

**Identification of significant coronary artery disease by the measurement of H-FABP (Heart-type Fatty Acid Binding Protein) for patients with normal troponin concentrations**

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**Dedicated  
To My Parents**

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## Abstract

**Objective:** We intend to investigate the ability of H-FABP levels to identify significant coronary artery disease in low- to intermediate-risk patients with suspected acute coronary syndrome (ACS).

**Background:** H-FABP is a small intracellular protein involved in the transport and buffering of fatty acids in cardiac myocytes. There is an increasing evidence base for the utility of H-FABP as a cardiac biomarker for the early diagnosis of ACS. Levels have been shown to provide incremental prognostic information about future risk of myocardial infarction (MI) and death, independent of traditional risk factors and troponin levels.

**Methods:** 238 patients presenting with possible ACS were recruited as they attended the Emergency Department (ED) of a major UK teaching hospital. Venous blood samples were drawn at presentation, 90 minutes later, and after 12 hours from index symptoms. H-FABP was measured using the RANDOX Immunoturbidometric Immunoassay. Computerised axial tomography coronary artery calcium scoring (CTCS) was used as a marker of coronary atheroma burden, combined with clinical assessment for the detection of obstructive coronary artery disease (CAD).

**Results:** There was a statistically significant positive correlation between H-FABP and CTCS, independent of age and renal function.

Used on its own as a continuous variable, H-FABP was poor at predicting Agatston CTCS >10, and the clinical demonstration of obstructive coronary disease (areas under the ROC curve 0.6-0.7). When used as part of a multifactorial model to predict these endpoints H-FABP was, however, a more powerful predictor than some traditional risk factors such as smoking status, diabetes, hypertension, as well as renal function.

H-FABP showed the greatest promise when used as a dichotomous variable within what we termed a 'triple rule-out' strategy. Here, H-FABP was used alongside the ECG and troponin to help identify patients at low risk of

obstructive coronary artery disease, and tertiary events within a median follow-up period of 406 days (death from a cardiovascular cause, myocardial infarction, coronary revascularisation). Using a H-FABP cut-off at the 50<sup>th</sup> centile (3.16 µg/l), the triple rule-out strategy had a 96.4% negative predictive value (95% CI 86.6%-99.3%), and sensitivity of 83.3% (95% CI 50.8%-97.1%). 31% of the recruited population fell into the rule-out group.

The triple rule-out strategy was validated for mortality on a cohort of 483 patients from the FAB2 study (Viswanathan et al., 2010). 19% of patients met rule-out criteria, with a 2.2% all-cause six year mortality, as compared to a 20.6% all-cause six year mortality for patients that did not. In the rule-out group no deaths were seen within the first 18 months. This equates to an all-cause six year mortality sensitivity of 97.5% (95% CI 90.5%-99.6%) and negative predictive value of 97.8% (95% CI 91.4%-99.6%).

**Conclusion:** The analyses demonstrate the potential for H-FABP to be used as part of a triple rule-out strategy, guided by clinical assessment, alongside the ECG and troponin to identify low risk patients as they present to the ED. The strategy would benefit from external validation.

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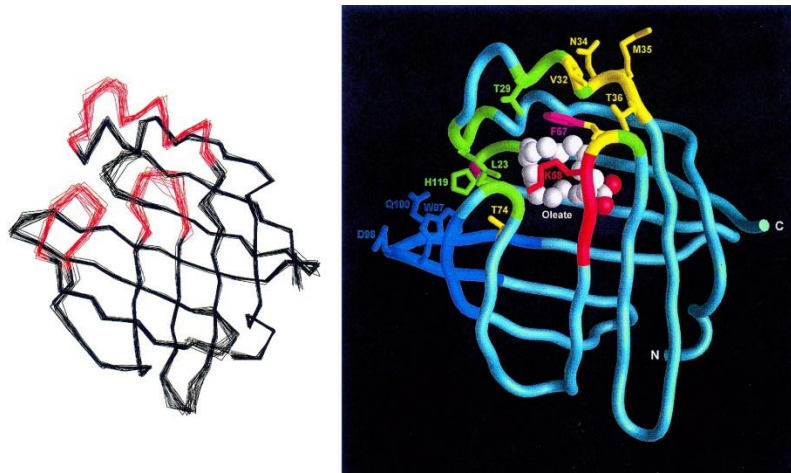
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## 1- Introduction

Chest pain presentations represent a significant healthcare burden, locally accounting for 6% of emergency department attendances, and 27% of acute medical hospital admissions (Goodacre et al., 2005). When a cardiac cause is felt possible, current UK practice is to measure troponin, an 'intermediate / late' cardiac biomarker. If early samples are normal, patients are kept in hospital and troponin levels are re-assessed at least 10-12 hours after the onset of pain (NICE CG95). There is a growing interest in 'early' cardiac biomarkers that could allow the risk stratification of these patients at a much earlier stage, facilitating discharge from hospital sooner if negative, and potentially allowing earlier treatment when positive.

Heart-type fatty acid binding protein (H-FABP) is a cytoplasmic protein found abundantly within cardiac myocytes (Glatz et al., 1997). Its small size (15 Kd) allows it to be readily released into the circulation after both myocardial ischaemia and infarction. Following myocardial infarction serum levels peak after around 6-8 hours, and return to normal within about 24 hours (Glatz et al., 2002; Glatz et al., 1994). Although highly cardiac-specific, small concentrations are found in tissues outside the heart. Other limitations include its renal excretion (like troponin) potentially making it less useful in patients with renal impairment, and the relatively narrow diagnostic time window. Elevated H-FABP levels are reported in patients with atrial fibrillation, atrial flutter, and after electrical cardioversion. This will need to be taken into consideration if being used as a biomarker in these patient groups (Mazovets et al., 2006).



**Figure 1.1** Three-dimensional structure of human H-FABP.

Biochemical Journal (2001) 354, 259-266.

There is a growing evidence base supporting the high potential of H-FABP levels in the early diagnosis of ACS, the risk assessment of patients presenting with chest pain, and emerging evidence suggesting that H-FABP levels are closely related to angiographic findings in patients presenting with ACS.

A Medline search was conducted for full reports of original research between January 2000 and January 2014 using the search terms “heart-type fatty acid-binding protein”, “fatty acid-binding protein”, “H-FABP”, “FABP”. The references of papers identified were explored to identify research not identified in the original search. Studies including less than 100 patients were excluded, as were studies solely using point-of-care methods.

### **1.1 H-FABP as a Diagnostic Marker**

There are now a number of studies published in this area, although many include fewer than 100 patients, and used different H-FABP assays. The conclusions of meta analyses are mixed. The systematic review and critical appraisal by Dekker *et al* published in 2010 concluded that H-FABP seemed to be a promising biomarker in the early assessment of ACS (Dekker *et al.*, 2010). Slot *et al* examined 16 studies (3709 patients, prevalence of MI 13–74%, male gender 49–84%, median age 64–76 years). For the diagnosis of MI the summary estimate was a sensitivity of 84% (95% CI 76% to 90%) and specificity of 84% (95% CI 76% to 89%). They conclude that H-FABP does

not fulfil the requirements needed for a safe and early diagnosis of AMI when used as a stand-alone test (Slot et al., 2010).

The met-analysis by Carroll *et al* included eight studies of quantitative H-FABP and nine studies of qualitative H-FABP. The summary estimates of sensitivity and specificity were 81% (95% CI 50% to 95%) and 80% (26% to 98%) respectively for the quantitative assays and 68% (11% to 97%) and 92% (20% to 100%) respectively for the qualitative assays. Four studies reported the sensitivity of troponin combined with H-FABP at presentation in which the combination was considered positive if either test was positive. The addition of H-FABP to troponin increased sensitivity from 42–75% to 76–97% but decreased specificity from 94–100% to 65–93%. They conclude that H-FABP may have a role alongside troponin in improving early sensitivity but comparison with high sensitivity troponin assays is required (Carroll et al., 2012).

In a study of 705 patients presenting with suspected cardiac chest pain, in blood drawn at the time of presentation (median of 3.5 hours after symptom onset), H-FABP was demonstrated to be superior to CKMB, myoglobin and troponin for the detection of MI (area under ROC curve (AUC) 0.86 (95% CI 0.82-0.90)) (Body et al., 2011b). Although in this study no single biomarker could enable clinically acceptable exclusion of MI on its own, the combination of troponin I and H-FABP was found to be the optimal combination. Similarly, in 485 patients Gururajan *et al* demonstrated H-FABP, measured 3-6 hours after the onset of pain, to be a good discriminator between patients with ACS as compared with those felt to have non-cardiac symptoms and normal controls (AUC 0.97, 95% CI 0.95-0.98) (Gururajan et al., 2010).

Collinson *et al* randomised 850 low-risk patients presenting with chest pain to either diagnostic assessment by a cardiac panel measured by point-of-care testing or to diagnosis when biomarker measurement was based on central laboratory testing. Patients with ECG changes of myocardial infarction or high-risk ACS were excluded. Blood was drawn on admission and 90 minutes from admissions for point-of-care measurement of Tnl, CKMB, and myoglobin. Samples were drawn at the same time for central



laboratory analysis of TnI, TnT, H-FABP, and copeptin. The authors conclude that the measurement of Tn was the single best test, but admission measurement alone did not achieve adequate diagnostic efficiency for rule-out. Diagnostic sensitivity was improved by measurement of H-FABP in addition to Tn (Collinson et al., 2014).

