2015).

This study aimed to determine if routine quantification of plasma high sensitivity cardiac troponin I concentrations could improve estimation of the pre-test probability of obstructive coronary artery disease in patients with suspected stable angina.
5.3 METHODS

5.3.1 STUDY DESIGN

The Scottish COmputed Tomography of the Heart (SCOT-HEART) trial was a prospective, multi-centre, randomised controlled study that investigated the role of coronary computed tomography angiography (CCTA) in patients referred to a specialist clinic with suspected angina due to coronary heart disease. The study design (The SCOT-HEART investigators 2012) and principal findings (The SCOT-HEART investigators 2015) have previously been reported. Briefly, participants were recruited from 12 cardiology chest pain clinics across Scotland and those randomised to the intervention arm underwent CCTA imaging at one of 3 sites in addition to routine clinical assessment. There was a pre-specified biomarker sub-study which obtained blood samples from those participants where the CCTA was performed at the Clinical Research Imaging Centre in Edinburgh, UK. Recruitment began November 18, 2010 and follow-up of clinical outcomes continued until June 30, 2016. The study was performed in accordance with the Declaration of Helsinki and with research ethics committee approval.

5.3.2 HIGH-SENSITIVITY CARDIAC TROPONIN I MEASUREMENT

Venous blood samples for biomarker testing were obtained immediately prior to CCTA imaging. Blood was processed and stored at –80°C until analysed. Plasma high-sensitivity cardiac troponin I concentrations were measured using a high-sensitivity single molecule counting assay on the Erenna platform (Singulex Inc, Alameda, California, USA) which has a limit of detection (LoD) of 0.1 ng/L, a limit of quantification (LOQ, co-efficient of variation <10%) of 0.4 ng/L and a 99th centile
upper reference limit (URL) of 10.9 ng/L (Apple and Collinson 2012, Wu, Estis et al. 2015). To facilitate internal validation of this measurement with a clinically available assay, a secondary analysis was performed wherein the samples were analysed using the ARCHITECT STAT high-sensitive troponin I assay (Abbott Laboratories, Abbott Park, Illinois, USA) which has a limit of detection of 1.2 ng/L and coefficient of variation <10% at 3.0 ng/L (Apple and Collinson 2012).

5.3.3 CORONARY COMPUTED TOMOGRAPHY ANGIOGRAPHY

Participants underwent coronary artery calcium scoring and CCTA using a 320-detector scanner (Aquilion One, Toshiba Medical Systems, Nasushiobara, Japan). Computed tomography images were analysed by 2 trained observers with excellent reproducibility (Williams, Golay et al. 2015). Differences in categorisation were resolved by consensus. Coronary artery calcium scoring was performed using dedicated software (VScore, Vital Images, Minnetonka, USA). Agatston score was calculated using a threshold of 130 HU (Hounsfield units) for each vessel and summed to give a total score. The coronary arteries were assessed using a 15-segment model with each segment classified into one of five categories dependent on the degree of luminal cross-sectional area stenosis: normal (<10% stenosis), mild non-obstructive (10-49% stenosis), moderate non-obstructive (50-69% stenosis), obstructive (70-99% stenosis) or total/sub-total occlusion (100% stenosis). For the purposes of the primary outcome, obstructive coronary artery disease was defined prior to this analysis within the published SCOT-HEART trial protocol, as a luminal cross-sectional area stenosis of ≥70% (approximating to a 50% diameter stenosis) in at least one major epicardial vessel or ≥50% in the left main stem (The SCOT-HEART investigators 2012). Using
previously described methods (Min, Shaw et al. 2007), the segment stenosis score (SSS) was quantified as a measure of overall atherosclerotic burden. All image analysis was performed blinded to the biomarker results.

5.3.4 THE CORONARY ARTERY DISEASE CONSORTIUM MODEL

The Coronary Artery Disease Consortium (CADC) is part of the European network for the Assessment of Imaging in Medicine (EuroAIM). In 2011 the CADC updated and extended the earlier Diamond-Forrester model to estimate more accurately the pre-test probability (PTP) of obstructive coronary artery disease identified on invasive coronary angiography in patients with suspected stable angina (CAD Consortium 2011). The CADC model incorporates age, sex and chest pain characteristics and underpins the risk tables included in the current European Society of Cardiology guideline on the management of stable coronary artery disease (European Society of Cardiology Task Force 2013). The guideline uses three thresholds in order to stratify patients into four testing pathways. Patients with a PTP less than 15% are deemed low-risk and no further testing for coronary artery disease is recommended; those with a PTP 15% to 65% should undergo myocardial ischaemia testing with either exercise electrocardiography (ECG), or non-invasive stress imaging; the group with a PTP 65% to 85% should undergo non-invasive stress imaging; and those with a PTP greater than 85% are considered high risk and do not require further testing to confirm a diagnosis of coronary artery disease although coronary angiography may be indicated for prognostic stratification or to facilitate therapeutic revascularisation (European Society of Cardiology Task Force 2013).
5.3.5 Validation cohort

External validation of the revised model was performed in a previously described study population (Hosbond, Diederichsen et al. 2014, Madsen, Diederichsen et al. 2017) comprising 487 patients with suspected stable angina who underwent biomarker sampling in addition to coronary imaging (CTCA in 336, invasive angiography in 151) at the Odense University Hospital, Denmark. Troponin concentrations were determined using the Abbott Architect assay.

5.3.6 Statistical analysis

Statistical analysis was performed using R version 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria). Summary statistics for patient characteristics were estimated, by quintile of cardiac troponin concentration, with Chi-squared and ANOVA tests being used to compare categorical and continuous variables, respectively. In logistic regression models, the probability of each patient having obstructive coronary artery disease was estimated. Cardiac troponin concentration and coronary artery calcium scores were log-transformed as linearising transformations. Associations were estimated unadjusted, and after adjusting for age, sex, chest pain characteristics, cardiovascular risk factors and non-invasive test results. The baseline CADC model and CADC model with the addition of cardiac troponin were also fitted. In both cases the model intercept was estimated from the sample data (with the coefficients for age, sex and chest pain typicality fixed) to allow fair comparison of model performance. Discrimination and calibration were compared for the current CADC model, and the CADC model with troponin, using the DeLong method (DeLong, DeLong et al. 1988) and the Hosmer-Lemeshow (H-L) goodness of fit test.
(p-value <0.05 defined as poor calibration) respectively. The coefficient of discrimination (D) was calculated according to the method proposed by Tjur (Tjur 2009). The categorical net reclassification improvement index was estimated using the ESC-recommended PTP thresholds of 15% and 65%. The association between troponin assays was assessed using the Pearson correlation coefficient.

The performance, in terms of discrimination and net reclassification, of the new model incorporating troponin concentration was also compared to the existing CADC model in an independent cohort. Neither the intercept nor the coefficients were re-estimated for either model.

Figure 5.1 Consort diagram of biomarker substudy population

CCTA, coronary computed tomography angiography; CRIC, clinical research imaging centre.
5.4 RESULTS

5.4.1 DATA COLLECTION AND STUDY POPULATION

The study population of the SCOT-HEART trial has previously been described (The SCOT-HEART investigators 2015). Between Nov 18th 2010 and Sept 24th 2014, 4,146 participants were recruited of whom 2,073 were randomly assigned to standard care plus CCTA and 1,778 of these underwent CCTA at one of three sites. Blood samples were obtained from 987 participants at the time of CCTA imaging at a single centre and 943 had plasma cardiac troponin I concentrations measured. CCTA image quality was non-diagnostic in 6 cases resulting in an analysis set comprising 937 participants (Figure 5.1). The baseline characteristics were similar between all participants who underwent CCTA and those with troponin concentrations available for analysis (Table 5.1).
Table 5.1 Baseline characteristics of biomarker sub-study and intervention arm of the SCOT-HEART trial

<table>
<thead>
<tr>
<th></th>
<th>All Participants with CCTA</th>
<th>CCTA + Troponin</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1781</td>
<td>937</td>
<td>0.57</td>
</tr>
<tr>
<td>Age (mean (SD))</td>
<td>57.57 (9.47)</td>
<td>57.79 (9.55)</td>
<td>0.57</td>
</tr>
<tr>
<td>Male (%)</td>
<td>998 (56.0)</td>
<td>533 (56.9)</td>
<td>0.702</td>
</tr>
<tr>
<td>BMI (mean (SD))</td>
<td>29.63 (5.64)</td>
<td>29.62 (5.54)</td>
<td>0.985</td>
</tr>
<tr>
<td>Pre-existing CHD (%)</td>
<td>162 (9.1)</td>
<td>78 (8.3)</td>
<td>0.547</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>612 (34.7)</td>
<td>336 (36.2)</td>
<td>0.485</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
<td>1,083 (60.8)</td>
<td>565 (60.3)</td>
<td>0.828</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>196 (11.0)</td>
<td>98 (10.5)</td>
<td>0.711</td>
</tr>
<tr>
<td>Smoking habit* (%)</td>
<td>928 (52.1)</td>
<td>514 (54.9)</td>
<td>0.19</td>
</tr>
<tr>
<td>Family history of CHD (%)</td>
<td>770 (43.6)</td>
<td>394 (42.5)</td>
<td>0.629</td>
</tr>
<tr>
<td>Predicted 10-year CHD risk‡</td>
<td>17.9 (11.0)</td>
<td>18.2 (11.1)</td>
<td>0.407</td>
</tr>
<tr>
<td>Anginal Symptoms§ (%)</td>
<td></td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td>Atypical angina</td>
<td>436 (24.5)</td>
<td>221 (23.6)</td>
<td></td>
</tr>
<tr>
<td>Non-anginal</td>
<td>687 (38.6)</td>
<td>317 (33.8)</td>
<td></td>
</tr>
<tr>
<td>Typical angina</td>
<td>658 (36.9)</td>
<td>399 (42.6)</td>
<td></td>
</tr>
<tr>
<td>Exercise ECG Performed (%)</td>
<td>1512 (85.1)</td>
<td>770 (82.3)</td>
<td>0.059</td>
</tr>
<tr>
<td>Normal</td>
<td>925 (65.2)</td>
<td>475 (66.2)</td>
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</tr>
<tr>
<td>Inconclusive</td>
<td>257 (18.1)</td>
<td>113 (15.7)</td>
<td></td>
</tr>
<tr>
<td>Abnormal˚</td>
<td>236 (16.6)</td>
<td>130 (18.1)</td>
<td></td>
</tr>
</tbody>
</table>

CCTA, coronary computed tomography angiography; SD, standard deviation; BMI, body mass index; CHD, coronary heart disease; *Current and ex-smokers; ‡ASSIGN score; §European Society of Cardiology Criteria, ECG, electrocardiography.

5.4.2 CORONARY COMPUTED TOMOGRAPHY ANGIOGRAPHY

The median interval between randomisation and CCTA was 13 days (interquartile range [IQR] 7 to 18 days). The median coronary artery calcium score was 31 (IQR 0 to 281) Agatston Units (AU). CCTA demonstrated normal coronary arteries in 322 (34%), mild to moderate non-obstructive disease in 348 (37%), and obstructive disease in 267 (28%) participants.

5.4.3 HIGH-SENSITIVITY CARDIAC TROPONIN I CONCENTRATIONS

Cardiac troponin I concentrations were above the LoD in 934/937 (99.6%) patients. The three samples with concentrations below this limit were assigned a value of 0.1 ng/L. The median concentration of hs-cTnl was 1.41 (IQR 0.89 to 2.28) ng/L with 907
(96.8%) and 27 (2.9%) of patients above the limit of quantification (0.4 ng/L) and 99th centile URL (10.9 ng/L) respectively.

Higher cardiac troponin quintiles were associated with increasing age, male sex and a number of cardiovascular risk factors (Table 5.2). The majority (82.3%) of patients underwent exercise electrocardiography and this test was more likely to demonstrate inducible ischaemia in those with higher cardiac troponin concentrations (Table 5.3).

Higher cardiac troponin quintiles were associated with greater coronary atherosclerotic burden as determined by coronary artery calcium score or segment stenosis score. They were also more likely to have obstructive coronary disease with a five-fold increase between the first and fifth quintiles (9.3% to 47.5%; Table 5.3). Each 2-fold increment in troponin concentration was associated with a 1.71-fold increment (95% confidence intervals (CI), 1.60-1.83) in the odds of identifying obstructive coronary artery disease on CCTA. This association was moderately attenuated after adjusting for age and sex (OR 1.39; 95% CI, 1.29-1.49), but persisted on further adjustment for chest pain description, cardiovascular risk factors, exercise ECG findings and the coronary calcium score (OR 1.27; 95% CI, 1.17-1.39; Table 5.4).

Troponin testing with a second high-sensitivity cardiac troponin I assay (Abbott Diagnostics) was performed on 931 samples and demonstrated good agreement with the Singulex assay (r=0.88). The median troponin concentration was 2.1 ng/L (95% CI, 1.2-3.5 ng/L) and a number of samples reported results below the LoD (200, 21.5%). Despite this, the overall findings were consistent with the primary analysis (Tables 5.5-5.6).
Cardiac troponin I concentrations by quintile (range [ng/L])

<table>
<thead>
<tr>
<th>Quintile</th>
<th>Q1 (≤0.82)</th>
<th>Q2 (0.83-1.16)</th>
<th>Q3 (1.17-1.61)</th>
<th>Q4 (1.62-2.66)</th>
<th>Q5 (&gt;2.66)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>193</td>
<td>186</td>
<td>183</td>
<td>192</td>
<td>183</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>51.8 (9.4)</td>
<td>57.0 (8.3)</td>
<td>59.0 (9.5)</td>
<td>60.7 (8.8)</td>
<td>60.7 (8.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Male, %</td>
<td>60 (31.1)</td>
<td>93 (50.0)</td>
<td>113 (61.7)</td>
<td>132 (68.8)</td>
<td>135 (73.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Chest pain symptom, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typical angina</td>
<td>54 (28.0)</td>
<td>64 (34.4)</td>
<td>81 (44.3)</td>
<td>101 (52.6)</td>
<td>99 (54.1)</td>
<td></td>
</tr>
<tr>
<td>Atypical angina</td>
<td>65 (33.7)</td>
<td>39 (21.0)</td>
<td>46 (25.1)</td>
<td>32 (16.7)</td>
<td>39 (21.3)</td>
<td></td>
</tr>
<tr>
<td>Non-anginal</td>
<td>74 (38.3)</td>
<td>83 (44.6)</td>
<td>56 (30.6)</td>
<td>59 (30.7)</td>
<td>45 (24.6)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>29.6 (6.2)</td>
<td>29.1 (5.5)</td>
<td>30.1 (5.1)</td>
<td>30.0 (5.5)</td>
<td>29.4 (5.4)</td>
<td>0.429</td>
</tr>
<tr>
<td>Pre-existing CHD, %</td>
<td>12 (6.2)</td>
<td>6 (3.2)</td>
<td>19 (10.4)</td>
<td>20 (10.4)</td>
<td>21 (11.5)</td>
<td>0.018</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>36 (18.7)</td>
<td>54 (29.3)</td>
<td>70 (38.5)</td>
<td>92 (48.7)</td>
<td>84 (46.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hyperlipidemia, %</td>
<td>95 (49.2)</td>
<td>109 (58.6)</td>
<td>117 (63.9)</td>
<td>126 (65.6)</td>
<td>118 (64.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>20 (10.4)</td>
<td>17 (9.1)</td>
<td>13 (7.1)</td>
<td>23 (12.0)</td>
<td>25 (13.7)</td>
<td>0.285</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>46 (23.8)</td>
<td>45 (24.2)</td>
<td>33 (18.0)</td>
<td>31 (16.2)</td>
<td>30 (16.4)</td>
<td>0.113</td>
</tr>
<tr>
<td>Family history of CHD, %</td>
<td>95 (50.0)</td>
<td>85 (46.4)</td>
<td>81 (44.8)</td>
<td>71 (37.4)</td>
<td>62 (34.1)</td>
<td>0.010</td>
</tr>
<tr>
<td>10-year CHD risk*</td>
<td>11.0 [6.0, 16.0]</td>
<td>15.0 [9.0, 22.8]</td>
<td>17.0 [11.0, 24.0]</td>
<td>19.0 [14.0, 27.0]</td>
<td>19.0 [14.0, 27.5]</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Table 5.2 Baseline characteristics of patients with suspected angina stratified by cardiac troponin

Data are mean (standard deviation), median [IQR], or value (%); BMI, body mass index; CHD, coronary heart disease.