The absolute reported sensitivity and specificity of H-FABP for the early diagnosis of ACS varies in the literature. This may reflect the different assays used, diverse characteristics of the recruited populations, combined with variations in the way ACS is categorised and the time blood samples were drawn. As an example, in the study by Charpentier *et al* using samples from 677 patients with suspected ACS on admission to the ED, H-FABP was predictive of the diagnosis of ACS with an OR of 4.65 (95% CI 2.39-9.04), specificity of 96.8% (95% CI 95.4%-98.1%), but low sensitivity of 13.5% (95% CI 10.9%-16.1%) (Charpentier et al., 2010). In this study H-FABP, measured using an immunodetection with CardioDetect assay, was not felt to add significantly to standard diagnostic tools. Figiel *et al* demonstrated a similar specificity for diagnosing non-ST segment elevation MI in 100 patients, using blood drawn at presentation, although sensitivity in this study was found to be much higher at 94.7% (CI not available) (Figiel et al., 2008). This study also used the CardioDetect assay, although the recruited population was quite different: in this study all patients had a confirmed rather than suspected diagnosis of ACS. Cavus *et al* reported a sensitivity of 97.6% on samples taken during the first hour from presentation (CI not available) (Cavus et al., 2006).

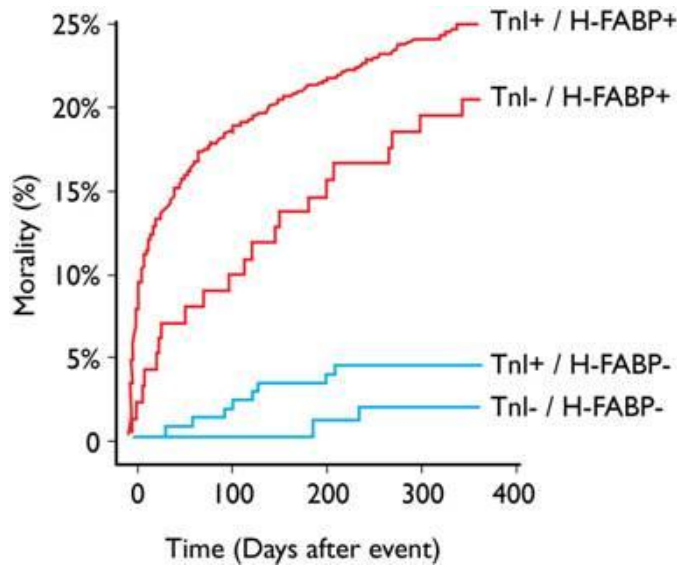
In the study by McMahon *et al* H-FABP was found to have a high negative predictive value (NPV) for MI, 93% at 0 to 3 hours from pain onset, increasing to 97% at 3 to 6 hours (CI not available for this study). Combining H-FABP with troponin increases this to 94 and 98% respectively. In this study, which also assessed CKMB, myoglobin, and TnI, H-FABP had the greatest sensitivity at 0 to 3 hours (64.3%), 3 to 6 hours (85.3%), and 6 to 12 hours (89.9%) after chest pain onset (an average superiority of 13.6% over Tn). The combination of TnI measurement with H-FABP increased this sensitivity to 71.4% at 0 to 3 hours, 88.2% at 3 to 6 hours, and 92.4% at 6 to 12 hours demonstrating an increase in sensitivity of 20.6% for the

combination marker approach at 3 to 6 hours. Troponin, on the other hand, demonstrated a higher specificity than H-FABP at 0 to 3 hours (93.3% compared with 84.2%) and at 3 to 6 hours (94.3% compared with 88.7% for H-FABP). At 6 to 12 hours, the specificity of H-FABP was comparable with that of TnI (93.5% for H-FABP compared with 94.2% for TnI) (McMahon et al., 2012).

Numerous other studies support the use of H-FABP for the early diagnosis of MI (Nakata et al., 2003; Okamoto et al., 2000; Orak et al., 2010; Ruzgar et al., 2006; Valle et al., 2008; Kim et al., 2011; Kim et al., 2010).

## **1.2 H-FABP as a Prognostic Marker**

One of the largest published studies on H-FABP, conducted by our department, examined the ability of H-FABP levels to predict long term mortality in patients with confirmed ACS. This used samples taken at 12-24 hours after chest pain from 1448 consecutive consenting patients with a confirmed diagnosis of ACS. Specifically patients were included in the study if they fulfilled the revised European Society of Cardiology / American College of Cardiology definition of MI: raised troponin value taken 12-24 hours after the onset of symptoms accompanied by at least one of the following: 1) ischaemic symptoms; 2) development of pathological Q waves on the ECG; 3) ECG changes indicative of ischaemia; 4) delivery of primary coronary angioplasty and 5) compatible post-mortem findings. Some unstable angina patients were also included on the basis of clinical and ECG diagnosis. H-FABP was strongly predictive of all-cause mortality at one year after index hospital admission, independent of traditional clinical risk factors, and across the whole spectrum of ACS (unstable angina, NSTEMI and STEMI presentations). Of great interest, was the ability of H-FABP levels to separate troponin negative patients into a low and high risk group. Unstable angina patients with a low H-FABP level (<5.8 µg/l) had a 1-year all-cause mortality of 2.1%, whereas patients with levels above this cut off had a mortality of 22.9%. The occurrence of a negative test for both H-FABP and troponin was associated with zero mortality prior to six months (Kilcullen et al., 2007).



**Figure 1.2** All-cause mortality by troponin and H-FABP clinical cut-offs. Note the ability of H-FABP levels to separate patients with negative troponin into low and high risk groups. TnI = troponin I, - = below clinical cut-off, + = above clinical cut-off. JACC (2007) 50, 2061-7.

A follow-on study further examined the role of H-FABP in the low to medium risk population by using 1080 consecutive presenting patient with suspected rather than confirmed ACS (Viswanathan et al., 2010). All individuals aged 18 years or above with chest pain of possible or definite cardiac aetiology were deemed eligible for recruitment, irrespective of ECG changes. 79% of these patients had negative troponin results  $\geq 12$  hours from pain. The risk of death or MI increased with increasing H-FABP levels, confirming the ability of H-FABP to predict events independent of other clinical risk factors, including troponin (Advia TnI-Ultra assay). The findings from these two studies are in keeping with those reported by O'Donoghue *et al* using samples taken from patients recruited into the OPUS-TIMI 16 trial (O'Donoghue et al., 2006).

Garcia-Valdecasas *et al* demonstrated that H-FABP, measured within the first 3-6 hours after the onset of chest pain, was not only more sensitive than TnI in the early diagnosis of MI, but was an independent predictor of events within a 6-month follow-up (Garcia-Valdecasas et al., 2011). Similarly, the study by Inoue *et al* utilising blood drawn at presentation from 432 patients,

concluded that H-FABP offered similar diagnostic performance to hsTnT for the rule-out of ACS (Inoue et al., 2011).

Numerous other studies confirm the prognostic potential of H-FABP (Suzuki et al., 2005; Erlikh et al., 2005).

### **1.3 Relating raised H-FABP to Stenotic Atherosclerosis**

My literature search revealed only one study examining the correlation between H-FABP levels and coronary artery stenosis. In a study of 93 patients with ACS presenting within two hours of onset of chest pain, H-FABP was measured at 2, 4, and 6 hours from symptom onset. All patients underwent coronary angiography. Peak H-FABP levels were seen at 4 hours. Measured at 2 hours, the sensitivity of H-FABP for at least 50% stenosis was 70%, rising to 85% at four hours for  $\geq 50\%$  stenosis, and 88% at four hours for  $\geq 70\%$  stenosis. The sensitivity and positive predictive value for revascularisation was 89 and 80% respectively at four hours (Kalay et al., 2010). There are no published studies examining the correlation between H-FABP and total atherosclerosis burden (i.e. a non-obstructive and obstructive coronary artery disease end-point).

### **1.4 Rationale for Avenue of Original Research Chosen**

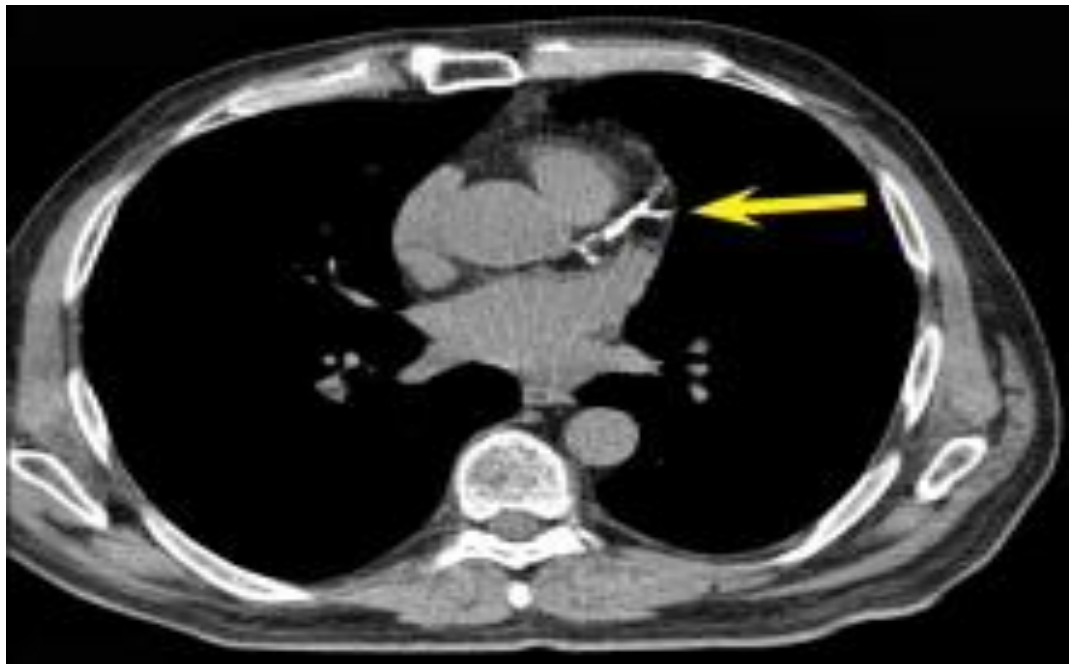
The majority of patients presenting to the ED with chest pain have not had a MI and are troponin negative (Goodacre et al., 2005). Within this large group most patients have relatively little short- to medium-term event risk, yet there are a small number of patients with a very high relative risk approaching 27% at one year (Viswanathan et al., 2010; Kilcullen et al., 2007). I believe that one of the primary potential uses of H-FABP is to differentiate between these two groups, perhaps facilitating discharge from hospital sooner if negative, and detecting high risk patients in need of further assessment.

Research undertaken in our department has already demonstrated the powerful potential of H-FABP as an independent prognostic marker in patients presenting with chest pain. Despite the high potential of H-FABP as a cardiac biomarker, it is yet to be used on any significant scale in the UK. **A study exploring a potential link between H-FABP's prognostic ability**

**and coronary artery disease would substantially strengthen the case for the use of H-FABP measurement in clinical practice.**

The FAB3-CT study was designed to examine the relationship between H-FABP levels and coronary artery disease in low-moderate risk patients presenting to the ED with suspected cardiac chest pain. All patients underwent a combination of clinical assessment and investigation as appropriate under the supervision of a Consultant Cardiologist to exclude obstructive coronary artery disease. In addition, CT Coronary Calcium Scoring was used to look for non-obstructive coronary atheroma.

### **1.5 CT Coronary Calcium Scoring as a Marker of Coronary Atheroma Burden**



**Figure 1.3** CT scan showing coronary artery calcification.

Left main stem, left anterior descending and circumflex artery calcification (arrow).

Many coronary events occur in the territory of coronary arteries that demonstrate only mild-moderate, rather than high grade stenosis (Schroeder and Falk, 1995). It is not surprising therefore that coronary plaque burden strongly relates to the risk of coronary events (Schmermund et al., 2003). Atherosclerosis is the only disease process associated with calcification

within the coronary arteries (Figure 1.3). The extent of calcification demonstrated by CT is therefore reflective of the overall atherosclerotic plaque burden (Rumberger, 2003), and large studies including more than 25,000 patients have demonstrated the ability of CT calcium scoring to provide incremental and independent predictive value over traditional risk factors (Budoff et al., 2007). It must be remembered, however, that although the risk of stenotic lesions is higher with increasing calcium scores, there is not a one-to-one correlation. A zero calcium score is associated with a less than 5% probability of obstructive coronary disease, and a good prognosis (event rate less than 0.2% per year), but does not exclude coronary stenosis (Haberl et al., 2001).

## **1.6 Accelerated Discharge Protocols for Patients with Chest Pain**

Chest pain is the second most common presenting complaint amongst adults attending the ED. The risk-adjusted mortality for patients with acute myocardial infarction (AMI) that are not hospitalised is 1.9, and for unstable angina 1.7 (Pope et al., 2000). As a consequence, formal diagnostic testing is undertaken on even very low risk patients, with the potential for false positive tests and unwarranted treatment, investigations and cost.

The yield of routine provocative cardiac testing has been evaluated in a study involving 4181 patients. These patients attended the ED with signs or symptoms of possible ACS, but were not found to have resting ECG ischaemia or positive biomarkers. They were evaluated with either exercise stress testing or myocardial perfusion imaging. A subset with positive provocative tests underwent invasive angiography. The therapeutic yield was low at 0.7%, suggesting that this may not be the best approach.

Most currently used discharge protocols rely on the results of a troponin measurement taken 12 hours from the index episode of chest pain.

Accelerated Discharge Protocols (ADPs) rely on ECG findings, clinical risk characteristics, and cardiac biomarkers, or a combination of these, to allow the identification of low risk patients at an earlier stage. These patients may be considered suitable for early discharge from hospital, occasionally with

further investigations planned on an outpatient basis. The introduction of chest pain pathways has been shown to reduce hospital length of stay, while improving patient satisfaction (Siebens et al., 2010).

It is worth bearing in mind that there is no universally accepted definition of a low-risk patient for ACS. The safety of an ADP relates to the number of missed events within the early discharge group. As previously mentioned, the risk-adjusted mortality for patients with AMI that are not hospitalised is 1.9, and for unstable angina 1.7 (Pope et al., 2000). Although debate exists as to the acceptable level of discharge risk, this is likely to be 1-2% (threshold for death, AMI, revascularisation). Kline *et al* described a test threshold representing a pre-test probability of <2% for ACS: the point at which the risks of a false positive test outweigh the risks of untreated disease (Kline et al., 2005).

In this section, I explore the current evidence base regarding ADPs. A Medline search was conducted for full reports of original research between January 2000 and January 2014 using the search terms “accelerated”, “chest pain”, “discharge protocol”, “diagnostic protocol”, “rule” and “critical pathway”. Accepting that some papers may not be indexed under terms related to an ‘ADP’, the references of papers identified were explored to identify research not identified in the original search. The studies are explored individually, then summarised in Table 1.1.

### **ASPECT Study (Than et al., 2011)**

This was an observational multi-centre study prospectively validating the safety of a predefined 2-hour accelerated diagnostic protocol. Patients were defined as ADP negative (low risk) if the following criteria were met: TIMI score 0 (Thrombolysis in Myocardial Infarction), ECG showing no ischaemia, combined with a satisfactory point-of-care (POC) multi biomarker panel (MMP) performed at 0 and 2 hours (troponin I, CKMB and myoglobin). The primary endpoint was major adverse cardiac events (MACE) within 30 days after initial presentation, defined as death (not clearly non-cardiac), acute myocardial infarction, cardiac arrest, emergency revascularisation, cardiogenic shock, ventricular arrhythmia and high-degree atrioventricular block needing intervention. Of the 3582 patients recruited 9.8% were

classified as ADP low risk (352 patients). Within this group the MACE rate was 0.9% (3 patients). The sensitivity for 30-day MACE was 99.3% and specificity 11%. Cardiac troponin as a sole biomarker was sufficient alone to produce a sensitivity of 98.6%, although this was not an a-priori hypothesis. Although the ADP was able to identify low-risk patients for discharge, no long-term outcome data is given, and the investigators do not make suggestions about what to do with the patients post-discharge.

A sub-study including 1000 of the ASPECT patients recruited in New Zealand, examined the impact of altering the original ADP, using point-of-care troponin I only, high sensitivity troponin T only, including patients with a TIMI risk score of 0 and 1, or a combination of these strategies. The primary endpoint was defined as AMI and ACS (i.e. unstable angina) within 30 days, including prevalent events (i.e. events being the cause for the patient's initial presentation). Unstable angina was defined as ischaemic symptoms with objective evidence of ischaemia on ECG, stress testing, or the finding of significant coronary artery disease. Of the 1000 patients, the diagnosis of ACS was made in 343 patients (34.3%), 235 with NSTEMI, and 108 with unstable angina. The use of early biomarker (POC-MMP, POC TnI, hsTnT) and ECG results alone missed many patients with ACS (sensitivity 71.5-87.6%). Incorporating a TIMI score with any of the biomarker strategies improved sensitivity. The 0- to 2-hour ECG/point-of-care multi-marker panel (POC-MMP)/TIMI score 0 (the original ASPECT ADP) was more sensitive than the ECG/POC-MMP/TIMI 0-1, with a sensitivity of 99.2% for the diagnosis of ACS, as compared to 97%. 12.3% of patients fell into the very low risk ASPECT ADP category. ECG/TIMI 0/POC-TnI and ECG/TIMI 0/hsTnT had similar sensitivities to the ASPECT ADP, but identified more patients as low risk (15.3 vs. 12.3%).

### **ADAPT Study (Than et al., 2012)**

Another study, also by Than *et al* examined an ADP using serial troponin as the only biomarker. This ADP also included a TIMI score and ECG, combined with 0- and 2-hour laboratory troponin I. Most participants were also part of the ASPECT study. MACE data was collected at 30 days, 45 days, and after one year. Patients were defined as ADP negative if their TIMI score was 0, no ischaemic ECG changes were present, and central



laboratory troponin I was below the institutional cut off at 0 and 2 hours. MACE was defined as death (not clearly non-cardiac), cardiac arrest, emergency revascularisation, cardiogenic shock, ventricular arrhythmia or high-degree atrioventricular block needing intervention, and acute myocardial infarction.

Of the 1975 consenting patients, 302 patients (15.3%) had a MACE within 30 days. 20% of patients were classified as low risk by the ADP. Only 1 patient classified as low risk had a MACE within 30 days, giving the ADP a sensitivity of 99.7% (95% CI 98.1%-99.9%). By including TIMI 1 patients in the ADP, the proportion of patients classified as low risk increased to 38.4%, at the expense of a reduction in MACE sensitivity to 97%.

This was an observational study. The majority of ADP negative patients did in fact go on to have further investigation within 30 days (81.1% had a stress test). Therapeutic and procedural interventions occurred in 18.3% and 2.0% of ADP negative patients, respectively. This indicates that although the ADP could be used to discharge some patients earlier, they would still need to complete outpatient investigation and review. Indeed the high level of interventions may have secured a better outcome for the ADP. The low specificity (23.5%) highlights the potential of this ADP as a rule-out rather than a rule-in tool. 45 day and one-year MACE event rates were not given in this paper.

### **ADAPT (sub-group) / APACE**

Cullen *et al* (Cullen et al., 2013), using two patient cohorts for validation purposes, tested the performance of this ADP using high sensitivity troponin. For the first cohort (1635 patients), the sensitivity, specificity and negative predictive value were 100% (95% CI 98.5-100%), 23.1% and 100% respectively. Amending the ADP to classify TIMI  $\leq 1$  (instead of TIMI 0) as low risk increased the proportion of patients falling into the low risk group from 19.6 to 41.5%, with sensitivity remaining high at 99.2%. Findings were equivalent for the second cohort. hsTn was regarded as elevated if above a 99<sup>th</sup> centile cut-off. Serial changes in troponin (deltas) were not used.