*ASSIGN Score (see http://assign-score.com/)
### Cardiac troponin I concentrations by quintile (ng/L)

<table>
<thead>
<tr>
<th>Quintile</th>
<th>Exercise ECG performed, %</th>
<th>Exercise ECG outcome</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1 (&lt;0.82)</td>
<td>162 (83.9)</td>
<td>Normal, %</td>
<td>123 (84.2)</td>
</tr>
<tr>
<td>Q2 (0.83-1.16)</td>
<td>161 (86.6)</td>
<td>109 (71.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Q3 (1.17-1.61)</td>
<td>153 (84.1)</td>
<td>Inconclusive, %</td>
<td>24 (15.7)</td>
</tr>
<tr>
<td>Q4 (1.62-2.66)</td>
<td>149 (77.6)</td>
<td>Abnormal, %</td>
<td>12 (8.2)</td>
</tr>
<tr>
<td>Q5 (&gt;2.66)</td>
<td>145 (79.2)</td>
<td>Coronary calcium score</td>
<td>0.0 [0.0, 31.0]</td>
</tr>
</tbody>
</table>

#### Coronary disease on CT, %

<table>
<thead>
<tr>
<th>CHD</th>
<th>No significant</th>
<th>Non-obstructive</th>
<th>Obstructive</th>
<th>SSS Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD</td>
<td>107 (55.4)</td>
<td>67 (34.7)</td>
<td>18 (9.3)</td>
<td>0.0 [0.0, 2.0]</td>
</tr>
<tr>
<td>CHD</td>
<td>78 (41.9)</td>
<td>69 (37.1)</td>
<td>39 (21.0)</td>
<td>1.0 [0.0, 6.0]</td>
</tr>
<tr>
<td>CHD</td>
<td>55 (30.1)</td>
<td>75 (41.0)</td>
<td>53 (29.0)</td>
<td>3.0 [0.0, 10.0]</td>
</tr>
<tr>
<td>CHD</td>
<td>44 (22.9)</td>
<td>78 (40.6)</td>
<td>70 (36.5)</td>
<td>5.0 [1.0, 12.0]</td>
</tr>
<tr>
<td>CHD</td>
<td>37 (20.2)</td>
<td>59 (32.2)</td>
<td>87 (47.5)</td>
<td>7.0 [1.0, 14.0]</td>
</tr>
</tbody>
</table>

<0.001

---

**Table 5.3 Exercise electrocardiography and coronary computed tomography findings by troponin quintile**

Data are median [IQR], or value (%); ECG, electrocardiography; IQR, interquartile range; CHD, coronary heart disease; SSS, segment stenosis score.
Table 5.4 Multi-variable predictors of obstructive coronary artery disease

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
<th>Model 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log₂ (hs-cTnI)</td>
<td>1.71</td>
<td>1.39</td>
<td>1.35</td>
<td>1.35</td>
<td>1.3</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>(1.51-1.96)</td>
<td>(1.21-1.6)</td>
<td>(1.17-1.56)</td>
<td>(1.17-1.57)</td>
<td>(1.11-1.55)</td>
<td>(1.08-1.52)</td>
</tr>
<tr>
<td>Age</td>
<td>1.07</td>
<td>1.05</td>
<td>1.06</td>
<td>1.04</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.05-1.09)</td>
<td>(1.03-1.07)</td>
<td>(1.03-1.08)</td>
<td>(1.02-1.07)</td>
<td>(0.97-1.02)</td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>3.86</td>
<td>3.88</td>
<td>4.04</td>
<td>3.67</td>
<td>1.64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2.72-5.57)</td>
<td>(2.7-5.65)</td>
<td>(2.78-5.97)</td>
<td>(2.37-5.8)</td>
<td>(1.05-2.58)</td>
<td></td>
</tr>
<tr>
<td>Chest pain symptom</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atypical angina</td>
<td>1.82</td>
<td>1.47</td>
<td>1.11</td>
<td>1.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.11-3.00)</td>
<td>(0.88-2.47)</td>
<td>(0.61-2.03)</td>
<td>(0.73-2.34)</td>
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<td></td>
</tr>
<tr>
<td>Typical angina</td>
<td>4.28</td>
<td>3.13</td>
<td>2.05</td>
<td>2.78</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>(2.85-6.53)</td>
<td>(2.03-4.9)</td>
<td>(1.21-3.49)</td>
<td>(1.69-4.62)</td>
<td></td>
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</tr>
<tr>
<td>Diabetes</td>
<td>0.83</td>
<td>1.08</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.48-1.42)</td>
<td>(0.56-2.03)</td>
<td>(0.31-1.08)</td>
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</tr>
<tr>
<td>Hypertension</td>
<td>1.03</td>
<td>1.08</td>
<td>0.98</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>(0.73-1.46)</td>
<td>(0.71-1.62)</td>
<td>(0.66-1.45)</td>
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<td></td>
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</tr>
<tr>
<td>Hyperlipidemia</td>
<td>2.43</td>
<td>2.16</td>
<td>1.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.65-3.63)</td>
<td>(1.35-3.51)</td>
<td>(1.16-2.82)</td>
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<tr>
<td>Smoking status</td>
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<tr>
<td>Ex-smoker</td>
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<td>1.02</td>
<td>0.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.58-1.25)</td>
<td>(0.65-1.59)</td>
<td>(0.42-1.00)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.4</td>
<td>1.56</td>
<td>1.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.87-2.22)</td>
<td>(0.9-2.68)</td>
<td>(0.62-1.83)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of CHD</td>
<td>1.26</td>
<td>1.47</td>
<td>1.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.89-1.79)</td>
<td>(0.97-2.22)</td>
<td>(0.69-1.55)</td>
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<tr>
<td>Exercise ECG result</td>
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<tr>
<td>Inconclusive</td>
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<tr>
<td>Abnormal</td>
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<td>(1.77-4.98)</td>
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<tr>
<td>Log₂ (Calcium score)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>1.53</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>(1.42-1.65)</td>
</tr>
</tbody>
</table>

Table 5.4 Multi-variable predictors of obstructive coronary artery disease

CAD, coronary artery disease; CCTA, coronary computed tomography angiography; CI, confidence interval; hs-cTnI, high-sensitivity cardiac troponin I; CHD, coronary heart disease; ECG, electrocardiography.
### Cardiac troponin I concentrations by quintile (range [ng/L])

**Abbott hs-cTnI Assay**

<table>
<thead>
<tr>
<th>Quintile</th>
<th>Q1 [≤1ng/L]</th>
<th>Q2 [1.1-1.9ng/L]</th>
<th>Q3 [2-2.5ng/L]</th>
<th>Q4 [2.6-4.1ng/L]</th>
<th>Q5 [≥4.2ng/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>198</td>
<td>188</td>
<td>183</td>
<td>178</td>
<td>184</td>
</tr>
<tr>
<td>Age, years</td>
<td>54.25 (9.40)</td>
<td>55.67 (10.33)</td>
<td>58.28 (9.05)</td>
<td>59.80 (9.04)</td>
<td>60.51 (8.68)</td>
</tr>
<tr>
<td>Male, %</td>
<td>62 (31.3)</td>
<td>103 (54.8)</td>
<td>104 (56.8)</td>
<td>123 (69.1)</td>
<td>139 (75.5)</td>
</tr>
<tr>
<td>Chest pain symptom, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-anginal</td>
<td>72 (36.4)</td>
<td>75 (39.9)</td>
<td>64 (35.0)</td>
<td>58 (32.6)</td>
<td>46 (25.0)</td>
</tr>
<tr>
<td>Atypical angina</td>
<td>55 (27.8)</td>
<td>47 (25.0)</td>
<td>48 (26.2)</td>
<td>32 (18.0)</td>
<td>39 (21.2)</td>
</tr>
<tr>
<td>Typical angina</td>
<td>71 (35.9)</td>
<td>66 (35.1)</td>
<td>71 (38.8)</td>
<td>88 (49.4)</td>
<td>99 (53.8)</td>
</tr>
<tr>
<td>BMI</td>
<td>29.20 (5.72)</td>
<td>29.68 (5.87)</td>
<td>29.93 (5.64)</td>
<td>29.57 (5.17)</td>
<td>29.64 (5.50)</td>
</tr>
<tr>
<td>Pre-existing CHD, %</td>
<td>10 (5.1)</td>
<td>16 (8.5)</td>
<td>14 (7.7)</td>
<td>20 (11.2)</td>
<td>19 (10.3)</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>55 (27.8)</td>
<td>50 (27.0)</td>
<td>62 (34.1)</td>
<td>82 (46.3)</td>
<td>85 (47.0)</td>
</tr>
<tr>
<td>Hyperlipidemia, %</td>
<td>101 (51.0)</td>
<td>103 (54.8)</td>
<td>122 (66.7)</td>
<td>109 (61.2)</td>
<td>125 (67.9)</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>23 (11.6)</td>
<td>18 (9.6)</td>
<td>21 (11.5)</td>
<td>15 (8.4)</td>
<td>22 (12.0)</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>37 (18.7)</td>
<td>46 (24.5)</td>
<td>34 (18.6)</td>
<td>26 (14.6)</td>
<td>31 (16.8)</td>
</tr>
<tr>
<td>Family history of CHD, %</td>
<td>89 (45.9)</td>
<td>93 (49.7)</td>
<td>83 (45.9)</td>
<td>67 (37.9)</td>
<td>62 (33.9)</td>
</tr>
<tr>
<td>10-year CHD risk*</td>
<td>11.00 [6.00, 18.00]</td>
<td>14.50 [8.75, 23.00]</td>
<td>16.00 [10.50, 23.00]</td>
<td>17.00 [13.00, 25.00]</td>
<td>19.00 [15.00, 28.25]</td>
</tr>
</tbody>
</table>

### Table 5.5 Baseline characteristics of patients with suspected angina stratified by cardiac troponin using the Abbott hs-cTnI assay

Data are mean (standard deviation), median [IQR], or value (%); BMI, body mass index; CHD, coronary heart disease.

*ASSIGN Score (see http://assign-score.com/)
### Table 5.6 Exercise electrocardiography and coronary computed tomography findings by troponin quintile using the Abbott hs-cTnI assay

Data are median [IQR], or value (%); ECG, electrocardiography; IQR, interquartile range; CHD, coronary heart disease; SSS, segment stenosis score.
5.4.4 UPDATE AND EXTENSION OF THE CADC MODEL

Compared to the cohort used to develop the CADC model, participants in our cohort were younger and less likely to have typical angina or obstructive disease on coronary imaging (Table 5.7). Goodness-of-fit for the baseline CADC model was adequate (p=0.324). On adding cardiac troponin concentrations, the model fit improved (difference in deviance 20.3, 1 degrees of freedom, p<0.001).

The addition of cardiac troponin concentration improved overall model performance (D 0.215 to 0.246; Table 5.8) including discrimination (c-statistic: 0.785 to 0.800, p=0.003; Figure 5.2). The addition of cardiac troponin concentration also improved classification of patients into ESC risk categories (Table 5.9, Figure 5.3). In patients with and without obstructive coronary disease, 10.1% and 4.5% were appropriately reclassified respectively, whilst 0.3% were inappropriately reclassified (net reclassification index (NRI), 0.143 [95% CI, 0.093-0.193]). Similar findings to the primary analysis were seen when samples were analysed with the Abbott Architect high sensitivity assay (Tables 5.10-5.11, Figures 5.4-5.5).
Novel approaches to the diagnostic and prognostic assessment of coronary heart disease

<table>
<thead>
<tr>
<th>Performance measure</th>
<th>CADC Model</th>
<th>CADC Model with troponin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.215</td>
<td>0.246</td>
</tr>
<tr>
<td>Coefficient of Discrimination</td>
<td>0.164</td>
<td>0.159</td>
</tr>
<tr>
<td>C-statistic [95% CI]</td>
<td>0.785</td>
<td>0.800</td>
</tr>
<tr>
<td>[0.755-0.816]</td>
<td>[0.770-0.829]*</td>
<td></td>
</tr>
<tr>
<td>Calibration (Hosmer-Lemeshow Test)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chi-square</td>
<td>9.221</td>
<td>14.746</td>
</tr>
<tr>
<td>P-value</td>
<td>0.324</td>
<td>0.064</td>
</tr>
<tr>
<td>NRI (Categorical) [95% CI]</td>
<td>NA</td>
<td>0.143</td>
</tr>
<tr>
<td>[0.093 – 0.193]</td>
<td>[1.8-6.6]</td>
<td></td>
</tr>
<tr>
<td>NRI for patients with CAD</td>
<td>10.1%</td>
<td></td>
</tr>
<tr>
<td>NRI for patients without CAD</td>
<td>4.2%</td>
<td></td>
</tr>
<tr>
<td>Statistics at 15% PTP threshold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.930</td>
<td>0.936</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.408</td>
<td>0.430</td>
</tr>
<tr>
<td>PPV</td>
<td>0.475</td>
<td>0.519</td>
</tr>
<tr>
<td>NPV</td>
<td>0.910</td>
<td>0.910</td>
</tr>
</tbody>
</table>

Table 5.7 Characteristics of SCOT-HEART biomarker sub-study cohort and CAD Consortium cohort

Values are percentage or mean ± standard deviation. CAD, coronary artery disease; SD, standard deviation; CA, coronary angiography; CCTA, coronary computed tomography angiography.

Table 5.8 Coronary Artery Disease Consortium (CADC) model statistics

CADC, Coronary Artery Disease Consortium; CI, confidence interval; NRI, net reclassification improvement; PTP, pre-test probability; PPV, positive predictive value; NPV, negative predictive value; *p = 0.003 that true difference in AUC is not equal to 0.
Figure 5.2 Receiver operating characteristic curve for the prediction of obstructive coronary artery disease

CADC, Coronary Artery Disease Consortium; AUC, area under curve; hs-cTnI, high-sensitivity cardiac troponin I.
### Table 5.9 Net reclassification with the addition of cardiac troponin I to the CADC model

CADC, Coronary Artery Disease Consortium; NRI, net reclassification improvement; CI, confidence interval.
Figure 5.3 Cardiac troponin improves predicted risk of obstructive coronary artery disease in patients with suspected angina

The red dots represent the risk of obstructive CAD as estimated by the established CAD Consortium model accounting for age, sex and symptom description. The blue dots represent the revised risk estimates with the addition of cardiac troponin quintiles. The shaded regions correspond to the risk groups and associated recommendations for further investigations as described in the ESC guidelines on the management of stable CAD.

CAD, coronary artery disease; ESC, European Society of Cardiology; hs-cTnI, high-sensitivity cardiac troponin I; ECG, electrocardiography; y, years.
<table>
<thead>
<tr>
<th>Performance measure</th>
<th>CADC Model</th>
<th>CADC Model with troponin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient of Discrimination</td>
<td>0.214</td>
<td>0.255</td>
</tr>
<tr>
<td>Brier score</td>
<td>0.161</td>
<td>0.155</td>
</tr>
<tr>
<td>Discrimination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-statistic [95% CI]</td>
<td>0.787 [0.756-0.834]</td>
<td>0.805 [0.775-0.834]*</td>
</tr>
<tr>
<td>Calibration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Hosmer-Lemeshow Test)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chi-square</td>
<td>9.064</td>
<td>6.529</td>
</tr>
<tr>
<td>P-value</td>
<td>0.337</td>
<td>0.588</td>
</tr>
<tr>
<td>NRI (Categorical) [95% CI]</td>
<td>N/A</td>
<td>0.117 [0.058-0.176]</td>
</tr>
<tr>
<td>NRI for patients with CAD</td>
<td>N/A</td>
<td>8.4% [3.1-13.7]</td>
</tr>
<tr>
<td>NRI for patients without CAD</td>
<td>N/A</td>
<td>3.3% [0.6-5.9]</td>
</tr>
<tr>
<td>Statistics at 15% PTP threshold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.935</td>
<td>0.929</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.405</td>
<td>0.421</td>
</tr>
<tr>
<td>PPV</td>
<td>0.472</td>
<td>0.514</td>
</tr>
<tr>
<td>NPV</td>
<td>0.916</td>
<td>0.900</td>
</tr>
</tbody>
</table>

**Table 5.10 Model statistics using the Abbott ARCHITECT STAT assay**

CADC, Coronary Artery Disease Consortium; CI, confidence interval; NRI, net reclassification improvement; PTP, pre-test probability; PPV, positive predictive value; NPV, negative predictive value; *p = 0.003 that true difference in AUC is not equal to 0.
### Outcome: No Obstructive Disease

<table>
<thead>
<tr>
<th>CADC Model</th>
<th>Low Risk (&lt;15%)</th>
<th>Intermediate Risk (15-65%)</th>
<th>High Risk (≥65%)</th>
<th>Reclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Risk</td>
<td>298</td>
<td>16</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Intermediate Risk (15-65%)</td>
<td>44</td>
<td>269</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>High Risk (≥65%)</td>
<td>0</td>
<td>7</td>
<td>19</td>
<td>27</td>
</tr>
</tbody>
</table>

### Outcome: Obstructive Disease

<table>
<thead>
<tr>
<th>CADC Model</th>
<th>Low Risk (&lt;15%)</th>
<th>Intermediate Risk (15-65%)</th>
<th>High Risk (≥65%)</th>
<th>Reclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Risk</td>
<td>19</td>
<td>3</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Intermediate Risk (15-65%)</td>
<td>7</td>
<td>151</td>
<td>34</td>
<td>21</td>
</tr>
<tr>
<td>High Risk (≥65%)</td>
<td>0</td>
<td>8</td>
<td>40</td>
<td>17</td>
</tr>
</tbody>
</table>

NRI(Categorical) [95% CI]: 0.1170 [0.0579-0.1761]
NRI(Continuous) [95% CI]: 0.5366 [0.3979-0.6752]

**Table 5.11 Net reclassification with the addition of cardiac troponin I to the CADC model (Abbott ARCHITECT STAT assay)**

CADC, Coronary Artery Disease Consortium; NRI, net reclassification improvement; CI, confidence interval.
Figure 5.4 Updated Risk Model for Obstructive CAD Incorporating Troponin Quintiles (Abbott ARCHITECTSTAT assay)

The red dots represent the risk of obstructive CAD as estimated by the established CAD Consortium model accounting for age, sex and symptom description. The blue dots represent the revised risk estimates with the addition of cardiac troponin quintiles. The shaded regions correspond to the risk groups and associated recommendations for further investigations as described in the ESC guidelines on the management of stable CAD.