**Ng *et al* (Ng *et al.*, 2001)**

Ng *et al* describe a ninety-minute accelerated pathway. This relied on the use of multi-biomarker point-of-care measurements at 0, 30, 60 & 90 minutes, and is unlikely to be feasible in most busy EDs.

**Aldous *et al* (Aldous *et al.*, 2012a)**

Aldous *et al* assessed the use of hsTnT in 385 patients. 21.3% had AMI. hsTnT had a sensitivity of 95.1% (95% CI 88.7-98.1%) by 2 hours, with a specificity of 75.6% (95% CI 73.8-76.5%). The sensitivity was not statistically different between peak values at 2h and 24h. The addition of a  $\geq 20\%$  delta criterion between 0 and 2h samples (with one value  $\geq 99^{\text{th}}$  centile) increased specificity to 92.4% (95% CI 90.2-94.3%) but reduced sensitivity to 56.1%. Taking ECG results into account reduced the false negative rate to 1.2%. The investigators concluded that although the measurement of hsTnT at 0 and 2h after presentation is not sufficiently sensitive to identify all cases of AMI, in combination with the ECG it could potentially safely identify patients suitable for early functional testing.

<b>Study</b>	<b>Year of Recruitment</b>	<b>N</b>	<b>Entry Criteria</b>	<b>ADP</b>	<b>Low Risk Classification</b>	<b>Outcome</b>
<b>ASPECT</b>	11/2007 – 07/2010	3582	>18 years Symptoms suggestive of ACS	TIMI 0 No new ECG ischaemia Normal point of care biomarker panel at 0 & 2h (Tnl, CKMB, myoglobin)	9.8%	Sensitivity 99.3 NPV 99.1 Specificity 11 PPV 12.9
<b>ADAPT</b>	11/2007 – 02/2011	1975	>18 years Symptoms suggestive of ACS	TIMI 0 No new ECG ischemia Normal Tnl at 0 and 2 hours	20%	Sensitivity 99.7 NPV 99.7 Specificity 23.4 PPV 19
<b>ADAPT (sub-group)</b>	11/2007 – 02/2011	1635	>18 years Symptoms suggestive of ACS	TIMI 0 No new ECG ischemia Normal hsTnl at 0 and 2 hours	19.6%	Sensitivity 100 NPV 100 Specificity 23.1 PPV 18.8

**Table 1.1** Accelerated Discharge Protocols – Summary Table.

<b>APACE</b>	11/2007 – 02/2011	909	>18 years Symptoms suggestive of ACS	TIMI 0 No new ECG ischemia Normal hsTnl at 0 and 2 hours	25.3%	Sensitivity 100 NPV 100 Specificity 30.5 PPV 23
<b>ADAPT (sub- group)</b>	11/2007 – 02/2011	1635	>18 years Symptoms suggestive of ACS	TIMI ≤1 No new ECG ischemia Normal hsTnl at 0 and 2 hours	41.5%	Sensitivity 99.2 NPV 99.7 Specificity 48.7 PPV 25.6
<b>APACE</b>	11/2007 – 02/2011	909	>18 years Symptoms suggestive of ACS	TIMI ≤1 No new ECG ischemia Normal hsTnl at 0 and 2 hours	38.6%	Sensitivity 99.4 NPV 99.7 Specificity 46.5 PPV 27.8

**Table 1.1** Accelerated Discharge Protocols – Summary Table.

<b>Christenson et al</b>	06/2000 – 01/2003	769	>25 years Chest pain	Age <40, normal initial ECG & no prior history of ischaemic chest pain  Age ≥40 normal initial ECG, no prior history of ischaemic chest pain, low risk pain characteristics, initial CKMB <3.0µg/l, or ≥3.0µg/l with no change in ECG or CKMB or Tn from 0 to 2 hours.	32.5%  (3.2% in a validation study – applied to a higher risk cohort)	Sensitivity 98.8%  Specificity 32.5%
<b>APACE (sub-group) Reichlin et al</b>	04/2006 - 06/2009	872	>18 years Symptoms suggestive of ACS	No STE on ECG  Baseline hsTnT <12 ng/l and an absolute change within the first hour of <3 ng/l	60%	Sensitivity 100%  NPV 100%
<b>Hess et al</b>	07/2007- 02/2010	2718	>24 years Chest pain  No STE or haemodynamic instability	NONE of - New ischaemia on initial ECG - History of CAD - Pain typical for ACS - Presentation Tn positive  AND Age ≤40 years or 41-50 years with negative Tn six hours from symptoms	18%	Sensitivity 100%

**Table 1.1** Accelerated Discharge Protocols – Summary Table.

<b>Body et al</b>	Testing completed 08/2009	703	>25 years Chest pain within 24 hours	Presentation hsTnT below detection limit for assay (99 <sup>th</sup> centile: 14 ng/l, coefficient of variation <10% at 9 ng/L)	27.7%	Sensitivity of 100% (95% CI 97.2 to 100%) and NPV of 100%
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**Table 1.1** Accelerated Discharge Protocols – Summary Table.

**Table 1.1** Accelerated Discharge Protocols – Summary Table.

### **The Vancouver Chest Pain Rule (Christenson et al., 2006)**

Christenson *et al* studied a population of 769 patients, and derived a clinical prediction rule for the exclusion of 30 day ACS, that was 98.8% sensitive. 10% of the population had a diagnosis of MI, and a further 11.4% unstable angina at adjudication based on assessment of cases at 30 days. They conclude that patients are at very low risk of ACS if they have a normal initial ECG, no previous ischaemic chest pain, and are younger than 40 years. Patients aged 40 years or older were considered low risk if they met the same criteria, have low risk pain characteristics (pain not radiating to the arm/neck/jaw, pleuritic pain or pain increasing with palpation) and have an initial CKMB  $<3.0 \mu\text{g/l}$  or an initial CKMB  $\geq 3.0 \mu\text{g/l}$  but no ECG or serum marker increase at 2 hours (CKMB or Tn). 32.5% of patients fell into the low-risk group.

Aldous *et al* validated the sensitivity, but only 3.2% of patients fell into the low risk category when it was applied to their recruited population. The recruited population in this case was higher risk, 36.2% having an eventual diagnosis of ACS. The authors attribute this to their system of pre-hospital screening that excludes patients with atypical symptoms (Aldous et al., 2012b).

The Vancouver Chest Pain Rule was also validated by Greenslade *et al* using hsTnI as the only biomarker (Greenslade et al., 2013). The rule was applied to the ADAPT study population previously mentioned. This included 1635 patients, 20.4% of whom had ACS. 7.4% of patients fell into the low risk group on presentation, with a further 25.6% after 2-hour ECG and troponin. Of the 33% of patients assigned to the low risk group (539 patients), 5.6% had ACS. The sensitivity for ACS at 30 days was 91%, NPV 94.4%. The authors comment that the sensitivity would have been no higher if CKMB rather than troponin had been used. The sensitivity was insufficient to enable safe early discharge. An earlier validation of the chest pain rule in its original form by Jalili *et al* found the sensitivity to be 95.1% (95% CI 88.0%-98.7%), using a cohort of 593 patients recruited in Tehran (Jalili et al., 2012).

**Sebbane et al (ultrasensitive copeptin combined with hsTnT)  
(Sebbane et al., 2013)**

Sebbane *et al* examined the value of combined hsTnT and ultrasensitive copeptin drawn at admission for the early rule out of AMI in 194 consecutive patients presenting to the ED with chest pain. The combination of biomarkers significantly improved the receiver operating characteristic area under the curve (AUC) from 0.89 (95% CI 0.85-0.92) for hsTnT alone to 0.93 (95% CI 0.89-0.97),  $P = .18$ . The sensitivity of the dual biomarker strategy was 96% for the detection of NSTEMI, with a negative predictive value of 95%.

The study by Reichlin *et al* demonstrated similar findings. This recruited 487 consecutive patients presenting to the ED with symptoms suggestive of AMI. 17% of the recruited cohort had a final diagnosis of AMI. The diagnostic accuracy of TnT at presentation in the diagnosis of AMI as quantified by AUC was 0.86 (95% CI 0.80-0.92), which was significantly higher than the diagnostic accuracy of copeptin at presentation (AUC 0.75; 95% CI 0.69-0.81;  $p = .009$ ). However, the combination of the 2 markers significantly increased the diagnostic accuracy provided by TnT alone, with an AUC of 0.97 (95% CI 0.95-0.98;  $p < 0.001$ ). The authors conclude that AMI would have been correctly ruled out at presentation in 65% of patients, with a sensitivity of 98.8%, a negative predictive value of 99.7% and a specificity of 77.1%. One major limitation is the finding that patients with unstable angina, which accounted for 17% of the recruited population after adjudication, had similar copeptin levels to patients with other causes of chest pain. Copeptin therefore cannot discriminate patients with unstable angina from patients with non-ischaemic chest pain (Reichlin *et al.*, 2009).

Maisel *et al* also used a copeptin-troponin biomarker combination, coupled with the finding of a non-diagnostic ECG. 1967 patients presenting to the ED with chest pain were recruited in a multi-site study. Patients were followed up for 180 days, with the primary outcome being a diagnosis of AMI. 7.9% of patients had a final diagnosis of AMI. A negative copeptin and TnI ruled out AMI for 58% of patients, with a negative predictive value of 99.2% (95% CI 98.5-99.6) (Maisel *et al.*, 2013).



A fourth study took samples from 641 consecutively presenting patients with chest pain within the last 12 hours, without ST elevation. Blood was drawn at presentation. The sensitivity for the detection of NSTEMI for the combination of copeptin and TnI was 90.4%, insufficient to safely rule out NSTEMI at presentation (Charpentier et al., 2012). The overall picture for copeptin based strategies is therefore mixed.

### **High-sensitivity troponin**

Recently, we have seen the development of high-sensitivity troponin, with limits of detection around 10-fold lower than conventional assays. Although the higher sensitivity allows the detection of myocardial necrosis earlier, clinicians have been challenged to decide how best to integrate these assays into clinical decision making given their low specificity. In the study by Reichlin *et al* upon adjudication 12 out of 72 patients were felt to have been falsely ruled in for AMI based on hsTnT: the corrected diagnoses assigned being arrhythmia, myocarditis, pulmonary embolism, hypertensive crisis, heart failure, and chest pain of unknown origin (Reichlin et al., 2012). Other studies have suggested possible susceptibility of hsTn to biological variability across age and sex, and reported elevations in patients with stable CAD and heart failure.

### **Reichlin et al (Reichlin et al., 2012)**

Reichlin *et al* studied a one-hour rule-in rule-out of AMI using hsTnT. This study included 872 patients from the APACE study cohort. Blood samples were drawn at presentation and after one hour. An algorithm for the use of hsTnT was developed in a randomly selected 436 patients of the total group, and then validated in the other 50% of patients. The adjudicated final diagnosis was AMI in 17% of patients, and unstable angina in 12%. Classification and regression tree analysis (CART) was used to help develop the algorithm. Entered into this were baseline hsTnT, absolute hsTnT change within the first hour, age, sex, ECG features, and time since onset of symptoms. For rule-out of AMI, the optimal thresholds were selected to allow for a 100% sensitivity and NPV. The rule-out criteria were defined as a baseline hsTnT <12 ng/l and an absolute change within the first hour of <3 ng/l. Rule-in was defined as a baseline hsTnT at presentation ≥52 ng/L or an

absolute change within the first hour of  $\geq 5$  ng/L. The other variables entered into the CART analysis did not improve accuracy.

After applying the hsTnT algorithm to the validation cohort, 60% of patients were classified as rule-out with a sensitivity and NPV of 100%. 17% (72) of patients were classified as rule-in, with 12 of these being a false rule-in as mentioned above. The remaining 23% of patients were classified into an 'observational zone'.

There were 12 deaths in the whole cohort within 30 days, and 55 within 24 months. For the rule-out group cumulative survival rates were 99.8% at 30 days, and 98.1% at 24 months. This study demonstrated a simple algorithm for safe rule-out within 1 hour of presentation. There are many questions to be explored however: how to deal with the 23% of patients falling into the observational zone, and how to assess the resource implications for the false rule-in group. The authors raise two notable points: 1- that hsTn should be interpreted as a quantitative rather than a qualitative measure of risk due to the observation that the proportion of patients with adjudicated AMI rose continuously with increasing hsTnT values. In the context of hsTn the terms 'troponin positive' and 'troponin negative' should be avoided. Furthermore, 2- they highlighted the usefulness of quantitative baseline measures of hsTn combined with changes over a given time.

Body *et al* also investigated the ability of presentation hsTnT to exclude AMI, but used an undetectable hsTnT level as the cut-off (Body et al., 2011a). A fifth generation hsTnT was used (99<sup>th</sup> centile: 14 ng/l, coefficient of variation <10% at 9 ng/L). The recruited cohort, which included 703 patients, had an adjudicated AMI rate of 18.5%. No patients with a hsTnT value of <3 ng/l at the time of presentation had an AMI, giving a sensitivity of 100% (95% CI 97.2 -100%) and negative predictive value of 100%. 27.7% of patients fell into this 'rule-out' group, 2 (1%) of whom died or had AMI within 6 months (1 peri-procedural AMI, 1 non-cardiac death due to carcinoma). Similar findings were seen upon audit after introducing hsTnT into their clinical service. It must be highlighted that the hsTnT cut-off was chosen to rule-out AMI. The specificity at this level was low (34%, 95% CI 30.2-38.1%). Patients should not be labelled as abnormal at values of less than the 99<sup>th</sup> centile for the

reference population. These patients would need to undergo further evaluation. There is still a lot of debate about how best to use hsTn to rule-in patients given the low specificity.

**Hess et al (Hess et al., 2012)**

This study sought to develop a clinical prediction rule to identify ED patients at very low risk of 30-day cardiac events (AMI, revascularisation, death), who might be suitable to discharge for out-patient follow-up. 2,718 patients were enrolled following presentation with chest pain without ECG STE or haemodynamic instability. 64 variables were recoded, with a decision rule generated by statistical analysis. 16.2% of patients suffered a MACE event by 30 days. A patient was said to have met the discharge rule if 1- there were no new ECG changes, 2- there was no history of CAD, 3- the pain was felt to be non-cardiac on clinical assessment, 4- the presentation cardiac troponin was negative, and 5- the patient was aged  $\leq 40$  years, or aged 41-50 years with a negative troponin at six hours from symptoms. A contemporary troponin assay was used. The sensitivity of the chest pain rule was found to be 100% for MACE at 30 days.

I have discussed a large number of biomarker strategies presented in the literature. These have been summarised in Table 1.1. All have their limitations. Some require biomarker assessment at multiple time points, which may not be practicable in a busy ED; others are highly sensitive for rule-out but only capture a small number of the presenting population within the rule-out; some are complex; and some leave many patients in an indeterminate diagnostic zone which places the clinician in a management quandary. A common theme is the ability to detect AMI as diagnosed by a positive troponin, by the measurement of troponin. One should not be surprised if the two correlate well. It is important that strategies are also assessed on their ability to prognosticate. Unfortunately most of the studies do not provide outcome data beyond 30 days. Each healthcare provider will need to explore the available options, and apply a strategy that suits their pre-existent experience, infrastructure, and biomarker availability.

## **2- Aims and Objectives of the Research**

I have presented a summary of the evidence base underlying the clinical utility of H-FABP measurement. H-FABP levels are predictive of the risk of death or MI independent of other clinical risk factors, including troponin, in patients presenting with possible ACS. The potential for the use of H-FABP levels to aid with the risk stratification of patients presenting to hospital with chest pain is of great interest, particularly in the majority group of patients who have a negative troponin.

### **Research Hypothesis**

**We hypothesise that raised H-FABP levels are a marker for significant coronary artery disease in patients presenting with chest pain with normal troponin concentrations. More specifically:**

- That H-FABP is higher in patients with new onset chest pain that are shown to have significant coronary artery disease, and
- That for patients with new onset chest pain, measurement of H-FABP provides information that is additive to the clinical criteria recommended in the NICE risk of CAD tables (NICE CG95).

My MD thesis will be based around the design, implementation, results, and analysis of an original prospective observational clinical research study conducted to test this hypothesis. In the spirit of the Translational Research Grant awarded by Heart Research UK in support of this study, I will explore the potential to use the information obtained to generate an early discharge rule that could be applied to patients to identify those at very low risk of adverse outcomes, that may be suitable for early discharge from the Emergency Department. I will attempt to validate this by applying the rule to previously recruited patient cohorts on which we have long term follow-up data.

### 3- Methods

This was a prospective observational study. We recruited 238 patients presenting to the ED of the Leeds General Infirmary, a major university-affiliated teaching hospital in Northern England, between October 2011 and November 2012. All patients were considered eligible if their presenting symptoms were felt to be suggestive of possible ACS by the assessing ED physician in conjunction with the cardiology research physician, or specialist research nurse. When in doubt, the final judgment regarding the appropriateness of inclusion into the study was made following discussion of the case with the principal investigator (STA). Inclusion criteria were pragmatic to ensure that the recruited cohort was reflective of the lower risk portion of patients presenting to the ED. Appendix A contains details of the power calculations undertaken to determine the number of study participants required.

#### 3.1 Inclusion Criteria

- I. Chest pain suggestive of ACS.
- II. Lack of acute ischaemic ECG changes.
- III. Absence of a prior history of coronary artery disease, coronary artery bypass surgery, or percutaneous coronary revascularisation.

For ECG interpretation, criteria set out in the ESC/ACCF/AHA/WHF Expert Consensus Document on the Universal Definition of Myocardial Infarction were used to define acute ischaemic changes (Thygesen et al., 2007). In the absence of LVH and LBBB the criteria were:

- ST elevation - new ST elevation at the J-point in two contiguous leads with the cut-off points:  $\geq 0.2$  mV in men or  $\geq 0.15$  mV in women in leads V2–V3 and/or  $\geq 0.1$  mV in other leads.
- ST depression and T-wave changes - new horizontal or down-sloping ST depression  $\geq 0.05$  mV in two contiguous leads; and/or T inversion  $\geq 0.1$  mV in two contiguous leads with prominent R-wave or R/S ratio  $> 1$ .

A prior history of coronary artery disease was defined as: a known diagnosis of angina, prior abnormal cardiac functional assessment, or a prior abnormal coronary angiogram.

### **3.2 Exclusion Criteria**

- I. Obvious non-cardiac cause diagnosed.
- II. Troponin >99<sup>th</sup> centile at any stage (see below).
- III. Patient unable (e.g. in cases of cognitive impairment), or unwilling to consent.
- IV. Serious co-morbidities (e.g. disseminated cancer) or social problems preventing participation.
- V. Presentation >24h after onset of index symptoms.
- VI. Pregnancy.

After obtaining patient consent, venous blood samples were drawn at admission, 90 minutes later, and also at 12 hours from index symptom onset.

With reference to exclusion criterion II. Troponin levels were not known at the time of patient screening. Recruited patients who developed a troponin >99<sup>th</sup> centile at any stage, were excluded from subsequent parts of the study, apart from monitoring for the occurrence of major adverse cardiovascular events. All patients had a  $\geq 12$  hour troponin taken as part of routine clinical care.