CAD, coronary artery disease; ESC, European Society of Cardiology; hs-cTnI, high-sensitivity cardiac troponin I; ECG, electrocardiography; y, years.
Figure 5.5 Receiver operating characteristic curve for the prediction of obstructive coronary artery disease (Abbott ARCHITECT STAT assay)

CADC, Coronary Artery Disease Consortium Risk Model; hs-cTnI, high-sensitivity cardiac troponin I; AUC, area under curve.
5.4.5 **EXTERNAL VALIDATION**

The validation cohort has been previously described (Hosbond, Diederichsen et al. 2014, Madsen, Diederichsen et al. 2017) and a summary of baseline characteristics is provided in Table 5.12. The overall prevalence of obstructive coronary disease was 19.3% and again, a five-fold increase was seen across troponin quintiles. The addition of cardiac troponin concentration improved overall model performance (D 0.188 to 0.241) including discrimination (c-statistic: 0.761 to 0.784, p=0.008; Figure 5.6). The addition of cardiac troponin concentration also improved classification of patients into ESC risk categories (Table 5.13).

| Cardiac troponin I concentrations by quintile (range [ng/L]) | (Abbott ARCHITECT STAT Assay) |
|---|---|---|---|---|---|
| n | 104 | 111 | 81 | 88 | 103 |
| Age, years | 53.4 (10.1) | 56.3 (9.3) | 58.6 (10.1) | 60.7 (10.9) | 62.8 (12.3) |
| Male, % | 40 (38.5) | 45 (40.5) | 46 (56.8) | 55 (62.5) | 76 (73.8) |
| Chest pain symptom, % | | | | | |
| Non-anginal | 32 (30.8) | 29 (26.1) | 25 (30.9) | 23 (26.1) | 29 (28.2) |
| Atypical angina | 33 (31.7) | 47 (42.3) | 24 (29.6) | 26 (29.5) | 28 (27.2) |
| Typical angina | 39 (37.5) | 35 (31.5) | 32 (39.5) | 39 (44.3) | 46 (44.7) |
| Obstructive CHD, % | 8 (7.7) | 15 (13.5) | 11 (13.6) | 20 (22.7) | 40 (38.8) |

**Table 5.12 Baseline characteristics in external validation cohort stratified by cardiac troponin using the Abbott ARCHITECT STAT assay**

Data are mean (standard deviation), median [IQR], or value (%); CHD, coronary heart disease.
Figure 5.6 Receiver operating characteristic curve for the prediction of obstructive coronary artery disease in the external validation cohort (Abbott ARCHITECT STAT assay)

CADC, Coronary Artery Disease Consortium Risk Model; hs-cTnl, high-sensitivity cardiac troponin I; AUC, area under curve.
### Outcome: No Obstructive Disease

<table>
<thead>
<tr>
<th>CADC Model with cardiac troponin</th>
<th>Low Risk (&lt;15%)</th>
<th>Intermediate Risk (15-65%)</th>
<th>High Risk (≥65%)</th>
<th>% Reclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Risk (&lt;15%) CADC Model</td>
<td>128</td>
<td>25</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Intermediate Risk (15-65%)</td>
<td>1</td>
<td>212</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>High Risk (≥65%)</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

### Outcome: Obstructive Disease

<table>
<thead>
<tr>
<th>CADC Model with cardiac troponin</th>
<th>Low Risk (&lt;15%)</th>
<th>Intermediate Risk (15-65%)</th>
<th>High Risk (≥65%)</th>
<th>% Reclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Risk (&lt;15%) CADC Model</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>Intermediate Risk (15-65%)</td>
<td>0</td>
<td>48</td>
<td>20</td>
<td>29</td>
</tr>
<tr>
<td>High Risk (≥65%)</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
</tbody>
</table>

NRI(Categorical) [95% CI]: 0.1672 [0.0714-0.263]
NRI(Continuous) [95% CI]: 0.0745 [-0.0001-0.1491]

Table 5.13 Net reclassification with the addition of cardiac troponin I to the CADC model in the external validation cohort (Abbott ARCHITECT STAT assay)

CADC, Coronary Artery Disease Consortium; NRI, net reclassification improvement; CI, confidence interval.
5.5 DISCUSSION

In the assessment of suspected stable angina, measurement of high-sensitivity cardiac troponin I improves the accuracy of the pre-test probability of obstructive coronary artery disease as estimated using the existing risk model endorsed by the European Society of Cardiology. Used in this manner, high-sensitivity troponin testing can appropriately reclassify one in seven patients. This simple investigation has potential to improve the appropriate use of diagnostic stress imaging tests in 10.1% of patients with obstructive coronary disease, and to reduce unnecessary testing in 4.2% of those without disease. We have developed a risk estimation tool that incorporates cardiac troponin I concentrations to allow clinicians to improve their estimation of pre-test probability for coronary disease.

Our study has a number of notable strengths. First, we chose to use a troponin assay with exceptional analytical characteristics (Apple and Collinson 2012), including a diagnostic sensitivity that outperforms other available platforms and that was able to detect cardiac troponin concentrations in 99.6% of our population, and to accurately quantify cardiac troponin concentrations in 96.8% of patients. Second, as this study was nested within a larger randomised trial of CCTA imaging in patients with suspected angina, we were able to minimise the potential for case ascertainment bias that can arise when the decision to proceed to coronary imaging is dependent on clinician perception of coronary disease risk. Third, we made use of state-of-the-art CT imaging using a 320-slice scanner to define the presence and extent of coronary artery disease in all patients. Fourth, the prospective nature of this study enabled detailed and accurate phenotypic characterisation of patients at baseline and
comprehensive clinical follow-up. Finally, we demonstrated the externally validity of the derived model in an independent cohort from a separate European country.

Current guidelines recommend a routine full blood count and measurement of renal function to identify drivers of myocardial ischaemia and improve risk prediction. They also encourage analysis of lipid profiles and glycaemic indices as these represent important cardiovascular risk factors. Whilst acknowledging that elevations in troponin have some prognostic value in stable patients, the consensus opinion in 2013 (European Society of Cardiology Task Force 2013) was that there was insufficient independent prognostic value to warrant routine measurement. This viewpoint is now being challenged by a growing body of evidence that demonstrates cardiac troponin does have independent prognostic value, and may even be a useful indicator of therapeutic response (Zeller, Tunstall-Pedoe et al. 2014, Everett, Brooks et al. 2015, Everett, Zeller et al. 2015).

Overall, our findings expand on this research demonstrating that troponin concentrations predict the presence of obstructive coronary artery disease in patients with suspected stable angina. The mechanisms behind this association, including ventricular strain (Chin, Shah et al. 2014), and myocardial ischaemia (Lee, Twerenbold et al. 2016) are now emerging. Additionally, it seems apparent from our study that atherosclerotic burden plays an important role. Whether these low concentrations of troponin reflect subclinical myocardial necrosis related to coronary plaque disruption and microvascular disease, or increased myocardial cell turnover remains to be determined. To our knowledge this is the first time a circulating biomarker has been shown to provide improved discrimination for the diagnosis of
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stable obstructive coronary artery disease beyond established risk factors. Importantly, this improvement results in successful reclassification of patients into more appropriate diagnostic pathways which could enable more rational use of subsequent investigations.

The high-sensitivity assay used in this study has particularly robust analytical characteristics but is presently available for research use only. We were able to measure troponin concentrations in more than 99% of the population across both sexes and a wide range of ages. Our internal validation demonstrated consistent results when using a commercially available test, but it is important to note the risk calculation will be assay specific. Whether our findings can be extrapolated to alternative clinical assays is unclear, but it would be prudent for manufacturers to validate each testing platform individually before considering use in this setting where troponin concentrations are approaching the limits of detection. Furthermore, we cannot be certain of how knowledge of troponin concentration may influence clinical management decisions as treating clinicians did not have access to the biomarker results during the conduct of the trial.

We made use of the latest generation of CT scanners developed with a focus on advancing the performance of coronary computed tomography angiography. Although some authors may suggest that invasive coronary angiography remains the reference standard, it seems unlikely that troponin would be related to CT-defined coronary artery disease independent of the presence and extent of true coronary artery disease. As such, any misclassification is likely to be non-differential with respect to troponin, and hence to cause us to underestimate the association between troponin and stable
coronary artery disease, and the predictive performance of the model. Moreover, the chosen criteria for defining significant coronary disease on CCTA has previously been shown to correlate well with invasive angiographic findings and with non-invasively determined myocardial ischaemia (Gaemperli, Schepis et al. 2008). Indeed, in the SCOT-HEART trial, CCTA was associated with a >60% reduction in the rate of normal coronary angiography and a 30% increase in obstructive disease when downstream invasive coronary angiography was performed (The SCOT-HEART investigators 2016). We also contend that a particular strength of this study arises from it being nested within a larger trial which randomised patients to coronary imaging, thereby minimising the case ascertainment bias inherent in earlier trials that only included patients referred for invasive coronary angiography. This applicability to the general population is reflected in the relatively lower rates of obstructive disease identified compared with previous reports.

We added a single additional continuous variable to an existing model. As such, the improvement in model performance by adding cardiac troponin is unlikely to have been substantially inflated by overfitting. Confirmation of this is demonstrated by our findings on applying the model to the external validation cohort. Indeed, it appears increasingly likely, given the potential prognostic and diagnostic information cardiac troponin offers, that indications for testing outside the acute coronary syndrome setting now exist.
5.6 Conclusion

Plasma high-sensitivity cardiac troponin I concentrations independently predict the presence of obstructive coronary disease in patients with suspected stable angina. Employing this test within the chest pain clinic may improve the selection of patients for further investigation and treatment of coronary artery disease.
CHAPTER 6

CARDIAC TROPOIN I AND RISK OF
CARDIOVASCULAR EVENTS IN PATIENTS WITH
COPD AND HEIGHTENED CARDIOVASCULAR RISK
CHAPTER 6 CARDIAC TROPNIN I AND RISK OF CARDIOVASCULAR EVENTS IN PATIENTS WITH COPD AND HEIGHTENED CARDIOVASCULAR RISK

6.1 SUMMARY

BACKGROUND
Patients with chronic obstructive pulmonary disease (COPD) are at increased risk of cardiovascular events. We evaluated the association between plasma high-sensitivity cardiac troponin I concentration and cardiovascular events in patients with COPD and heightened cardiovascular risk.

METHODS
In a double-blind randomised controlled trial, 16,485 patients with COPD and cardiovascular disease or risk factors were randomised to once daily inhaled placebo, fluticasone furoate (100 µg), vilanterol (25 µg), or their combination. Plasma high-sensitivity cardiac troponin I concentrations (Abbott Diagnostics) were measured in a subgroup of 1,599 patients. Outcomes were cardiovascular events or COPD exacerbations over a median follow up of 18 months.

RESULTS
Baseline plasma cardiac troponin I concentrations were above the lower limit of detection in 1,559 (97.5%) patients and were unaffected by inhaled therapies at 3 months (p>0.05 for all). Compared with the lowest tertile (cardiac troponin I concentrations ≤3.0 ng/L), patients in the highest tertile (≥5.5 ng/L) were at greater risk of cardiovascular events (hazard ratio [HR] 3.0, 95% confidence interval [CI] 1.5 to 6.2, p=0.002) and cardiovascular death (HR 9.6, 95% CI 2.6 to 35.6, p<0.001) after
adjustment for cardiovascular risk factors. In contrast, there were no differences in COPD exacerbations between tertiles (HR 1.0, 95%CI 0.8 to 1.3, p=0.923).

**CONCLUSION**

In patients with COPD and heightened cardiovascular risk, plasma cardiac troponin I concentrations are a specific and major predictor of future cardiovascular events and cardiovascular death. Inhaled therapies did not affect cardiac troponin I concentrations consistent with their neutral effect on all-cause mortality and cardiovascular outcomes.
6.2 Introduction

Cardiovascular disease, including ischaemic heart disease and stroke, accounts for 1 in 4 deaths globally and is increasing in prevalence (Lozano, Naghavi et al. 2012). Despite recent advances in our understanding of risk factors and therapeutic interventions, atherosclerotic events remain unacceptably common. Residual risk is particularly high amongst patients with pro-inflammatory comorbidities, such as chronic obstructive pulmonary disease (COPD) (Mullerova, Agusti et al. 2013). In some cases, it remains unclear whether it is the disease process itself, or the off-target effects of the pharmacological treatments, that contribute to this elevated risk (Woodruff 2013).

Notwithstanding prior major advances, future clinical trial conduct is hampered by several important and increasing challenges. It is well recognised that clinical trial participants represent a relatively low-risk subset of the real-world patient population. Consequently, modest event rates necessitate large and costly trials in order to demonstrate treatment efficacy. Conversely, this low event rate creates the potential for researchers to fail to recognise cardiovascular harms related to new medications prior to their clinical approval (Bresalier, Sandler et al. 2005). These challenges have contributed to growing interest in the search for better biomarkers suitable for use as a surrogate for treatment efficacy and safety.

An ideal cardiovascular biomarker needs to be a sensitive as well as specific indicator of cardiovascular risk. High-sensitivity cardiac troponin I is a potential suitable candidate to fill this role. Plasma concentrations can be reliably quantified in the vast majority of apparently healthy individuals and numerous studies have demonstrated
clear associations between elevated plasma troponin concentrations and cardiovascular events in both primary and secondary prevention populations (Omland, de Lemos et al. 2009, de Lemos, Drazner et al. 2010, deFilippi, de Lemos et al. 2010, Eggers, Venge et al. 2013, Omland, Pfeffer et al. 2013, Zeller, Tunstall-Pedoe et al. 2014, Everett 2017). Furthermore, plasma cardiac troponin I concentrations measured by a high-sensitivity assay have recently been shown to be modifiable, with statin-induced reductions in cardiac troponin I proving a more powerful indicator of treatment efficacy than changes in serum cholesterol (Ford, Shah et al. 2016). The role of serial testing with high-sensitivity cardiac troponin I to predict the effect of other therapies on cardiovascular outcomes has to date been unexplored in patients with more diverse multi-morbid conditions.

The Study to Understand Mortality and MorbidITy (SUMMIT) (Vestbo, Anderson et al. 2013, Vestbo, Anderson et al. 2016) assessed the efficacy and safety of inhaled corticosteroids and long-acting beta agonists in 16,485 patients with COPD and heightened cardiovascular risk. This was a multi-morbid population with interventions that could have both benefit (Calverley, Anderson et al. 2007) and harm (Salpeter, Ormiston et al. 2004). The present study contains post-hoc analyses aiming to determine whether plasma high-sensitivity cardiac troponin I concentrations could stratify cardiovascular risk, be modified by inhaled corticosteroids and bronchodilators, and predict outcomes within the context of SUMMIT.
6.3 METHODS

6.3.1 STUDY POPULATION

SUMMIT was a prospective, multi-centre, international randomised controlled trial to determine whether treatment with an inhaled long-acting beta-agonist (LABA), inhaled corticosteroid (ICS) or both in combination, could improve clinical outcomes in patients with moderate chronic obstructive pulmonary disease (COPD) and increased cardiovascular risk. Details regarding study design have been previously published (Vestbo, Anderson et al. 2013, Vestbo, Anderson et al. 2016). In brief, eligible participants included current or former smokers (≥10 pack-years) between the ages of 40 and 80 years, with a history of COPD and a post-bronchodilator FEV$_1$ ≥50 and ≤70% of the predicted value, a ratio of post-bronchodilator FEV$_1$ to forced vital capacity ≤0.70, and a score ≥2 on the modified Medical Research Council dyspnoea scale. Patients were additionally required to have a history, or be at increased risk, of cardiovascular disease. Cardiovascular disease was defined as coronary artery disease, peripheral arterial disease, prior stroke or myocardial infarction, or diabetes mellitus with target organ disease. Increased cardiovascular risk was defined as being ≥60 years and receiving medications for two or more of the following: hypercholesterolemia, hypertension, diabetes mellitus or peripheral vascular disease.