Demographic and relevant clinical baseline data (including known prognostic factors) were collected at the time of consent or during the hospital stay. This was facilitated by integration of the research documentation into the normal printed ED patient management protocol and record sheets (Appendix B). Patients were admitted to the ED Clinical Decisions Unit to be managed in line with standard local protocols, including any necessary initial investigations / specialty reviews, serial ECGs, and to await  $\geq 12$  hour troponin and further review under the care of the ED team.

Following discharge, all troponin negative patients (<99<sup>th</sup> centile) underwent CT scanning to obtain a coronary artery calcium score. In addition, these patients were seen (in the majority of cases within two weeks) in a specialised rapid access chest pain clinic under the supervision of a Consultant Cardiologist. Patients were investigated with reference to standard local and national clinical assessment and investigation protocols (NICE CG95), as deemed appropriate by the assessing physician. The out-patient clinical assessment formed part of the patient's standard care, aimed at ascertaining the cause of their original symptoms and excluding obstructive coronary artery disease where this was suspected clinically. From the study perspective, this follow-up was designed to look for both obstructive and non-obstructive coronary artery disease by combining a CT coronary calcium score, a marker of atheroma burden, with appropriate assessment to exclude obstructive coronary artery disease where this was clinically indicated.

All patients were followed up for the occurrence of major adverse cardiovascular events for a minimum of 6 months after index assessment – defined as death from cardiovascular cause, MI, or coronary revascularisation procedure. Monitoring of long-term survival status is planned through the UK Office of National Statistics. The hospital electronic records system was used to identify the occurrence of MI or coronary revascularisation procedure. This method was found to be robust and reproducible when used for the FAB2 study, during which it was validated on a random sample of patient hospital records and follow-up questionnaires (Viswanathan et al., 2010). As our hospital is the sole provider of emergency care within the city of Leeds, and the only tertiary referral site for primary angioplasty within West Yorkshire, it is expected that using this method we were able to identify the majority of cardiovascular events. In cases of patients who were not local to our area, records were sought from their local care provider if they had continued investigation elsewhere, and where necessary contact was made with the patient to ensure no events were missed.

### **3.3 Patient Consent**

Allowing for the busy ED environment and the fact that many patients are in discomfort on presentation, a two-stage consent process was deployed. This has been used successfully with ethical approval in previous studies undertaken in our department.

Verbal consent was taken for the initial set of presentation blood samples. Patients usually have blood tests taken at this stage anyway for their routine clinical care, and so this stage did not involve additional phlebotomy.

This was followed by full written consent prior to the 90 minute samples allowing sufficient time for patients to read the patient information sheet, and consider the matter further. In cases where the patient did not want to proceed further with the study, the initial research samples taken were destroyed. Appendix B contains copies of the Patient Information Sheet and Consent Form used.

At the time of their CT scan or clinic review, patients were asked to consider providing consent for long term storage and use of their samples in further studies (denoted the FAB3-CTx (extension) Study). A copy of the Patient Information Sheet and Consent Form used can be found in Appendix C.

### **3.4 Ensuring Safe Research Conduct**

A comprehensive education strategy was put in place to ensure that all members of the ED staff involved with the clinical care of recruited patients were familiar with pertinent aspects of the study. This took the form of informative e-mails and posters, combined with presentations at the mandatory ED staff induction sessions for each cohort of new ED doctors, and a presentation at the ED clinical governance meeting.

### **3.5 Measurement of Cardiac Biomarkers**

All venous blood samples were analysed for troponin I, H-FABP, B-type natriuretic peptide (BNP), and high-sensitivity C-reactive protein (hsCRP).

The routine clinical measurement of troponin I (TnI Ultra - Siemens Chemiluminescence Immunoassay) was used to define patients who were



“*troponin negative*”, i.e. those below the clinical decision limit. Troponin was the only biomarker measured live, and the results at each time point were made available to the clinical team to aid with patient management. The availability of troponin results at presentation and 90 minutes later was not standard practice within our ED at the time of initiating this study. The measurement of admission troponin for study patients brought practice in line with NICE guidelines (CG95). The release of 90 minute troponin results was felt necessary from an ethical perspective and is part of practice in some UK centres. As recruited patients who developed a troponin >99<sup>th</sup> centile at any stage were excluded from subsequent parts of the study, apart from monitoring, this is not expected to impact on study findings.

The availability of admission and 90 minute troponin results formed one of the focuses of the education strategy, in particular to ensure that patients could not be discharged based on a negative early troponin result. The release of early troponin results to the clinical team was intended to augment patient care, allowing ACS therapies to be commenced at the earliest opportunity following the diagnosis of ACS, in line with current NICE guidance. Furthermore, it was felt that this would be an aid to service development within the ED. As part of service development work stemming from this study, the routine measurement of admission troponin has subsequently been introduced across the Leeds Teaching Hospitals NHS Trust.

All FAB3-CT study blood samples were centrifuged and stored at -70°C for batch assays to be carried out for measurement of H-FABP, BNP, and hsCRP at a later stage. Sample stability tests previously conducted in our department showed no significant change in H-FABP concentrations after storage for 12 months using this method. H-FABP was measured using the RANDOX Immunoturbidometric Immunoassay, on a Siemens 1800 Advia Instrument platform. Assays used for the other biomarkers were:

- hsCRP - Siemens Immunoturbidometric Immunoassay, on a Siemens 1800 Advia instrument platform.
- TnI Ultra - Siemens Chemiluminescence Immunoassay, on the Siemens Advia Centaur platform.

- NTproBNP - Siemens Chemiluminescence Immunoassay on the Siemens Immulite 2000 platform.

### **3.6 ECG Interpretation**

For the purpose of patient management, ECGs were interpreted by the clinical team. For the purposes of the study all ECGs were assessed according to the pre-agreed criteria already stated. A report for each was recorded in the study database.

### **3.7 Genetic Samples**

A blood sample for DNA, and saliva sample for RNA were taken at the '90 minute' time point for processing and storage. It is our intent that these samples will be used for future collaborative research following further grant submissions. Similarly, BNP and hsCRP will not be a focus for this thesis.

### **3.8 CT Coronary Calcium Score**

A 64 slice Siemens scanner was used to take a total of 40 sections with a tomographic slice thickness of 3 mm covering the area between the carina and the diaphragm, proceeding in a caudal direction. Images were obtained at 100-ms/slice scanning time using ECG-triggered acquisition and sampled from 60 to 80% of the R-R interval. A calcified lesion is conventionally defined as >3 contiguous pixels with a peak attenuation of at least 130 Hounsfield units – being scored using the method of Agatston *et al* (Agatston *et al.*, 1990). Scans were reported by our collaborating Consultant Radiologist (MD).

### **3.9 Rapid Access Chest Pain Clinic Assessment**

In most cases clinical review was performed within 2 weeks of referral and involved “*one-stop*” assessment. Documentation of essential study data points was aided by the use of a purpose designed clinic document pack, which included a summary guide to current NICE guidelines (CG95) – see Appendix C. Additional investigations were arranged at the discretion of the assessing Cardiologist when clinically indicated. Progression to angiography

was dictated by these results combined with the judgement of the clinician, and expressed wishes of the patient. Consequently, we were able to obtain clinical information additional to that obtained at the time of index assessment in the ED.

Results of the CT coronary calcium score were made available to the assessing doctor to aid clinical management. The use of CTCS is part of NICE guidelines for the assessment of patients with estimated pre-test likelihood of CAD from 10-29% (CG95). The availability of the result for patients within other pre-test likelihood categories may have influenced secondary prevention measures and investigation decisions, but was felt to be an ethical requirement in this case. As most patients with CAD have coronary calcification, the availability of these results is likely to have increased the detection of significant CAD, which was an objective of the RACPC from the study perspective. Consideration of secondary prevention measures is routine for all patients seen in a RACPC, and unlikely to have affected event rates within the relatively short follow-up period of this study.

### **3.10 Study Endpoints**

These are the predetermined study endpoints, as defined in the study protocol.

**3.10.1 Primary Endpoint:** Presence of significant coronary artery disease defined as the finding of an Agatston CT coronary calcium score >10.

The cut-off of an Agatston calcium score >10 was a pragmatic decision. This was based on its use as a cut-off level in some of the larger coronary artery calcification studies. An example of this is the study by Blaha *et al*, which assessed annualised all-cause mortality rates in 44,052 consecutive asymptomatic patients referred for coronary artery calcium (CAC) testing. The mean follow-up of the cohort was 5.6 +/- 2.6 years. A total of 19,898 patients (45%) had no CAC on screening, whereas 5,388 (12%) had low levels (CAC 1-10), and 18,766 (43%) had CAC>10. Annualised all-cause

mortality rates for CAC = 0, CAC 1-10, and CAC >10 were 0.87, 1.92, and 7.48 deaths / 1000 person-years, respectively (Blaha et al., 2009).

**3.10.2 Secondary Endpoint:** Clinical demonstration of obstructive coronary artery disease based on clinical assessment of patients supported by a functional test of myocardial ischaemia and a stenosis of >50% in at least one artery at the time of coronary angiography.

**3.10.3 Tertiary Endpoint:** Occurrence of a major cardiovascular event in the first 6 months after index assessment – defined as death from a cardiovascular cause, myocardial infarction, or coronary revascularization procedure.

The study was powered to assess the primary endpoint. It was appreciated that the recruited cohort size may be inadequate to assess secondary and tertiary endpoints.

### **3.11 Data Security**

A System Level Security Policy is in place to ensure the security and safety of confidential patient information.

### **3.12 Ethics**

An Integrated Research Application was made and all necessary approvals obtained for this study, including approval from the East Yorkshire & North Lincolnshire Research Ethics Committee (REC Reference 10/H1304/36).

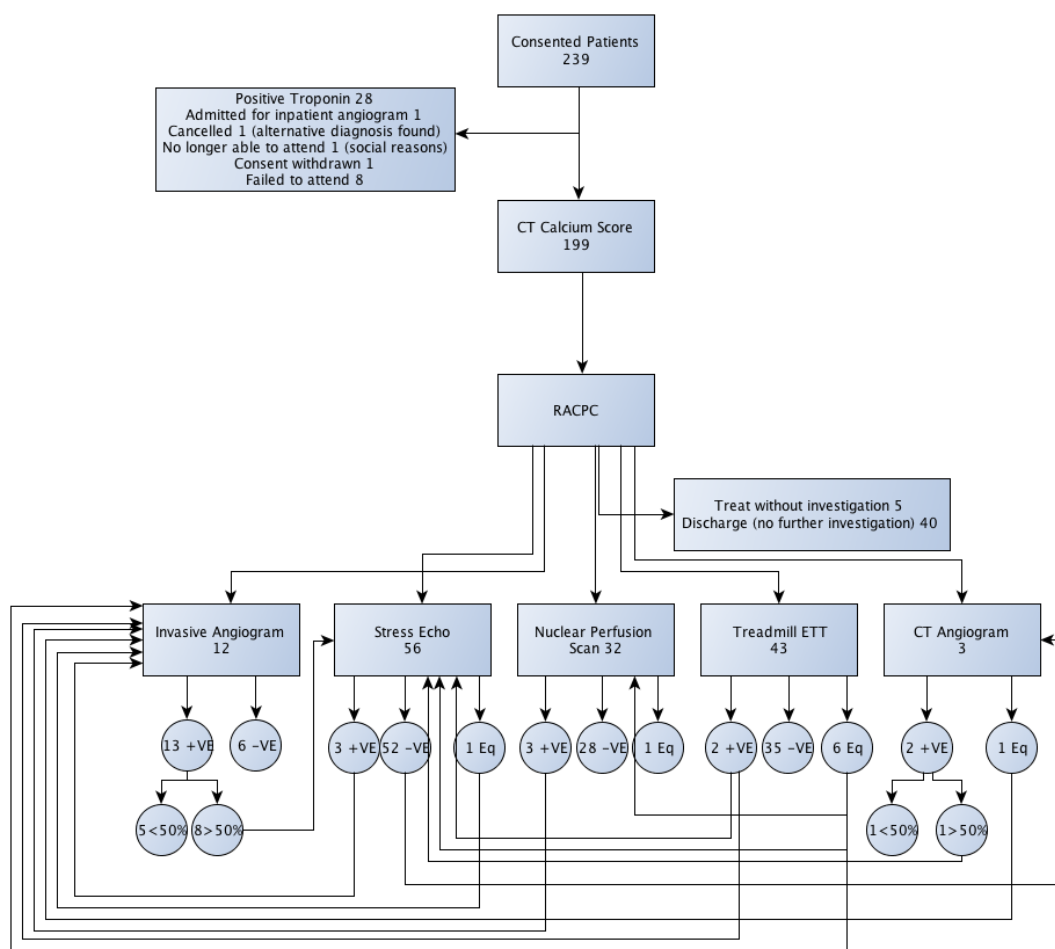
### **3.13 Funding**

Funding for this study was secured via a grant from Heart Research UK (Grant Reference RG2586). This grant included provision for specialist research nurse support. An additional grant was obtained from the Leeds Teaching Hospitals Charitable Foundation Research and Development Fund to support the involvement of a Clinical Research Fellow (STA). Successful

adoption was achieved on the UK Clinical Research Network Portfolio (UKCRN ID 9907).

## 4- Results

239 patients were recruited between 4<sup>th</sup> October 2011 and 23<sup>rd</sup> December 2012. The chart below summarises patient flow through the various stages of the study.



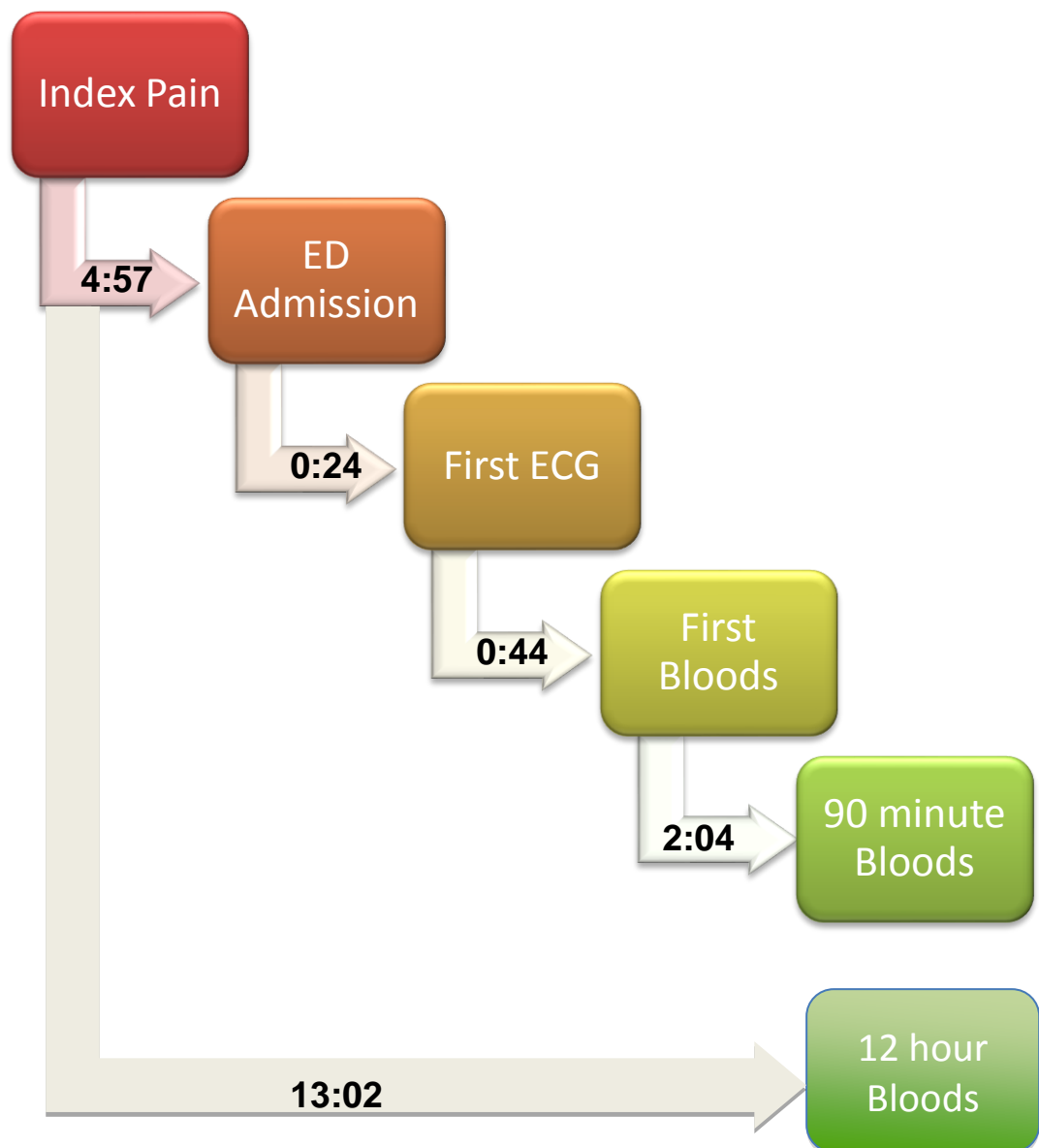
**Figure 4.1** Diagram depicting patient flow through the study.

RACPC – Rapid Access Chest Pain Clinic, ETT – exercise tolerance test, +ve – positive, -ve – negative, eq – equivocal.

8000 patients with chest pain present to the ED of the Leeds Teaching Hospitals each year – demographic data is not available for patients who were not included but who may have been eligible to participate.

### 4.1 The 'Average Patient' Journey

A record was made of the date and time of each study stage conducted in the ED. Using mean times, the diagram below demonstrates the typical journey through the ED for the 'average patient'.



**Figure 4.2** Flow diagram depicting the 'average' patient journey through the study (hh:mm).

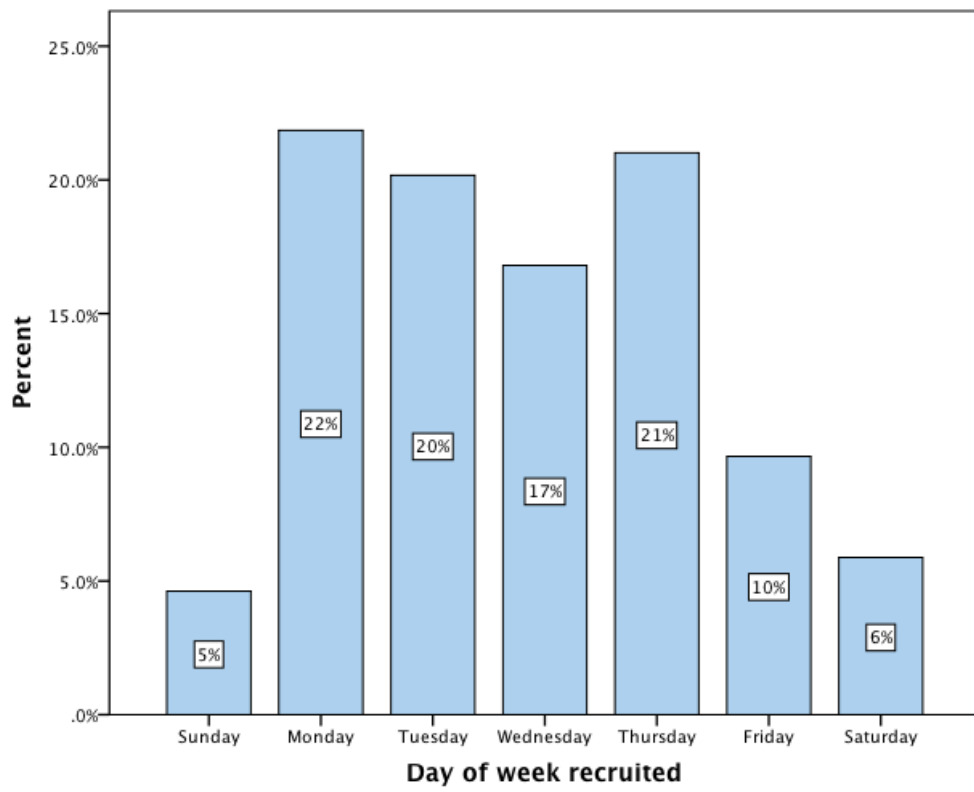
Acceptable time windows were considered to be 60 minutes from ED registration to phlebotomy for admission bloods, and 90-120 minutes later for 90 minute bloods. This allowed for the need for triage, clinical assessment, an ECG, and verbal consent prior to withdrawal of ED admission bloods. Written consent had to be taken prior to 90 minute bloods, and occasionally patients were in transit through the department or attending for a chest radiograph at the second phlebotomy time.