While prior inhaled corticosteroids and long-acting beta agonist treatments were discontinued before study entry, other COPD medications were permitted during the trial. Participants were then allocated equally to one of four randomised treatments: placebo, fluticasone furoate (FF, 100µg), vilanterol (VI, 25µg) or their combination
Novel approaches to the diagnostic and prognostic assessment of coronary heart disease

(FF/VI, 100/25µg) inhaled once daily as a dry powder. A total of 16,485 patients were enrolled and included in the final intention-to-treat efficacy population.

6.3.2 ENDPOINTS

In addition to the primary analysis of all-cause mortality by intention-to-treat analysis, the secondary cardiovascular endpoint was time to first-on-treatment cardiovascular event comprising cardiovascular death, myocardial infarction, stroke, unstable angina and transient ischaemic attack (Brook, Anderson et al. 2017). Categorisation of the cause of each death was adjudicated by a clinical endpoint committee blinded to the treatment allocation who also determined whether any reported cardiovascular event met the definition of the composite endpoint (Vestbo, Anderson et al. 2013). Individuals discontinuing study treatments could not be assessed for the adjudicated composite cardiovascular endpoint as follow-up visits were not performed and only data regarding mortality were available. The primary respiratory endpoint comprised moderate or severe exacerbations of COPD. Moderate exacerbations were defined as a symptomatic deterioration requiring treatment with antibiotic drugs or systemic corticosteroids, whereas severe exacerbations were defined as events leading to hospital admission.

6.3.3 HIGH-SENSITIVITY CARDIAC TROPONIN I

Venous blood samples were obtained before randomisation and at 3 months. Blood was processed and plasma stored at −80°C until analysed. Plasma high-sensitivity cardiac troponin I concentrations were measured at a single site using the ARCHITECT STAT high-sensitive cardiac troponin I assay (Abbott Laboratories, Abbott Park, Illinois, USA), which has a limit of detection of 1.2 ng/L, coefficient of variation
<10% at 4.7 ng/L and gender-specific 99\textsuperscript{th} centile upper reference limits of 16 and 34 ng/L in women and men respectively (Apple and Collinson 2012, Shah, Griffiths et al. 2015).

\textbf{6.3.4 Statistical analysis}

Forty patients with plasma cardiac troponin I concentrations <1.0 ng/L were imputed with a value of 0.5 ng/L. Cardiac troponin I concentrations were log-transformed prior to statistical modelling, and results transformed back to the original scale. To determine which patient characteristics were associated with baseline cardiac troponin I, regression modelling was performed. The final model was achieved using backwards selection where to remain in the model all variables needed \(p<0.1\). To test whether inhaled corticosteroid or long acting beta agonist therapy affected cardiac troponin I values at 3 months, an analysis of covariance (ANCOVA) was performed adjusting for baseline cardiac troponin I, age, sex, prior myocardial infarction and hypertension.

Patients were grouped into tertiles based on their baseline cardiac troponin I concentrations. To explore the effect of baseline cardiac troponin I tertile on each of the study endpoints (cardiovascular composite, cardiovascular death and COPD exacerbations), analysis of time-to-first event was performed using Cox proportional hazards regression modelling, adjusted for age, sex, study therapy and cardiovascular risk factors of prior myocardial infarction and hypertension.

Previous reports have identified adverse cardiovascular outcomes are associated with plasma troponin I concentrations \(\geq 5\) ng/L (Shah, Anand et al. 2015, Ford, Shah et al. 2016). To explore this association further, and investigate whether the predictive value of this threshold could be applied to the SUMMIT population, patients were grouped
into those who had plasma concentrations (i) <5 ng/L at both baseline and three months, (ii) <5 ng/L at baseline and $\geq 5$ng/L at three months, (iii) $\geq 5$ ng/L at baseline and <5 ng/L at three months, or (iv) $\geq 5$ng/L at both baseline and three months.

Scientific oversight of the trial was provided by a steering committee composed of academic experts and employees from GlaxoSmithKline, who were collectively responsible for the study design and analysis, and for the review and interpretation of the data. This study is registered with ClinicalTrials.gov, number NCT01313676.
6.4 RESULTS

The study population and principal findings of SUMMIT have previously been described (Vestbo, Anderson et al. 2016). Between January 2011 and March 2014, 16,485 participants were recruited and included in the primary intention-to-treat analysis. The majority of recruited patients had established cardiovascular disease (n=10,961; 67%) or diabetes mellitus with end-organ damage (n=701 [4%]), whilst a minority (n=4,641 [28%]) fulfilled the criteria for an increased risk of cardiovascular disease only and 182 (1%) did not meet the cardiovascular entry criteria (Brook, Anderson et al. 2017). Blood samples were taken prior to randomization from 1,673 patients based in the United States (SUMMIT biomarker population), of which baseline cardiac troponin I concentrations were assessed in 1,599 patients and 1,258 had a second troponin measurement performed 3 months after randomisation.

6.4.1 DISTRIBUTION OF HIGH-SENSITIVITY CARDIAC TROPONIN I AT BASELINE

Cardiac troponin I concentrations were ≥1.2 ng/L in 1,542 participants (96%) and above the sex-specific 99th centile (16 ng/L in women, 34 ng/L in men) in 42 participants (2.6%). The median cardiac troponin I concentration was 4.0 (interquartile range [IQR] 2.6 to 6.7) ng/L.

Although broadly similar, the patient characteristics in the biomarker sub-study population demonstrated some potentially important differences from the overall SUMMIT population. In particular, participants in the biomarker population were more likely to be female, had higher body-mass index, had fewer previous exacerbations and had differences in cardiovascular history and cardiovascular therapy (Table 6.1). Participants were stratified into tertiles by plasma cardiac troponin I
concentration from samples obtained prior to randomisation. Compared to the lowest tertile (≤3.0 ng/L), patients in the highest tertile (≥5.5 ng/L) were older, more likely to be male, former smokers, have higher systolic blood pressure, a history of ischemic heart disease, coronary artery disease, congestive heart failure, hypercholesterolemia, hypertension, diabetes mellitus, a family history of myocardial infarction or stroke and to be receiving treatment with anti-platelet and statin therapies.

A number of patient characteristics appeared associated with baseline plasma cardiac troponin I concentration (Table 6.2). In a multivariate linear regression model, higher baseline plasma cardiac troponin I concentrations were associated with increasing age, male gender and other cardiovascular risk factors. After adjustment for other variables, increased baseline FEV\textsubscript{1} appeared to be associated with lower baseline cardiac troponin I concentration.
Novel approaches to the diagnostic and prognostic assessment of coronary heart disease

<table>
<thead>
<tr>
<th></th>
<th>Tertile 1 (&lt;3 ng/L)</th>
<th>Tertile 2 (≥3 to &lt;5.5 ng/L)</th>
<th>Tertile 3 (≥5.5 ng/L)</th>
<th>Biomarker Sub-Study</th>
<th>ITT-E Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>515</td>
<td>546</td>
<td>538</td>
<td>1,673</td>
<td>16,485</td>
</tr>
<tr>
<td>Age, years</td>
<td>63 (8)</td>
<td>67 (8)</td>
<td>68 (7)</td>
<td>66 (8)</td>
<td>65 (8)</td>
</tr>
<tr>
<td>Female</td>
<td>266 (52%)</td>
<td>207 (38%)</td>
<td>137 (25%)</td>
<td>635 (38%)</td>
<td>4,196 (25%)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30 (7)</td>
<td>31 (7)</td>
<td>31 (7)</td>
<td>31 (7)</td>
<td>28 (6)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>128 (15)</td>
<td>131 (15)</td>
<td>133 (18)</td>
<td>131 (16)</td>
<td>135 (15)</td>
</tr>
<tr>
<td>Heart rate, beats/minute</td>
<td>74 (10)</td>
<td>73 (12)</td>
<td>73 (11)</td>
<td>73 (11)</td>
<td>76 (10)</td>
</tr>
<tr>
<td>Past medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior MI or revascularisation</td>
<td>126 (24%)</td>
<td>185 (34%)</td>
<td>268 (50%)</td>
<td>601 (36%)</td>
<td>3,436 (21%)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>200 (39%)</td>
<td>246 (45%)</td>
<td>332 (62%)</td>
<td>818 (49%)</td>
<td>8,379 (51%)</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>21 (4%)</td>
<td>36 (7%)</td>
<td>85 (16%)</td>
<td>146 (9%)</td>
<td>3,456 (21%)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>421 (82%)</td>
<td>481 (88%)</td>
<td>491 (91%)</td>
<td>1458 (87%)</td>
<td>11,518 (70%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>437 (85%)</td>
<td>508 (93%)</td>
<td>507 (94%)</td>
<td>1519 (91%)</td>
<td>14,851 (90%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>179 (35%)</td>
<td>213 (39%)</td>
<td>226 (42%)</td>
<td>642 (38%)</td>
<td>4,997 (30%)</td>
</tr>
<tr>
<td>Family history of CVD</td>
<td>216 (42%)</td>
<td>201 (37%)</td>
<td>246 (46%)</td>
<td>691 (41%)</td>
<td>3,429 (21%)</td>
</tr>
<tr>
<td>Respiratory history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former smoker</td>
<td>232 (45%)</td>
<td>293 (54%)</td>
<td>285 (53%)</td>
<td>845 (51%)</td>
<td>8,807 (53%)</td>
</tr>
<tr>
<td>Post-bronchodilator FEV₁, L</td>
<td>1.7 (0.4)</td>
<td>1.7 (0.4)</td>
<td>1.7 (0.4)</td>
<td>1.7 (0.4)</td>
<td>1.7 (0.4)</td>
</tr>
<tr>
<td>Exacerbations in 12 months before study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>370 (72%)</td>
<td>393 (72%)</td>
<td>403 (75%)</td>
<td>1,215 (73%)</td>
<td>10,021 (61%)</td>
</tr>
<tr>
<td>1</td>
<td>90 (17%)</td>
<td>97 (18%)</td>
<td>85 (16%)</td>
<td>290 (17%)</td>
<td>4,020 (24%)</td>
</tr>
<tr>
<td>2+</td>
<td>55 (11%)</td>
<td>56 (10%)</td>
<td>50 (9%)</td>
<td>168 (10%)</td>
<td>2,444 (15%)</td>
</tr>
<tr>
<td>Concomitant medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-platelet agent</td>
<td>301 (58%)</td>
<td>340 (62%)</td>
<td>397 (74%)</td>
<td>1,081 (65%)</td>
<td>8,517 (52%)</td>
</tr>
<tr>
<td>Statin</td>
<td>369 (72%)</td>
<td>416 (76%)</td>
<td>425 (79%)</td>
<td>1,263 (75%)</td>
<td>10,721 (65%)</td>
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<tr>
<td>Treatment allocation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>147 (29%)</td>
<td>131 (24%)</td>
<td>143 (27%)</td>
<td>439 (26%)</td>
<td>4,111 (25%)</td>
</tr>
<tr>
<td>Fluticasone furoate</td>
<td>115 (22%)</td>
<td>153 (28%)</td>
<td>121 (22%)</td>
<td>415 (25%)</td>
<td>4,135 (25%)</td>
</tr>
<tr>
<td>Vilanterol</td>
<td>141 (27%)</td>
<td>118 (22%)</td>
<td>139 (26%)</td>
<td>416 (25%)</td>
<td>4,118 (25%)</td>
</tr>
<tr>
<td>Combination therapy</td>
<td>112 (22%)</td>
<td>144 (26%)</td>
<td>135 (25%)</td>
<td>403 (24%)</td>
<td>4,121 (25%)</td>
</tr>
</tbody>
</table>
Table 6.1 Baseline characteristics split by cardiac troponin I tertile

*Of the 1,673 patients in the biomarker population, 74 did not have baseline cardiac troponin I measured and therefore not included in the cardiac troponin I tertiles and analyses. Data are mean (standard deviation), or n (%). ITT-E, intention-to-treat efficacy; BMI, body-mass index; CVD, cardiovascular disease; FEV₁, forced expiratory volume in 1 second; MI, myocardial infarction.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Effect</th>
<th>Univariate P-value</th>
<th>Multivariate Effect</th>
<th>Type III P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, 10 years</td>
<td>1.256</td>
<td>&lt;0.001</td>
<td>1.221</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender, male</td>
<td>1.430</td>
<td>&lt;0.001</td>
<td>1.435</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, 5 kg/m²</td>
<td>1.045</td>
<td>0.004</td>
<td>1.046</td>
<td>0.002</td>
</tr>
<tr>
<td>Heart rate, 10 bpm</td>
<td>0.973</td>
<td>0.146</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, 10 mmHg</td>
<td>1.063</td>
<td>&lt;0.001</td>
<td>1.056</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Past medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior myocardial infarction or coronary revascularisation</td>
<td>1.404</td>
<td>&lt;0.001</td>
<td>1.306</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>1.338</td>
<td>&lt;0.001</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>1.716</td>
<td>&lt;0.001</td>
<td>1.485</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>1.302</td>
<td>&lt;0.001</td>
<td>1.169</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.506</td>
<td>&lt;0.001</td>
<td>1.297</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.095</td>
<td>0.034</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Family history of CVD</td>
<td>1.068</td>
<td>0.119</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Respiratory history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status, former smoker</td>
<td>1.104</td>
<td>0.018</td>
<td>0.918</td>
<td>0.041</td>
</tr>
<tr>
<td>Post-bronchodilator FEV₁, L Exacerbations in 12 months before study</td>
<td>0.980</td>
<td>0.657</td>
<td>0.864</td>
<td>0.007</td>
</tr>
<tr>
<td>1</td>
<td>0.919</td>
<td>0.131</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>2+</td>
<td>0.959</td>
<td>0.556</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Concomitant cardiovascular therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Platelet therapy</td>
<td>1.247</td>
<td>&lt;0.001</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Statin therapy</td>
<td>1.107</td>
<td>0.036</td>
<td>0.910</td>
<td>0.084</td>
</tr>
</tbody>
</table>

Table 6.2 Variables associated with baseline cardiac troponin I concentration

*P-value after adjusting for all other covariates first.
BMI, body-mass index; CVD, cardiovascular disease; FEV₁, forced expiratory volume in 1 second.
6.4.2 Baseline cardiac troponin and risk of clinical events

In the biomarker sub-study, during a median on-treatment follow-up of 1.5 (IQR 0.8 to 2.5) years, there were 74 (4.6%) patients with a composite cardiovascular event and 587 (36.7%) patients with a moderate or severe exacerbations of COPD. During a median on and post-treatment follow-up of 2.3 (IQR 1.6 to 3.1) years, there were 25 cardiovascular deaths.

Compared to the lowest tertile, participants in the highest tertile were at greater risk of experiencing a cardiovascular composite event (**Figure 6.1A**). This difference persisted after adjustment for confounding variables including cardiovascular risk factors (**Table 6.3**; HR 3.08, 95% confidence interval 1.52 to 6.26, p=0.002). Similarly, there was a marked increased risk for cardiovascular death in the highest tertile (**Table 6.3**; HR 9.64, 95% CI 2.61 to 35.61, p<0.001; **Figure 6.1B**). In contrast, there was no difference between the highest and lowest tertiles in the risk of moderate or severe COPD exacerbations (HR 1.01, 95% CI 0.81 to 1.26, p = 0.923; **Figure 6.1C**).
Hazard Ratios:

**Tertile 3 vs Tertile 1**: 3.08 (95% CI 1.52, 6.26)

**Tertile 2 vs Tertile 1**: 1.88 (95% CI 0.90, 3.89)

**Hazard Ratios**:

**Tertile 3 vs Tertile 1**: 9.64 (95% CI 2.61, 35.61)

**Tertile 2 vs Tertile 1**: 1.98 (95% CI 0.46, 8.53)
Figure 6.1 Kaplan–Meier plots

(A) cardiovascular composite events endpoint, (B) cardiovascular death and (C) COPD exacerbations. CI, confidence interval; CV, cardiovascular.

**Table 6.3 Time to first cardiovascular composite event and time to cardiovascular death by baseline cardiac troponin tertiles**

*Composite cardiovascular event comprising any of: cardiovascular death, myocardial infarction, stroke, unstable angina and transient ischaemic attack. CV, cardiovascular; CI, confidence interval.*
6.4.3 **Effect of treatment on cardiac troponin at 3 months**

Plasma cardiac troponin I concentrations at 3 months were unchanged from baseline (p>0.05 for all treatments; *Table 6.4*). There were no treatment-related differences in the change in cardiac troponin I concentration at 3 months (p>0.05 for all treatments). This was consistent with the lack of treatment effect on the cardiovascular composite endpoint (p>0.05 for all active treatments vs placebo, *Table 6.5*).

6.4.4 **Change in cardiac troponin and cardiovascular events**

Of the 1,258 patients with baseline and 3 month measurements, 673 (53%) had cardiac troponin concentrations below 5 ng/L on both occasions. Compared with this group, patients who had a plasma troponin ≥5 ng/L at either time point appeared to have increased rates of the composite cardiovascular endpoint (*Figure 6.2*).
Novel approaches to the diagnostic and prognostic assessment of coronary heart disease

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>FF 100</th>
<th>VI 25</th>
<th>FF/VI 100/25</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>314</td>
<td>311</td>
<td>319</td>
<td>314</td>
</tr>
<tr>
<td>Baseline troponin*, ng/L</td>
<td>4.4</td>
<td>4.2</td>
<td>4.2</td>
<td>4.0</td>
</tr>
<tr>
<td>3-month troponin*, ng/L</td>
<td>4.4</td>
<td>4.3</td>
<td>4.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Adjusted ratio^ to baseline</td>
<td>1.02</td>
<td>1.02</td>
<td>0.98</td>
<td>1.02</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.96, 1.08)</td>
<td>(0.96, 1.08)</td>
<td>(0.93, 1.04)</td>
<td>(0.97, 1.09)</td>
</tr>
<tr>
<td>Ratio of 3-month cardiac troponin I in active treatment vs placebo</td>
<td>1.00</td>
<td>0.96</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.92, 1.09)</td>
<td>(0.89, 1.05)</td>
<td>(0.92, 1.09)</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.947</td>
<td>0.404</td>
<td>0.893</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.4 Effect of inhaled therapy on cardiac troponin I concentration at 3 months

Model is ANCOVA of log transformed cardiac troponin I, adjusted for baseline cardiac troponin I, age, gender, previous MI history and previous hypertension history

*Geometric mean

^The geometric means displayed are unadjusted while the ratio is based on the model

FF, fluticasone furoate; VI, vilanterol; CI, confidence interval.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (N=439)</th>
<th>FF 100 (N=415)</th>
<th>VI 25 (N=416)</th>
<th>FF/VI 100/25 (N=403)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients experiencing a CV Event</td>
<td>20 (5%)</td>
<td>20 (5%)</td>
<td>19 (5%)</td>
<td>15 (4%)</td>
</tr>
<tr>
<td>Active vs. Placebo Hazard Ratio</td>
<td>0.89 (0.48, 1.67)</td>
<td>0.91 (0.49, 1.72)</td>
<td>0.67 (0.34, 1.32)</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.723</td>
<td>0.778</td>
<td>0.242</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.5 Effect of inhaled therapies on cardiovascular composite endpoint in the SUMMIT biomarker population

FF, fluticasone furoate; VI, vilanterol; CV, cardiovascular; CI, confidence interval.
Figure 6.2 Kaplan–Meier plot for cardiovascular composite events endpoint by cardiac troponin I groupings of <5ng/L and ≥5ng/L at baseline and 3 months

Groups determined by cardiac troponin I concentration at baseline and 3 months. e.g. <5 : <5 represents the grouping of patients with cardiac troponin I <5 ng/L at baseline and also <5 ng/L at 3 months.

CV, cardiovascular.
6.5 **DISCUSSION**

We have identified a strong association between plasma high-sensitivity cardiac troponin I concentration and cardiovascular outcomes in patients with COPD at heightened cardiovascular risk. Importantly, this relationship was specific for cardiovascular events, particularly cardiovascular death, with no demonstrable association with the risk of COPD exacerbations. In addition, we have shown that the presence of dynamic troponin concentrations over a 3-month period also confers increased risk of cardiovascular events, perhaps reflecting underlying atherosclerotic instability. Furthermore, there was no treatment-related change in plasma troponin concentrations, consistent with the overall neutral effect on all-cause mortality and cardiovascular outcomes reported in the primary trial analysis (Vestbo, Anderson et al. 2016). These findings highlight the potential use of high-sensitivity cardiac troponin concentrations as a surrogate endpoint in clinical trials of cardiovascular efficacy and safety.

This study has a number of notable strengths that distinguish it from previous reports on the use of plasma cardiac troponin within the outpatient setting. First, trial participants comprised a broad spectrum of risk, including primary and secondary prevention populations. Second, as a sub-study within the context of a large international randomised trial, we ensured comprehensive follow-up and rigorous adjudication of clinical events. Third, the troponin assay chosen for this analysis is both widely available and analytically robust with less than 5% of samples below the limit of detection. Finally, the availability of paired plasma samples pre-treatment and after 3 months of therapy allowed assessment of any potential relationship between
treatment-related changes in plasma troponin concentration and modification of clinical risk.

Chronic obstructive pulmonary disease is an important risk for the onset of cardiovascular disease. Whilst there is a clear correlation between both these conditions and established predisposing factors such as age and smoking history, it appears that the chronic inflammatory milieu that exists in patients with COPD provides additional pro-atherosclerotic impetus (Decramer and Janssens 2013). A number of studies have begun to explore this association (Maclay and MacNee 2013, Rajagopalan and Brook 2015), but questions have persisted regarding the cardiovascular safety of inhaled therapies for patients with COPD at increased cardiovascular risk (Gershon, Croxford et al. 2013, Woodruff 2013). The SUMMIT investigators addressed this uncertainty with a large prospective superiority trial using the primary endpoint of all-cause mortality. Notwithstanding its nature as the largest ever randomised placebo controlled trial in the treatment of COPD, the apparent 12% relative risk reduction was not statistically significant. Although commendable, this major endeavour could perhaps have been obviated had a suitably specific and broadly accepted surrogate indicator of treatment efficacy been available. Candidate biomarkers for the assessment of both pulmonary and cardiovascular risk are plentiful and represent a rich vein of research interest. However, most are non-specific in nature and very few have been demonstrated to hold promise in quantifying treatment efficacy (Mannino 2015). In contrast, cardiac troponin arises solely from the myocardium (Sharma, Jackson et al. 2004), has consistently demonstrated a strong association with cardiovascular outcomes (Everett 2017), is modifiable with medications (Paradigm-HF Investigators and Coordinators 2015), and has shown
robust correlation between treatment-related concentration change and clinical events (Everett, Brooks et al. 2015, Ford, Shah et al. 2016). When considered alongside this evidence, our findings provide additional support for the hypothesis that plasma cardiac troponin offers a role in the assessment of the efficacy and safety of novel pharmacological therapies.

Within this study, the troponin concentration that determined the upper tertile was 5.5 ng/L. Compared to the lowest tertile (<3 ng/L), we identified a 3-fold increased risk of all cardiovascular events and a nearly 10-fold increased risk of cardiovascular death. This cut-point and magnitude of risk is remarkably consistent with previous descriptions (Zeller, Tunstall-Pedoe et al. 2014, Everett, Zeller et al. 2015, Lipid study investigators 2015, Shah, Anand et al. 2015, Ford, Shah et al. 2016), and lends further support to the concept of a risk threshold above which event rates rise substantially. The threshold has not only potentially important clinical implications, but is also of clear research-related significance. Specifically, it could be used to more reliably identify and recruit high-risk individuals into pharmacological intervention trials, reducing required sample sizes and avoiding the paradox, whereby novel therapies are studied in low risk populations but subsequently prescribed for those at much greater risk. Furthermore, the finding that dynamic troponin concentrations have powerful prognostic value supports the value of repeated measurements throughout the follow-up periods of clinical trials.

Our study does have some limitations. Due to the additional requirement for specimen collection and storage at baseline and 3 months, this sub-study contained only 1,599 (10%) of the total number of participants included in the primary SUMMIT analysis.
and there are some differences in the baseline characteristics of the respective patient cohorts. However, the clinical outcomes appear broadly consistent with the full study analysis. Despite employing a robust and analytically precise assay, the average troponin concentrations were modest and close to the 10% coefficient of variation threshold. This reinforces the need for physician awareness regarding the analytical characteristics of the locally available troponin assay before implementing troponin monitoring in clinical practice. Nevertheless, the repeated concordant prognostic implications of high-sensitivity plasma cardiac troponin concentrations reported in previous studies (Zeller, Tunstall-Pedoe et al. 2014, Everett, Zeller et al. 2015, Lipid study investigators 2015, Shah, Anand et al. 2015, Ford, Shah et al. 2016) involving this assay support the validity of our findings.

6.6 Conclusion

In patients with combined respiratory and cardiovascular diseases, high-sensitivity plasma cardiac troponin I concentration is a prognostic marker that is specific to cardiovascular, but not respiratory, events. Plasma troponin concentrations were not modified by the inhaled therapies for COPD, suggesting neither a beneficial nor hazardous effect on cardiovascular health. These findings are consistent with the absence of a demonstrable effect on therapeutic efficacy or safety in SUMMIT. As such, high-sensitivity cardiac troponin I may represent a reliable surrogate indicator for predicting the cardiovascular consequences of novel medical therapies and interventions.
CHAPTER 7

OPTIMISATION AND REPRODUCIBILITY OF IMAGING CORONARY ATHEROSCLEROSIS WITH 18F-FLUORIDE POSITRON EMISSION TOMOGRAPHY
CHAPTER 7  OPTIMISATION AND REPRODUCIBILITY OF IMAGING CORONARY ATHEROSCLEROSIS WITH 18F-FLUORIDE POSITRON EMISSION TOMOGRAPHY

7.1 SUMMARY

BACKGROUND
18F-fluoride positron emission tomography (PET) and computed tomography (CT) coronary angiography offers a non-invasive method for identifying active atherosclerotic microcalcification, an important feature in coronary plaque vulnerability. The optimal approach and reproducibility of image analysis techniques for determining coronary tracer uptake remains uncertain.

METHODS
Twenty patients with established, stable multi-vessel coronary artery disease underwent repeated cardiac imaging with 18F-fluoride PET-CT within an interval of 11.5±4.5 days. 18F-fluoride uptake within the coronary arteries was determined visually and quantitatively with single measures of maximum and peak standardised uptake value (SUV) and the same measures normalised to blood pool or a referent coronary segment, known as the target to background ratio (TBR). We assessed interobserver and scan-rescan agreement for 3 independent observers reporting all scans on a per-patient and per-segment basis.

RESULTS
Scan analysis using the currently accepted approach of normalisation to a referent coronary segment (TBR_{REFERENT}) identified 10 (50%) patients with evidence of focal coronary 18F-fluoride uptake and demonstrated moderate agreement across observers.
on a per-patient level (κ = 0.56). This was similar to the level of agreement achieved with visual assessment alone (κ = 0.64). Reproducibility was improved by semi-quantitative reporting combining visual assessment with a threshold uptake value for determining the presence of tracer uptake (κ = 0.84). Using the optimised approach achieved excellent agreement on overall segmental uptake counts (intra-class correlation = 0.97).

CONCLUSION
In the assessment of clinically stable coronary artery disease, 18F-fluoride PET-CT imaging is highly reproducible over repeated scans and between multiple observers. A semi-quantitative approach achieves repeatable metrics than can now be assessed in prospective trials to determine their clinical significance.
7.2 BACKGROUND

Positron emission tomography (PET) using 18F-fluoride has been an established imaging technique for the assessment of physiological and pathological skeletal processes for several decades (Hoegerle, Juengling et al. 1998, Cook, Blake et al. 2002). More recently, the hybridisation of computed tomography (CT) and PET has enabled the simultaneous assessment of anatomy and disease activity within extra-skeletal disorders including the cardiovascular system (Dweck, Jones et al. 2012, Dweck, Jenkins et al. 2014, Joshi, Vesey et al. 2014, Irkle, Vesey et al. 2015, Jenkins, Vesey et al. 2015, Vesey, Jenkins et al. 2017). In this context, uptake of 18F-fluoride within valvular or atherosclerotic tissues reflects an underlying process of active calcification that may represent areas of disease progression or greater risk of clinical events (Adamson, Williams et al. 2016). This hypothesis is currently being investigated in at least 2 large scale, prospective clinical trials of coronary 18F-fluoride PET-CT imaging (NCT02110303 and NCT02278211).

Compared to established applications within fields such as oncology or neurology, cardiac PET-CT imaging poses unique challenges related to motion and spatial resolution that must be addressed in order to optimise the reproducibility of scan findings and facilitate more widespread adoption of this diagnostic investigation (Huet, Burg et al. 2015). Although we recently demonstrated the feasibility of 18F-fluoride PET-CT to reproducibly localise calcification activity within the aortic valve (Pawade, Cartlidge et al. 2016), imaging this process within the smaller structures of coronary plaque lies at the limit of PET spatial resolution related to the physical properties of positron emission. Furthermore, uncertainty persists regarding the
optimal metric to apply to quantifying disease activity and how to reliably discriminate increased 18F-fluoride uptake.

This study aimed to quantify scan reproducibility and establish a standardised approach to the assessment of disease activity within atherosclerotic coronary arteries using 18F-fluoride PET-CT.
7.3 METHODS

7.3.1 STUDY POPULATION

This investigation was conducted as a substudy of the ongoing Dual antiplatelet therapy to Inhibit coronary Atherosclerosis and Myocardial injury in patients with Necrotic high-risk coronary plaque Disease (DIAMOND) prospective randomised controlled trial. The study population comprised patients \( \geq 40 \) years of age with established, multi-vessel coronary artery disease that were recruited at least 12 months after any previous acute coronary syndrome or at least 3 months after any coronary revascularisation. Full inclusion and exclusion criteria are described in Table 7.1, but principally relate to concurrent therapy with antiplatelet or anticoagulant agents other than aspirin, or an inability to undergo PET-CT imaging using intravenous contrast (e.g. renal failure or pregnancy).

The study was approved by the Scottish Research Ethics Committee and United Kingdom (UK) Administration of Radiation Substances Advisory committee. It also has Clinical Trial Authorisation from the UK Medicines and Healthcare Regulatory Authority and was performed in accordance with the Declaration of Helsinki. All patients gave written informed consent.
Inclusion Criteria (all criteria must be met):
- Age ≥40 years
- Angiographically proven multi-vessel coronary artery disease defined as at least two major epicardial vessels with any combination of either:
  - >50% luminal stenosis, or
  - previous revascularization (percutaneous coronary intervention or coronary artery bypass graft surgery).
- Receiving aspirin
- Provision of informed consent prior to any study specific procedures

Exclusion Criteria:
- Inability or unwilling to give informed consent
- An acute coronary syndrome within the last 12 months
- An indication for dual anti-platelet therapy, such as drug eluting stent
- Receiving thienopyridine therapy such as clopidogrel or prasugrel
- Receiving oral anticoagulants including warfarin, rivaroxaban, dabigatran or apixaban
- Percutaneous coronary intervention or coronary artery bypass graft surgery within the last 3 months
- Renal dysfunction (eGFR ≤30 mL/min/1.73 m²)
- Contraindication to iodinated contrast agents
- Women who are pregnant, breastfeeding or of child-bearing potential (women who have experienced menarche, are pre-menopausal and have not been sterilised)
- Known hypersensitivity to ticagrelor or one of its excipients
- Active pathological bleeding or bleeding diathesis
- Significant thrombocytopenia: platelets <100 x 10⁹/L
- History of intracranial haemorrhage
- Moderate to severe liver impairment (Child’s Grade B or C)
- Maintenance therapy with strong CYP3A4 inhibitors, such as ketoconazole, nefazodone, ritonavir, indinavir, atazanavir, or clarithromycin
- Major intercurrent illness or life expectancy <1 year
- Planned coronary revascularization or major non-cardiac surgery in the next 12 months
- Maintenance therapy with simvastatin or lovastatin at doses greater than 40mg daily

Table 7.1 Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Condition</th>
<th>Inclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate.</td>
</tr>
</tbody>
</table>
7.3.2 Scan Acquisition

Twenty patients underwent cardiac 18F-fluoride PET-CT scanning on 2 occasions within 2 weeks. Both scans were performed prior to starting treatment with the investigational medical product.

Participants were administered a target dose of 250 MBq 18F-fluoride intravenously and subsequently rested in a quiet environment for 60 min prior to scanning. Additionally, those patients with a heart rate exceeding 65 beats/min received intravenous or oral metoprolol aiming to achieve a heart rate < 60 beats/min. An attenuation correction CT scan was performed before electrocardiographic (ECG)-gated positron emission tomography of the thorax in list-mode for 30 min within a hybrid scanner (64-multidetector Biograph mCT, Siemens Medical Systems, Erlangen, Germany). This was immediately followed by ECG-gated coronary CT calcium scanning and contrast-enhanced CT coronary angiography (CTCA) during held expiration. All patients received sublingual glyceryl trinitrate prior to the CTCA. To correct for cardiac motion, both PET and CT scans were reconstructed in multiple phases of the cardiac cycle, with the mid-diastolic phase used for analysis.

7.3.3 Image Analysis

Analysis of the CT images was performed by an experienced operator using dedicated software (Vitrea Advanced, Toshiba Systems) with multi-planar reformatting used as necessary. The coronary arteries were assessed according to the 15-segment model. Using previously described methods, the segment stenosis score (SSS) (Min, Shaw et al. 2007) and CT-adapted Leaman score (de Araujo Goncalves, Garcia-Garcia et al. 2013) were quantified as measures of overall atherosclerotic burden.
Qualitative and quantitative analysis of the PET images from all 40 scans was performed independently by 3 trained observers (PA, AM, MD) using an OsiriX workstation (OsiriX version 3.5.1 64-bit; OsiriX Imaging Software, Geneva, Switzerland). Images were anonymised and presented to the observers randomly with separate blinded study codes used for initial and repeat scans. Careful co-registration of PET and CT images was undertaken prior to image analysis using residual blood pool 18F-fluoride activity on the PET scan to align with contrast enhanced CT images of the cardiac chambers (Figure 1). Scans were reported on both a per-patient and per-segment basis. Coronary assessment began with visual inspection of tracer localisation to confirm its origin from within the coronary artery, that uptake followed the course of the coronary artery in 3 dimensions and to exclude signal arising from nearby structures such as the aortic valve or mitral valve annulus. Quantitative PET analysis was undertaken of all proximal coronary segments in addition to any atherosclerotic segments with suspected focal 18F-fluoride tracer uptake. Measurements were performed by drawing a volume of interest encompassing the site of maximal tracer uptake and the maximum standardised uptake value (SUV\text{MAX}) recorded. The peak SUV (SUV\text{PEAK}), defined as the mean SUV for all voxels within 5mm of the hottest voxel, was also recorded. Finally, as previously recommended (Dweck, Chow et al. 2012, Joshi, Vesey et al. 2014), a referent plaque without visual tracer uptake was also measured to enable semi-quantitative categorisation of plaques as 18F-fluoride positive lesions (those with visual tracer localisation and SUV\text{MAX} at least 25% greater than that measured within a proximal reference lesion). Measurement of blood pool activity was undertaken as previously described (Pawade, Cartlidge et al. 2016) with elliptical regions of interest drawn within the brachiocephalic vein, superior vena cava,
the interventricular septum, and all 4 cardiac chambers with mean and maximum SUV recorded for each region. Coronary SUV$_{\text{MAX}}$ and SUV$_{\text{PEAK}}$ were divided by the blood pool SUV$_{\text{MEAN}}$ to calculate coronary target to background ratios (TBR$_{\text{MAX}},$ TBR$_{\text{PEAK}}$ respectively), or the coronary SUV$_{\text{MAX}}$ was divided by the referent plaque SUV$_{\text{MAX}},$ (TBR$_{\text{REFERENT}}$). We compared the variability of these measurements between observers and repeated scans and explored potential quantitative thresholds that could optimise the overall repeatability and reproducibility of final diagnosis of PET positivity of patients and coronary segments. Finally, we investigated the scan-rescan repeatability of a per patient count of 18F-fluoride positive segments and a calculated score determined by incorporating the coronary artery segment weightings proposed by Leaman et al (Leaman, Brower et al. 1981).

### 7.3.4 Statistical Analysis

Continuous variables are reported as mean ± standard deviation (SD) or median and interquartile range (IQR). Categorical variables are reported as total and percentage. Coefficients of variation were calculated as the SD divided by the mean with 6 observations for each measurement (i.e. 3 observers each reporting 2 scans) and expressed as percentage. Linear regression with a 3-knot spline function was used to determine the change in coronary SUV$_{\text{PEAK}}$ coefficient of variation at increasing absolute values. Paired t-tests were used to determine statistical significance for comparisons of means with normal distribution. Bland-Altman analysis was used to determine intra-observer reproducibility of 2 observers both reporting the baseline scan and scan-rescan reproducibility with the same observer reporting both the baseline and repeat scans for all patients. The mode across 6 visual assessments of
coronary segment tracer localisation was used to adjudicate a positive diagnosis of focal tracer uptake and the presence of a single positive or ‘hot’ segment was sufficient for a patient level diagnosis of PET positivity. Binomial logistic regression was performed, using this segment based adjudication as the dependent variable and the mean of all 3 observers SUV\textsubscript{PEAK} measurements on scan 1 as the independent variable and receiver operating characteristic curves constructed. The optimal SUV\textsubscript{PEAK} threshold was determined according to the method of Youden (Youden 1950). Internal validation was undertaken by applying the derived model to SUV\textsubscript{PEAK} measurements made on scan 2. Kappa statistics (with 95% confidence intervals) were used to determine and to compare patient and segment level inter-rater agreement using the alternative approaches to PET analysis across both scans according to the method of Fleiss (Fleiss 1971). The $\kappa$ values were interpreted as follows: poor $\leq 0.20$, fair 0.21 to 0.4, moderate 0.41 to 0.60, good 0.61 to 0.80, and very good $\geq 0.81$. Agreement for count of positive plaques and segment weighted scores was reported by intra-class correlation (Shrout and Fleiss 1979). Statistical analysis was performed using R version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria).
7.4 Results

7.4.1 Patient and PET-CT scan characteristics

Twenty patients, mean age 69.5±7.3 years, underwent serial PET-CT imaging within an interval of 11.5±4.5 days. Thirteen patients (65%) had experienced a prior myocardial infarction and 19 (95%) had undergone prior coronary revascularisation. The mean segment stenosis score was 12±5.5 indicating extensive coronary atherosclerotic burden (*Table 7.2*).

Diagnostically acceptable image quality was obtained in all cases resulting in 20 scan pairs being available for analysis. Excellent co-registration of diastolic phase images was readily achieved by aligning residual blood pool 18F-fluoride activity identified on the PET scan with contrast enhanced CT images of the cardiac chambers (*Figure 7.1*). This was feasible because 18F-Fluoride uptake in the LV blood pool was consistently higher than that in the left ventricular myocardium (mean difference 0.341 [95% CI 0.317 to 0.365], p<0.001). The mean heart rate during both scans was 57 beats/min and there were no significant differences in scan parameters between the baseline and repeat scans (*Table 7.3*).
Novel approaches to the diagnostic and prognostic assessment of coronary heart disease

Table 7.2 Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
</tr>
<tr>
<td>Age, years</td>
<td>69.53 (7.32)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>17 (85.0)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.51 (3.97)</td>
</tr>
<tr>
<td>Previous MI (%)</td>
<td>13 (65.0)</td>
</tr>
<tr>
<td>Coronary revascularisation (%)</td>
<td></td>
</tr>
<tr>
<td>PCI</td>
<td>10 (50.0)</td>
</tr>
<tr>
<td>CABG</td>
<td>6 (30.0)</td>
</tr>
<tr>
<td>PCI + CABG</td>
<td>3 (15.0)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>14 (70.0)</td>
</tr>
<tr>
<td>High cholesterol (%)</td>
<td>20 (100.0)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>2 (10.0)</td>
</tr>
<tr>
<td>Previous stroke/TIA (%)</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>Atrial fibrillation (%)</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>Peripheral vascular disease (%)</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>Family history of CVD (%)</td>
<td>12 (60.0)</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
</tr>
<tr>
<td>Statin (%)</td>
<td>20 (100.0)</td>
</tr>
<tr>
<td>Beta-blocker (%)</td>
<td>9 (45.0)</td>
</tr>
<tr>
<td>ACE inhibitor (%)</td>
<td>17 (85.0)</td>
</tr>
<tr>
<td>Creatinine, umol/L</td>
<td>79.00 (12.24)</td>
</tr>
<tr>
<td>eGFR, mL/min</td>
<td>100.39 (16.68)</td>
</tr>
<tr>
<td>Scan separation, days</td>
<td>11.50 (4.48)</td>
</tr>
<tr>
<td>SSS</td>
<td>12.20 (5.50)</td>
</tr>
<tr>
<td>CT-adapted Leaman score</td>
<td>12.40 (5.34)</td>
</tr>
</tbody>
</table>

MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; TIA, transient ischaemic attack; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; SSS, segment stenosis score; CT, computed tomography.

Table 7.3 Performance characteristics of baseline and repeat PET-CT scans

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SCAN 1</th>
<th>SCAN 2</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Heart rate, /min</td>
<td>56.80 (8.00)</td>
<td>57.25 (10.29)</td>
<td>0.878</td>
</tr>
<tr>
<td>DLP, mGy</td>
<td>447.50 (259.31)</td>
<td>394.10 (226.05)</td>
<td>0.492</td>
</tr>
<tr>
<td>18F-fluoride dose, MBq</td>
<td>248.96 (8.86)</td>
<td>248.42 (8.59)</td>
<td>0.847</td>
</tr>
<tr>
<td>Tracer administration to scan delay, min</td>
<td>65.77 (7.26)</td>
<td>66.11 (10.47)</td>
<td>0.905</td>
</tr>
</tbody>
</table>

Min, minute; DLP, dose-length product; mGy, miliGray; MBq, megabecquerel.
Figure 7.1 Co-registration of PET and CT images

Co-registration of PET and CT images is aided by alignment of background blood pool 18F-fluoride activity with contrast-enhanced images of the cardiac chambers on CT (A and B). Achieving accurate co-registration of images ensures reproducible visual appearances on PET-CT scans performed 1 week apart (C and D). The importance of performing CT imaging during held expiration is demonstrated in images E-H. If the CT scan is performed during inspiration (E and G) the right coronary artery (white arrow) and aortic root (white ring) move anteriorly relative to the corresponding regions of 18F-fluoride activity on the PET scan (red arrow and red ring respectively). During held-expiration (F and H) these structures are seen to be well aligned on the CT and PET scans.

PET, positron emission tomography; CT, computed tomography.
7.4.2 **VISUAL AND QUANTITATIVE SCAN ANALYSIS**

Fourteen patients had visual evidence of tracer uptake within at least 1 coronary segment (*Table 7.4*). Following visual inspection, a total of 115 coronary segments had quantitative measurement of 18F-fluoride activity on both scans. Ten and 14 patients on the baseline and repeat scans respectively, fulfilled the established criteria of having at least 25% greater uptake in the plaque of interest compared to the chosen referent plaque (i.e. $TBR_{REFERENT} > 1.25$). On the baseline and repeat scans, there were 44 (38.3%) and 48 (41.7%) plaques with focal tracer localisation of which 36 (31.3%) and 42 (36.5%) met the above criteria of PET positivity (*Table 7.4*). The segmental distribution and mean SUV_{PEAK} of each plaque with focal tracer uptake is depicted in *Figure 7.2*.

With 6 measurements across 2 scans, the mean coefficients of variation for SUV_{MEAN} recorded in the left and right atria (LA and RA) were 5.9±2.8% and 6.5±2.5% respectively. The coefficients of variation in both of these regions were significantly lower when compared to the other areas of sampled blood pool activity (*Figure 7.3*). Intra-observer reproducibility for SUV_{MEAN} recorded from the left and right atria by 2 observers on a single scan demonstrated a percentage error of ±7.3% and ±9.2% respectively. The corresponding scan-rescan repeatability for a single observer reporting 2 scans demonstrated a percentage error of ±19.5% for the LA and ±22.8% for the RA (*Table 7.5*).
### PATIENT LEVEL ANALYSIS

<table>
<thead>
<tr>
<th></th>
<th>SCAN 1</th>
<th>SCAN 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Visually adjudicated coronary uptake (%)</td>
<td>14 (70.0)</td>
<td>14 (70.0)</td>
</tr>
<tr>
<td>TBR&lt;sub&gt;REFERENT&lt;/sub&gt; positive (%)</td>
<td>10 (50.0)</td>
<td>12 (60.0)</td>
</tr>
<tr>
<td>SUV&lt;sub&gt;PEAK&lt;/sub&gt; threshold positive (%)</td>
<td>11 (55.0)</td>
<td>10 (50.0)</td>
</tr>
<tr>
<td>Threshold positive plaques</td>
<td>1.65 (2.11)</td>
<td>1.55 (1.96)</td>
</tr>
<tr>
<td>Segment-weighted 18F-fluoride score</td>
<td>3.42 (4.41)</td>
<td>3.50 (4.45)</td>
</tr>
</tbody>
</table>

### PLAQUE LEVEL ANALYSIS

<table>
<thead>
<tr>
<th>Coronary plaques measured, n</th>
<th>115</th>
<th>115</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual 18F-fluoride uptake (%)</td>
<td>44 (38.3)</td>
<td>48 (41.7)</td>
</tr>
<tr>
<td>TBR&lt;sub&gt;REFERENT&lt;/sub&gt; positive (%)</td>
<td>36 (31.3)</td>
<td>42 (36.5)</td>
</tr>
<tr>
<td>SUV&lt;sub&gt;PEAK&lt;/sub&gt; threshold positive (%)</td>
<td>33 (28.7)</td>
<td>31 (27.0)</td>
</tr>
<tr>
<td><strong>Coronary artery with 18F-fluoride uptake (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMS</td>
<td>5 (11.4)</td>
<td>5 (10.4)</td>
</tr>
<tr>
<td>LAD</td>
<td>10 (22.7)</td>
<td>15 (31.2)</td>
</tr>
<tr>
<td>LCx</td>
<td>9 (20.5)</td>
<td>9 (18.8)</td>
</tr>
<tr>
<td>RCA</td>
<td>20 (45.5)</td>
<td>19 (39.6)</td>
</tr>
<tr>
<td><strong>Coronary location of 18F-fluoride uptake (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>27 (61.4)</td>
<td>27 (56.2)</td>
</tr>
<tr>
<td>Mid</td>
<td>10 (22.7)</td>
<td>13 (27.1)</td>
</tr>
<tr>
<td>Distal</td>
<td>7 (15.9)</td>
<td>8 (16.7)</td>
</tr>
</tbody>
</table>

**Table 7.4** Patient and plaque level analysis of 18F-fluoride PET-CT scans

TBR, target-to-background ratio; SUV, standardised uptake value; LMS, left main stem; LAD, left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery.
**Figure 7.2 Coronary distribution of 18F-fluoride uptake**

pRCA, proximal right coronary artery; mRCA, mid right coronary artery; dRCA, distal right coronary artery; LMS, left main stem; pLCx, proximal left circumflex artery; OM1, first obtuse marginal artery; pLAD, proximal left anterior descending artery; mLAD, mid left anterior descending artery; dLAD, distal left anterior descending artery.
Figure 7.3 Coefficients of variation for blood pool measurements of 18F-fluoride activity

NS, p ≥ 0.05; *, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001.
BCV, brachiocephalic vein; SVC, superior vena cava; RA, right atrium; LA, left atrium; IVS, interventricular septum.
<table>
<thead>
<tr>
<th>LOCATION</th>
<th>BLOOD POOL SUV&lt;sub&gt;MEAN&lt;/sub&gt;</th>
<th>DIFFERENCE</th>
<th>95% LIMITS OF AGREEMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OVERALL MEAN</td>
<td>MEAN</td>
<td>SD</td>
</tr>
<tr>
<td>(A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCV</td>
<td>0.903</td>
<td>-0.08</td>
<td>0.16</td>
</tr>
<tr>
<td>IVS</td>
<td>0.771</td>
<td>0.028</td>
<td>0.052</td>
</tr>
<tr>
<td>LA</td>
<td>1.159</td>
<td>-0.018</td>
<td>0.043</td>
</tr>
<tr>
<td>LV</td>
<td>1.142</td>
<td>-0.016</td>
<td>0.064</td>
</tr>
<tr>
<td>RA</td>
<td>1.13</td>
<td>-0.009</td>
<td>0.053</td>
</tr>
<tr>
<td>RV</td>
<td>1.124</td>
<td>-0.019</td>
<td>0.051</td>
</tr>
<tr>
<td>SVC</td>
<td>1.084</td>
<td>-0.013</td>
<td>0.174</td>
</tr>
<tr>
<td>(B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCV</td>
<td>0.863</td>
<td>-0.056</td>
<td>0.188</td>
</tr>
<tr>
<td>IVS</td>
<td>0.785</td>
<td>-0.038</td>
<td>0.098</td>
</tr>
<tr>
<td>LA</td>
<td>1.15</td>
<td>-0.024</td>
<td>0.114</td>
</tr>
<tr>
<td>LV</td>
<td>1.134</td>
<td>-0.048</td>
<td>0.119</td>
</tr>
<tr>
<td>RA</td>
<td>1.126</td>
<td>-0.029</td>
<td>0.131</td>
</tr>
<tr>
<td>RV</td>
<td>1.114</td>
<td>-0.022</td>
<td>0.134</td>
</tr>
<tr>
<td>SVC</td>
<td>1.078</td>
<td>-0.049</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 7.5 Bland-Altman limits of agreement for blood-pool measurements of 18F-fluoride activity

(A, Inter-observer; B, Scan-rescan)
SUV, standardised uptake value; SD, standard deviation; BCV, brachiocephalic vein; IVS, interventricular septum; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle; SVC, superior vena cava.
Compared with measurements made in coronary arterial segments without apparent 18F-fluoride localisation, the coefficient of variation was lower within regions with visual tracer uptake (p < 0.001). Within these regions of focal tracer localisation, measurement of SUV\textsubscript{PEAK} and SUV\textsubscript{MAX} demonstrated less variation than the established metric of TBR\textsubscript{REFERENT} (p <0.001 for both). There was no significant improvement in measurement reproducibility with target-to-background ratios employing blood pool SUV\textsubscript{MEAN} as the denominator (Figure 7.4). The intra-observer and scan-rescan percentage error for measurement of SUV\textsubscript{PEAK} was ±23% and ±32% respectively and these limits of agreement were not improved by use of alternative metrics such SUV\textsubscript{MAX}, or target-to-background ratios (Table 7.6).

![Figure 7.4 Coefficients of variation for coronary segment measurements of 18F-fluoride activity](image)

SUV, standardised uptake value; TBR, target-to-background ration; ref, referent; LA, left atrium.
Table 7.6 Bland-Altman limits of agreement for coronary segment measurements of 18F-fluoride activity
(A, Inter-observer; B, Scan-rescan).
SUV, standardised uptake value; SD, standard deviation; LA, left atrium; RA, right atrium; pk, peak.

<table>
<thead>
<tr>
<th>MEASURE</th>
<th>ALL PLAQUE</th>
<th>PLAQUE WITH VISUAL TRACER UPTAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OVERALL MEAN</td>
<td>DIFFERENCE MEAN</td>
</tr>
<tr>
<td>(A) SUV(_{\text{MAX}})</td>
<td>1.212</td>
<td>0.019</td>
</tr>
<tr>
<td>SUV(_{\text{PEAK}})</td>
<td>1.141</td>
<td>0.017</td>
</tr>
<tr>
<td>TBR(_{\text{REFERENT}})</td>
<td>1.336</td>
<td>-0.033</td>
</tr>
<tr>
<td>TBR(_{\text{MAX/RA}})</td>
<td>1.121</td>
<td>0.041</td>
</tr>
<tr>
<td>TBR(_{\text{Pk/Ra}})</td>
<td>1.053</td>
<td>0.037</td>
</tr>
<tr>
<td>TBR(_{\text{MAX/LA}})</td>
<td>1.085</td>
<td>0.035</td>
</tr>
<tr>
<td>TBR(_{\text{PK/LA}})</td>
<td>1.02</td>
<td>0.032</td>
</tr>
<tr>
<td>(B) SUV(_{\text{MAX}})</td>
<td>1.221</td>
<td>-0.01</td>
</tr>
<tr>
<td>SUV(_{\text{PEAK}})</td>
<td>1.149</td>
<td>-0.007</td>
</tr>
<tr>
<td>TBR(_{\text{REFERENT}})</td>
<td>1.318</td>
<td>0</td>
</tr>
<tr>
<td>TBR(_{\text{MAX/RA}})</td>
<td>1.14</td>
<td>0.048</td>
</tr>
<tr>
<td>TBR(_{\text{Pk/Ra}})</td>
<td>1.07</td>
<td>0.045</td>
</tr>
<tr>
<td>TBR(_{\text{MAX/LA}})</td>
<td>1.102</td>
<td>0.011</td>
</tr>
<tr>
<td>TBR(_{\text{PK/LA}})</td>
<td>1.035</td>
<td>0.013</td>
</tr>
</tbody>
</table>
7.4.3 Threshold Determination

Based on the above findings, plaque SUV\textsubscript{PEAK} was chosen as the optimal measurement for determining a suitable quantitative threshold to define 18F-fluoride positivity. The mean SUV\textsubscript{PEAK} measured by 3 observers reporting the baseline scan demonstrated excellent discrimination for visually adjudicated plaque uptake of 18F-fluoride (c-statistic, 0.92; 95% CI 0.87 to 0.98) with an optimal SUV\textsubscript{PEAK} threshold of 1.25 identified (Figure 7.5). Internal validity of SUV\textsubscript{PEAK} discrimination was confirmed on analysis of the repeat scan (c-statistic 0.91; 95% CI 0.85 to 0.97) where the threshold of 1.25 achieved a sensitivity and specificity of 0.85 and 0.86 respectively (Figure 7.6). The coefficient of variation for SUV\textsubscript{PEAK} measurements decreased at higher values, falling below 10% at 0.99, providing analytical support for the robust nature of this measurement at the 1.25 threshold (Figure 7.7).
Figure 7.5 Receiver-operating characteristic curve for threshold-based analysis (Scan 1)

$\text{SUV}_{\text{THRESHOLD}}$ of 1.25 measured on scan 1 for determining coronary segment 18F-fluoride positivity.

SUV, standardised uptake value; AUC, area under curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
Figure 7.6 Receiver-operating characteristic curve for threshold-based analysis (Scan 2)

$SUV_{\text{THRESHOLD}}$ of 1.25 measured on scan 2 for determining coronary segment 18F-fluoride positivity.

SUV, standardised uptake value; AUC, area under curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
Figure 7.7 Coefficient of variation for coronary segment measurement of 18F-fluoride SUV\(_{\text{PEAK}}\)

CV, coefficient of variation; SUV standardised uptake value.
7.4.4 Reproducibility of optimised approach

When assessed on a per-patient level, the overall agreement for visual assessment of coronary arterial uptake of 18F-fluoride was good ($\kappa = 0.64$) and this was not improved with the previously described metric of TBR$\text{REFERENT}$ ($\kappa = 0.56$). In contrast, very good reproducibility was achieved with the application of the SUV$\text{PEAK}$ threshold of 1.25 ($\kappa = 0.84$). This approach also resulted in excellent agreement between each observer and both scans for the total number of 18F-fluoride positive coronary plaques per patient (ICC = 0.97, 95% CI 0.94 to 0.99), and a segment-weighted 18F-fluoride score (ICC = 0.97, 95% CI 0.93 to 0.98; *Table 7.7*).

<table>
<thead>
<tr>
<th>(A) PATIENT LEVEL</th>
<th>AGREEMENT</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual tracer uptake</td>
<td>$\kappa = 0.64$</td>
<td>(0.52 - 0.75)</td>
</tr>
<tr>
<td>TBR$\text{REFERENT}$</td>
<td>$\kappa = 0.56$</td>
<td>(0.44 - 0.67)</td>
</tr>
<tr>
<td>SUV$\text{PEAK}$ $&gt;$ 1.25 (threshold)</td>
<td>$\kappa = 0.84$</td>
<td>(0.73 - 0.95)</td>
</tr>
<tr>
<td>18F-fluoride positive segments</td>
<td>ICC = 0.97</td>
<td>(0.94 - 0.99)</td>
</tr>
<tr>
<td>18F-fluoride weighted score</td>
<td>ICC = 0.97</td>
<td>(0.93 - 0.98)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(B) SEGMENT LEVEL</th>
<th>AGREEMENT</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual tracer uptake</td>
<td>$\kappa = 0.82$</td>
<td>(0.79 - 0.85)</td>
</tr>
<tr>
<td>TBR$\text{REFERENT}$</td>
<td>$\kappa = 0.79$</td>
<td>(0.76 - 0.82)</td>
</tr>
<tr>
<td>SUV$\text{PEAK}$ $&gt;$ 1.25 (threshold)</td>
<td>$\kappa = 0.91$</td>
<td>(0.88 - 0.94)</td>
</tr>
</tbody>
</table>

*Table 7.7 Patient and coronary segment level agreement statistics*

NB: 6 observations total across 2 scans with same 3 observers for each scan
$\kappa$, Fleiss Kappa; ICC, intra-class correlation; TBR, target-to-background ratio; SUV, standardised uptake value; CI, confidence interval.
7.5 DISCUSSION

In the assessment of clinically stable coronary artery disease, 18F-fluoride PET-CT imaging is a repeatable and reproducible diagnostic investigation. We have demonstrated how a semi-quantitative approach that combines visual assessment and a single radiological measurement (SUV\textsubscript{PEAK}) enables the reliable characterisation of tracer localisation within atherosclerotic lesions on both a per-patient and per-segment basis. This technique has direct application to ongoing clinical trials that promise novel insights regarding \textit{in vivo} pathological processes, cardiovascular prognostic stratification and emerging therapeutic interventions.

Our study has a number of notable strengths. First, to our knowledge this is the only prospective study to investigate the reproducibility of coronary imaging with serial 18F-fluoride PET-CT scans. Second, the study population comprising 20 patients with established, multi-vessel coronary disease represents a high-risk cohort for whom imaging biomarkers have genuine potential to improve prognostic stratification and guide therapeutic interventions. Third, we directly compared several proposed methods for quantifying focal tracer activity within coronary atherosclerotic lesions and determined the inter-observer and inter-scan agreement for these metrics. Finally, we derived an SUV\textsubscript{PEAK} threshold that may help standardise the existing operator dependent visual approach to reporting these images thereby improving generalisability of diagnostic findings.

Positron emission tomography employing selectively targeted tracers is gaining recognition for offering novel insights regarding pathophysiological processes in diverse disease states. Compared to use in other clinical fields, coronary PET-CT
imaging raises several technical challenges which have hindered widespread adoption but are beginning to be addressed. For example, although 18F-fluorodeoxyglucose (18F-FDG) represents a highly sensitive marker of plaque inflammation, non-specific myocardial uptake of this molecular marker has precluded reliable imaging beyond the largest and most proximal coronary segments (Joshi, Vesey et al. 2014, Tarkin, Joshi et al. 2017). In contrast, 18F-fluoride binds specifically to hydroxyapatite, is not taken up by the myocardium, and offers a non-invasive marker of atherosclerotic microcalcification, an important contributor to plaque vulnerability. Further difficulty arises from the 4-5mm fundamental limit of spatial resolution shared by all 18F-derived PET tracers and the challenge of continuous cardiac motion. Despite these potential limitations, a landmark paper by Rudd et al, demonstrated excellent reproducibility for 18F-fluoride PET imaging of peripheral arteries (Rudd, Myers et al. 2008) and we have previously established that the problem of intrathoracic motion can be addressed through ECG-gating in a similar manner to that employed in CT coronary imaging (Pawade, Cartlidge et al. 2016). Finally, although restricting the analysis of the PET signal to the third quarter of the cardiac cycle reduces the signal-to-noise ratio, we have now compensated for this by increasing tracer dose, increasing scan duration, and applying advanced image reconstruction algorithms.

One unexpected finding of this study relates to the failure to improve quantitative reproducibility using target-to-background ratios, particularly adjusting standardised uptake values in the plaque of interest to those measured in a proximal referent plaque without visual tracer uptake. Perhaps the most important factor contributing to this relates to the difficulty of determining exactly where to draw a volume of interest when measuring the absence of tracer uptake. Whilst it is relatively easy to agree on where
to centre a measurement within a visually apparent focal ‘hot’ spot, it is substantially more challenging to independently reach a consensus on the exact location for measurement of the signal nadir. Furthermore, it is possible to substantially change the resultant $TBR_{REFERENT}$ by shifting the site of measurement relatively short distances either proximally or distally along the coronary segment. In truth, this extra measurement intuitively seems unnecessary as it is likely that during the process of visual inspection of the PET-CT image the observer automatically detects focal regions of tracer uptake that are brighter than would be expected relative to the adjacent vascular structures. In contrast with use of the referent plaque approach, we did not demonstrate a significant difference in reproducibility between $SUV_{PEAK}$ alone, or when referenced against atrial blood pool activity. Although we chose to explore thresholds using $SUV_{PEAK}$, we note that the most recent European Association for Nuclear Medicine consensus paper on extra-cardiac vascular PET with 18F-FDG recommends the use $TBR_{MAX}$ due to the potential influence of scan protocols and reconstruction algorithms on $SUV_{MAX}$ alone (Bucerius, Hyafil et al. 2016). Whilst it is certainly plausible that the same applies to coronary imaging with 18F-fluoride, this will require reproducibility studies involving more than one PET-CT scanner platform.

7.5.1 RECOMMENDATIONS FOR FUTURE STUDIES

Although we have demonstrated coronary 18F-fluoride PET-CT imaging to be a robust and reproducible technique, its true value depends on whether clinical trials demonstrate worthwhile improvements in risk stratification or therapeutic targeting. We suggest such trials apply similar acquisition and image analysis protocols to that described here. Specifically, we believe there is little merit in measuring referent
plaque uptake in order to determine target to background ratios as this merely introduces a second opportunity for measurement error and does not improve the repeatability beyond a simple SUV measurement from the region of interest. We would also emphasise that future studies should continue to explore whether our proposed threshold carries clinical significance beyond being a reproducible metric.

7.5.2 LIMITATIONS

This study was undertaken in a centre with extensive experience in cardiac 18F-fluoride PET-CT imaging and scans were performed using a single PET-CT scanning system. It is important to acknowledge that many operator and system-dependent variables influence final image quality and these will necessitate careful consideration when applying our findings in different settings. Furthermore, the SUV\textsubscript{PEAK} value of 1.25 identified in this study reflects a threshold that optimised agreement with visual consensus and does not necessarily reflect the threshold most suitable for identifying a high-risk coronary lesion. Because of partial volume effects, standardised uptake values measured in more distal coronary segments are lower than those measured proximally and this will necessarily result in less recognition of tracer uptake in distal plaques if a single threshold is applied. Indeed, this concept is elegantly demonstrated in a recent paper investigating thresholds for 18F-FDG in extra-cardiac arteries which derived an optimal cutpoint for SUVmax of 1.85 in the carotid arteries or 2.38 in the (larger) aorta (van der Valk, Verweij et al. 2016). It remains to be determined if more advanced analytical techniques can correct for this phenomenon. In truth, the real-world significance of our findings can only be understood once clinical outcome trials have reported and it is expected that ongoing trials will begin to resolve this unknown.
Finally, it should be noted that the effective combined radiation dose patients received from both PET-CT scans was 14.4mSv, which is comparable with that received during coronary angioplasty procedures (Pantos, Patatoukas et al. 2009, Kuipers, Delewi et al. 2012, Arif, Bartus et al. 2014) and well within the acceptable radiation limits allowed for under the regulatory approvals of this trial.

7.6 CONCLUSION

In the assessment of clinically stable coronary artery disease, 18F-fluoride PET-CT imaging is highly reproducible over repeated scans and between multiple observers. A semi-quantitative approach achieves repeatable metrics than can now be assessed to determine their clinical significance. This approach to image acquisition and analysis provides strong support for the ongoing clinical studies investigating the value of 18F-fluoride PET-CT to inform cardiovascular prognostic assessment and guide novel therapeutic strategies.
CHAPTER 8

CONCLUSIONS AND FUTURE DIRECTIONS
CHAPTER 8    CONCLUSIONS AND FUTURE DIRECTIONS

8.1 SUMMARY OF THESIS FINDINGS

Despite recent advances in testing and treatment, coronary heart disease remains one of the largest causes of morbidity and mortality worldwide. In order to address this ongoing disease burden, there is a clear need to more effectively target the use of existing and novel diagnostic investigations and medical therapies. Improved targeting of diagnostic investigations requires more reliable estimation of pre-test probability of coronary disease whilst optimizing the use of pharmacological or interventional treatments requires more accurate prognostic stratification. Achieving both objectives in an equitable manner across all population groups will depend upon updated clinical guidelines containing improved risk models and enhanced management pathways.

Clinical guidelines for stable coronary heart disease abound but are imperfect solutions in their current state. To improve clinician engagement and adherence these guidelines must be demonstrated to improve patient outcomes in an efficient manner. Given the ongoing temporal and geographic changes in patient characteristics, risk scores ought to be revisited frequently and updated as necessary. Importantly, they must be calibrated to offer accurate risk estimation for an individual patient and not merely the average patient. New developments in circulating and imaging biomarkers hold great promise for improving the accuracy and applicability of such diagnostic and prognostic models.

The principal aim of this thesis was to investigate the potential clinical benefit of novel approaches to the diagnostic and prognostic assessment of coronary heart disease.
8.1.1 Evaluation of the 2016 National Institute for Health and Care Excellence Guidance on the Assessment of Suspected Stable Angina

Patients referred for assessment of suspected stable angina were randomized to an assessment pathway including computed tomography coronary angiography (CTCA) or standard care as part of the Scottish COmputed Tomography of the HEART (SCOT-HEART) trial. For the purposes of this analysis those patients without a prior history of coronary artery disease (n=3,770) were analysed according to the diagnostic groups described in the 2016 National Institute for Health and Care Excellence (NICE) guideline for the assessment of stable chest pain. The primary (diagnostic) endpoint was diagnostic certainty of angina at 6 weeks and the prognostic endpoint comprised fatal and non-fatal myocardial infarction.

Patient assessment according to the updated guideline improved the efficiency of patient assessment and maximises the benefits of CTCA on diagnostic certainty in those with possible angina. At 3.2 years of follow-up, fatal and non-fatal myocardial infarction was reduced in patients with possible angina but not in those with non-anginal symptoms, for whom CTCA would no longer be indicated.

8.1.2 Identification of Patients with Stable Chest Pain Deriving Minimal Value from Coronary Computed Tomography Angiography: An External Validation of the PROMISE Minimal-Risk Tool

The PROspective Multicenter Imaging Study for Evaluation of chest pain (PROMISE) minimal-risk tool was recently developed with the aim of improving the diagnostic and prognostic stratification of patients with suspected stable angina. The tool was applied to a subset of patients from the SCOT-HEART trial who had undergone CTCA as part of their diagnostic assessment and compared with the existing guideline-
endorsed CAD Consortium (CADC) risk score with tests of model discrimination and calibration.

Compared with the CADC model, the PROMISE minimal-risk tool improved discrimination of the pre-test probability of normal coronary arteries and no clinical events. However, model calibration was initially poor and required revision of the model intercept and covariate coefficients to be improved. This finding highlights the importance of rigorous external validation of prognostic models prior to widespread adoption.

8.1.3 **HIGH-SENSITIVITY CARDIAC TROPNON I IN THE DIAGNOSIS OF STABLE CORONARY ARTERY DISEASE**

Recent developments in immunoassays have enabled the reliable quantification of cardiac troponin I concentrations in most healthy individuals. When measured in the context of a clinically stable population, relative increases in troponin concentration are associated with increased risk of future cardiovascular events. The exact mechanisms underpinning this association are unclear but may in part reflect coronary atherosclerotic burden, raising the possibility that knowledge of troponin concentrations might improve the estimation of the pre-test probability for obstructive coronary disease. To test this hypothesis, plasma cardiac troponin was measured using a high-sensitivity assay in 943 adults with suspected stable angina who had undergone coronary computed tomography angiography as part of the SCOT-HEART trial. Rates of obstructive coronary disease were compared with the pre-test probability determined by the Coronary Artery Disease Consortium risk model with and without
cardiac troponin concentrations. External validation was undertaken in an independent study population from Denmark comprising 487 patients with suspected stable angina. Higher cardiac troponin concentrations were strongly and independently associated with obstructive coronary disease. Cardiac troponin concentrations improved model discrimination and net reclassification. Similar improvements in discrimination and net reclassification were demonstrated in the external validation cohort. Use of this test may improve the selection of patients for further investigation and treatment. Use of troponin testing may improve the selection of suspected angina patients for further investigation and treatment.

8.1.4 CARDIAC TROPONIN I AND RISK OF CARDIOVASCULAR EVENTS IN PATIENTS WITH COPD AND HEIGHTENED CARDIOVASCULAR RISK

The Study to Understand Mortality and Morbidity (SUMMIT) assessed the efficacy and safety of inhaled corticosteroids and long-acting beta agonists in 16,485 patients with chronic obstructive pulmonary disease (COPD) and heightened cardiovascular risk and failed to demonstrate any treatment-related improvement in mortality or cardiovascular outcomes. To investigate its utility as a surrogate marker of treatment efficacy, cardiac troponin I concentrations were measured in a biomarker sub-study cohort (n=1,559) and related to future risk of cardiovascular events.

Baseline plasma cardiac troponin I concentrations were found to be closely and specifically associated with future cardiovascular events including cardiovascular death but not respiratory endpoints or all-cause mortality. Inhaled therapies did not affect cardiac troponin I concentrations consistent with their neutral effect on all-cause mortality and cardiovascular outcomes in the main study. This finding lends strong
support to the potential use of troponin as a surrogate endpoint that could dramatically improve the efficiency of cardiovascular therapy trials.

8.1.5 Optimisation and Reproducibility of Imaging Coronary Atherosclerosis with 18F-fluoride Positron Emission Tomography

Positron emission tomography (PET) with 18F-fluoride offers the potential to non-invasively identify atherosclerotic microcalcification, an important hallmark of plaque vulnerability. To determine the reproducibility of coronary imaging with 18F-fluoride PET, 20 patients with established multivessel coronary artery disease underwent repeated imaging within a 2 week timeframe. Scans underwent qualitative and quantitative per-patient and per-segment level analysis by 3 independent observers.

In the assessment of clinically stable coronary artery disease, 18F-fluoride PET imaging was shown to be a repeatable and reproducible diagnostic investigation with excellent inter-observer and scan-rescan agreement on both a per-patient and per-plaque basis. Agreement was enhanced through the development of a semi-quantitative approach that combined visual assessment with an optimal threshold of measured tracer activity. This finding supports the ongoing use of 18F-fluoride PET imaging for cardiovascular risk assessment in larger clinical trials.
8.2 Future directions

The findings of this thesis have important implications for future investigations regarding the diagnostic and prognostic assessment of coronary heart disease. In particular, there is a clear need to incorporate the information offered by novel circulating and imaging biomarkers into existing risk models in order to improve their accuracy and clinical utility. A number of ongoing and future studies will be needed to inform how these new technologies can best be applied to clinical practice.

8.2.1 Pragmatic clinical trials

Randomised controlled trials have ballooned in number and size over recent decades. Such trials are designed as experiments with the ability to determine cause-effect relationships. By controlling for systematic bias and adopting strict inclusion and exclusion criteria they can identify the potential benefits or harms related to an intervention when applied to a clearly defined patient population. However, it has long been recognised that the results of trials conducted in ‘laboratory’ conditions may not correspond to the outcomes found in ‘normal’ conditions (Schwartz and Lellouch 1967), and there is increasing uncertainty as to how best to apply trial findings to the broader population (Weiss, Koepsell et al. 2008).

Pragmatic trials seek to address this uncertainty and better inform healthcare policy making by providing evidence for adoption of interventions in real-world settings (Patsopoulos 2011, Ford and Norrie 2016). They aim to test the effectiveness of specific therapies, or healthcare pathways, within a true all-comers population and principally report only those endpoints that are of important clinical relevance to patients and healthcare providers. These studies are often very large in scale,
substantially more cost effective than traditional randomised controlled trials and are
the ideal way to evaluate and validate new management guidelines or risk models prior
to universal adoption. The Centre for Cardiovascular Science within the University of
Edinburgh has a number of pragmatic trials underway in the field of coronary heart
disease.

**HIGH-SENSITIVITY TROPONIN IN THE EVALUATION OF PATIENTS WITH ACUTE
CORONARY SYNDROME (HIGH-STEACS)**

The arrival of novel high-sensitivity troponin assays resulted in more accurate
determination of the true 99th centile upper reference limit and subsequently the
recognition of the potential importance for sex-specific thresholds to be introduced
(Shah, Griffiths et al. 2015). The High-STEACS trial (clinicaltrials.gov identifier
NCT01852123) was established to provide the very large patient numbers needed to
test this hypothesis. It is designed as a stepped wedge, cluster randomised controlled
trial of the introduction of the Abbott ARCHITECT\textsuperscript{STAT} high-sensitivity troponin I
assay along with gender specific thresholds for the diagnosis of myocardial infarction.
It avoids individual patient enrolment, instead randomising each of the involved
hospitals with regards to the time of introduction of the new test and thereby intends
to consecutively recruit the 70,000 patients required for adequate statistical power with
a primary endpoint of recurrent myocardial infarction of cardiovascular death at 1 year.
Trial recruitment is now complete and results should be publicly available within the
next year.
High-sensitivity troponin assays allow the quantification of troponin concentrations well below the 99th centile upper reference limit. In a recent analysis, we identified a threshold for troponin I on presentation with suspected acute coronary syndrome of 5 ng/L, below which there was a very low rate of future myocardial infarction or cardiac death (negative predictive value 99.5%) (Shah, Anand et al. 2015). The findings of this study led to the development of an optimised assessment pathway with the potential to substantially reduce length of stay and rates of hospital admission in this patient cohort. As with the High-STEACS trial, we have now commenced a large, stepped wedge, cluster randomised controlled trial of the introduction of this pathway across Scotland (clinicaltrials.gov identifier NCT03005158). Again, trial recruitment is now complete and results should be publicly available within the next year.

Duration of dual anti-platelet therapy in acute coronary syndrome in Scotland (DUAL-ACS)

Previous research has demonstrated reductions in cardiovascular events when patients with recent acute coronary syndrome are treated with the combination of aspirin and a second anti-platelet agent (Wilson, Newby et al. 2017). However, these trials had significant differences with regards to duration of dual therapy and the greatest reduction in events appears to relate to the early months of treatment when risk of recurrent ischaemic events is high. Importantly, as with most randomised trials, these studies predominantly recruited patients at low risk for both ischaemic and bleeding events and it is unclear how applicable the findings are to the more complex patients typically seen in clinical practice. Clinical guidelines in North America (Amsterdam, Wenger et al. 2014) and Europe (Roffi, Patrono et al. 2016) currently recommend dual
anti-platelet therapy be continued for 12 months following myocardial infarction. In contrast, the recently revised Scottish Intercollegiate Guidelines Network now endorses 6 months of combination therapy in most and supports consideration of even shorter duration treatment in high bleeding risk individuals (Scottish Intercollegiate Guidelines Network 2016). DUAL-ACS is a pragmatic clinical trial designed to help resolve this uncertainty. Involving 21 hospitals across Scotland, it will recruit nearly 20,000 patients with recent acute coronary syndrome who require dual anti-platelet therapy. Patients will be individually randomised to dual anti-platelet therapy for a duration of 3 or 12 months with choice of second agent left to the discretion of the treating clinician. Follow-up will continue for 3 years with a primary endpoint of all-cause mortality. In keeping with the overarching objective of pragmatic trials and ensure a representative study population is enrolled, inclusion and exclusion criteria have been kept broad, an abbreviated consent process is in place and all endpoints will be determined from routinely collected national health care registries with no requirement for patients to attend additional study related appointments.

8.2.2 APPLICATIONS OF CARDIAC TROTONIN BEYOND THE ACUTE CORONARY SYNDROME

Alongside previous reports, the data presented in this thesis supports the assertion that troponin is not only detectable, but also prognostically informative in patients without clinical suspicion of acute coronary syndrome. Regardless of the setting, it is likely that elevated cardiac troponin concentrations correspond with increased risk of clinical events. Furthermore, we have shown in chapter 6 that this risk is specific to cardiovascular outcomes and not merely a marker of general co-morbidity. This feature of cardiac troponin has important implications with regards to guiding further
investigation and treatment decisions once an elevated troponin concentration is identified. It also lends itself to use as a potential surrogate endpoint within randomised controlled trials. Within the Centre for Cardiovascular Science at the University of Edinburgh we are exploring these potential applications in several ongoing clinical trials.

**CARDIAC CARE**

Cardiac CARE is a multicentre randomised controlled trial of high-sensitivity cardiac troponin I-guided combination angiotensin receptor blockade and beta blocker therapy to prevent cardiac toxicity in breast cancer patients receiving anthracycline adjuvant therapy. We have recently analysed data from a pilot study demonstrating that myocardial injury arising from anthracycline therapy is detectable with the Abbott ARCHITECT STAT high-sensitivity troponin I assay in the earliest stages of breast cancer treatment and is cumulative with each chemotherapy dose. Furthermore, the extent of myocardial injury varied substantially amongst patients, with a 3-fold difference in median troponin concentration after 5 treatment cycles between the lower and upper tertiles. In conjunction with other reports, this information has been used to design the Cardiac CARE trial which will randomise only those patients deemed at greatest risk of anthracycline-related cardiotoxicity because of high troponin concentrations to combination therapy comprising angiotensin receptor blockade and a beta blocker. The primary endpoint for this trial is change in left ventricular ejection as determined on cardiac magnetic resonance imaging performed at baseline and 6 months after initiation of therapy. This trial started recruitment in 2017.
**Dual Antiplatelet Therapy to Inhibit Coronary Atherosclerosis and Myocardial Injury in Patients with Necrotic High-Risk Coronary Plaque Disease (DIAMOND)**

The use of combined therapies to inhibit platelet function is well established in the treatment of acute myocardial infarction where it is typically continued for a period of 12 months (Yusuf, Zhao et al. 2001, Chen, Jiang et al. 2005, Wiviott, Braunwald et al. 2007, Wallentin, Becker et al. 2009). However, whether such therapy should be continued beyond this period remains unclear, as the bleeding risk associated with such treatment undermines the potential benefit with regards to reduced ischaemic events (Mauri, Kereiakes et al. 2014, Bonaca, Bhatt et al. 2015). The DIAMOND study is a randomised controlled trial investigating the ability of 18F-fluoride positron emission tomography to identify those patients with established coronary artery disease who are at greatest risk of cardiac events and therefore potentially most likely to derive net benefit from potent platelet inhibition. This trial has completed recruitment, enrolling 220 patients who have undergone baseline PET-CT imaging to determine the presence of coronary uptake of 18F-fluoride which reflects the presence of high-risk atherosclerotic plaque. Based on the results of this scan, patients have been grouped as at high or low-risk and each group was separately randomised to treatment with ticagrelor or placebo in addition to optimal medical therapies including aspirin. Patients will be followed for 12 months with serial monitoring of plasma cardiac troponin I concentrations providing the primary surrogate endpoint for determining treatment efficacy. We hypothesise that patients deemed at high risk according to the PET-CT scan will have higher baseline concentrations of troponin and will demonstrated a greater treatment-related reduction in troponin compared with those patients without coronary 18F-fluoride uptake. Patients will also undergo repeated CT
imaging at 12 months to investigate the possible association between baseline 18F-fluoride uptake and more rapid atherosclerotic disease progression.

8.2.3 18F-FLUORIDE POSITRON EMISSION TOMOGRAPHY FOR THE IDENTIFICATION OF HIGH-RISK CORONARY ATHEROSCLEROSIS

In chapter 7, we demonstrated the reproducibility of positron emission tomography with 18F-fluoride. Along with other groups we have previously demonstrated associations between vascular uptake of 18F-fluoride and risk of cardiovascular events (Dweck, Chow et al. 2012, Joshi, Vesey et al. 2014, Adamson, Hughes et al. 2015, Irkle, Vesey et al. 2015). The ability of this type of imaging to prospectively identify high-risk patients, independent of established risk factors requires further assessment in larger trials with sufficient statistical power to determine differences in clinical event rates.

PREDICTION OF RECURRENT EVENTS WITH 18F-FLUORIDE TO IDENTIFY RUPTURED AND HIGH-RISK CORONARY ARTERY PLAQUES IN PATIENTS WITH MYOCARDIAL INFARCTION (PREFFIR)

Risk for recurrent myocardial infarction following an index event remains increased relative to the patients with stable coronary artery disease. Previous studies have tried to improve and individualise risk assessment through the use of advanced coronary imaging techniques, such as intracoronary ultrasound (Calvert, Obaid et al. 2011, Stone, Maehara et al. 2011). To overcome the limitations inherent to invasive imaging, the PREFFIR trial (clinicaltrials.gov identifier NCT02278211) is a prospective, multicentre observational study involving clinical assessment and 18F-fluoride PET-CT imaging of patients with multi-vessel coronary disease in the early convalescent phase following an acute myocardial infarction. We have already recruited more than
300 patients to this study with a planned total enrolment of 700. Participants will be followed clinically for up to 5 years for the primary endpoint of cardiac death or recurrent myocardial infarction. Repeat coronary CT imaging will also be performed after 2 years to determine the differential rate of plaque progression related to 18F-fluoride uptake.

8.2.4 DETERMINING THE NATURAL HISTORY OF PLAQUE VULNERABILITY AS DETERMINED BY 18F-FLUORIDE POSITRON EMISSION TOMOGRAPHY

Whilst the PREFFIR trial will ultimately determine the clinical relevance of coronary uptake of 18F-fluoride, understanding how this information should change patient treatments requires greater understanding of the mechanisms underpinning 18F-fluoride uptake. To address this we are conducting natural history sub-studies of both the DIAMOND and PREFFIR trials whereby a total of 140 patients will undergo repeated 18F-fluoride PET-CT imaging at one of 4 time points following the baseline scan (6 weeks, 3 months, 6 months and 12 months).
8.3 CLINICAL PERSPECTIVE

Despite massive financial investment and dramatic technological advances, coronary heart disease remains a leading cause of death in the United Kingdom and globally. To address this ongoing challenge will require intelligent planning and coordination of ongoing research endeavours.

We have demonstrated the clear potential to improve the diagnostic and prognostic assessment of coronary heart disease with circulating and imaging biomarkers including high-sensitivity cardiac troponin and 18F-fluoride positron emission tomography. The simplicity of troponin testing clearly lends itself to broad application in primary and secondary prevention populations or as a surrogate endpoint in efficient randomised controlled trials. In contrast, positron emission tomography has complex infrastructural requirements and is consequently likely to have greatest value in individualising risk assessment in preselected patients, or in providing enhanced understanding of pathophysiological mechanisms in a non-invasive manner.

The promise these developments hold requires rigorous assessment in well-designed trials involving patients who closely reflect the population most likely to receive treatment. Such trials are difficult and costly to conduct with traditional methods and careful consideration should be given to more pragmatic approaches such as those employed in SCOT-HEART and DUAL-ACS. Finally, ongoing external validation of management pathways is vital to ensure appropriate translation of the results of these studies into future clinical guidelines.
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APPENDIX (SUPPLEMENTARY FILES ON CD)

A.1. DIAMOND TRIAL PROTOCOL

A.2. DIAMOND PATIENT INFORMATION SHEET – CONSENT FORM

A.3. DIAMOND SCOTLAND A RESEARCH ETHICS COMMITTEE APPROVAL

A.4. DIAMOND ADMINISTRATION OF RADIOACTIVE SUBSTANCES APPROVAL

A.5. DIAMOND NHS LOTHIAN RESEARCH AND DEVELOPMENT APPROVAL

A.6. DIAMOND SPONSORS AUTHORISATION TO RECRUIT

A.7. DIAMOND SPONSORS AUTHORISATION TO DOSE

A.8. DIAMOND LAB CERTIFICATE