## **4.2 Recruitment Characteristics**

As recruitment was being undertaken in a busy tertiary centre ED, it was agreed that most patients would be recruited within a 0900 – 1700 Monday to Friday time frame. This is reflective of the increased availability of research staff within day-time hours, and was designed to minimise impact on the ED.

89% of patients were recruited on week days, as opposed to 11% on Saturday / Sunday (Figure 4.3). This is not expected to affect the representative nature of the recruited patient population.

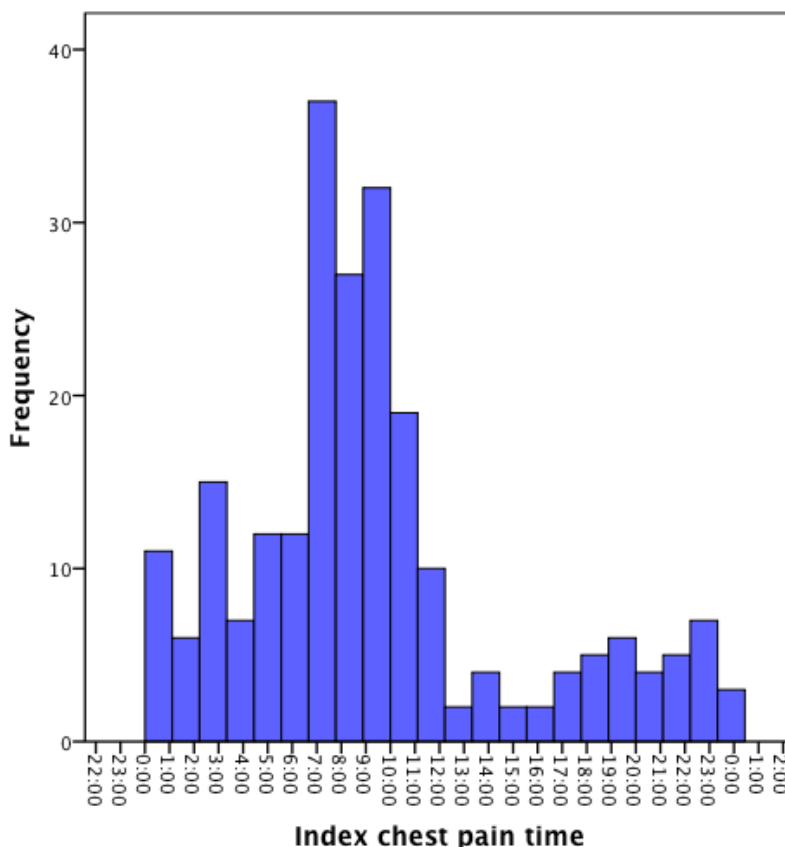




**Figure 4.3** Day of the week on which patients were recruited.

### 4.3 Patient Presentation Characteristics

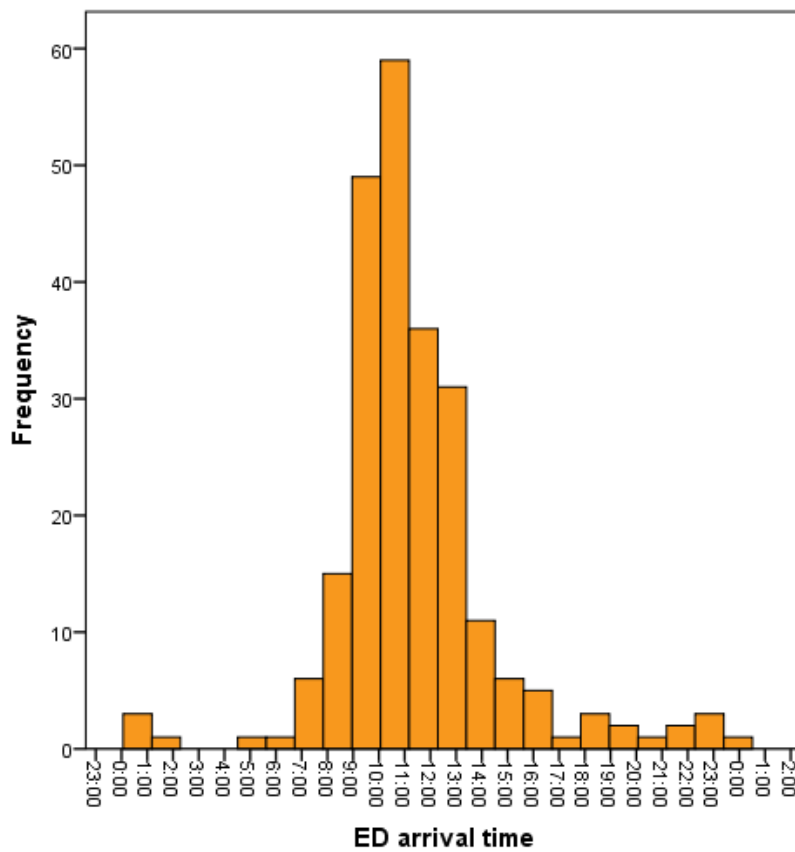
The recruited patients had index chest pain onset times spread throughout the day, but a majority experienced their pain in the early morning (between 0500 and 1100, see Figure 4.4). Reflective of this, the ED arrival time of patients also had a morning tendency, as shown in Figure 4.5. In this case the study recruitment patterns are likely to have contributed to this, although the findings are in keeping with other studies describing an early morning peak in chest pain onset times and ED chest pain presentations (Ekelund et al., 2012).



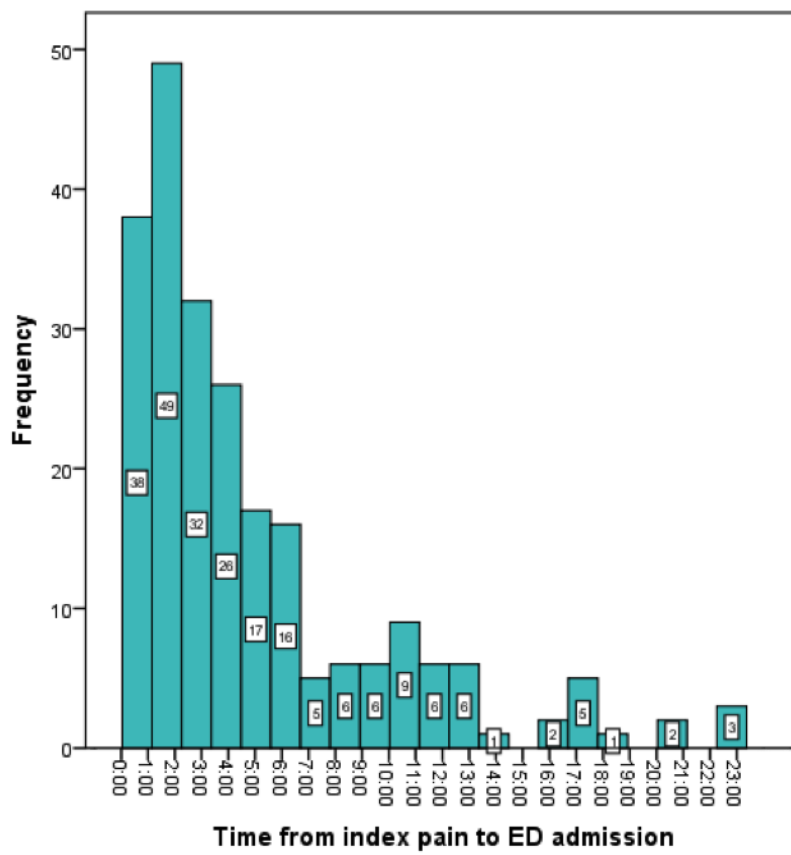
**Figure 4.4** Index chest pain onset time.

The assessing ED physician documented an index chest pain onset time for each patient. In the majority of cases this was the worst / most prolonged episode, occasionally the last episode if this was deemed significant. 77% of patients presented within 6 hours of their index chest pain, with a median presentation time of 3:09 hours, and range of 0:02 to 23:10 hours (Figure 4.6). Some patients reported on-going pain while en-route to the ED, and on

arrival to the ED. Patients who presented more than 24 hours after their index pain were excluded from participation: this was chosen as an exclusion criterion due to the potential for H-FABP levels to normalise after this duration from cardiac ischaemia.



**Figure 4.5** Patient ED arrival time.

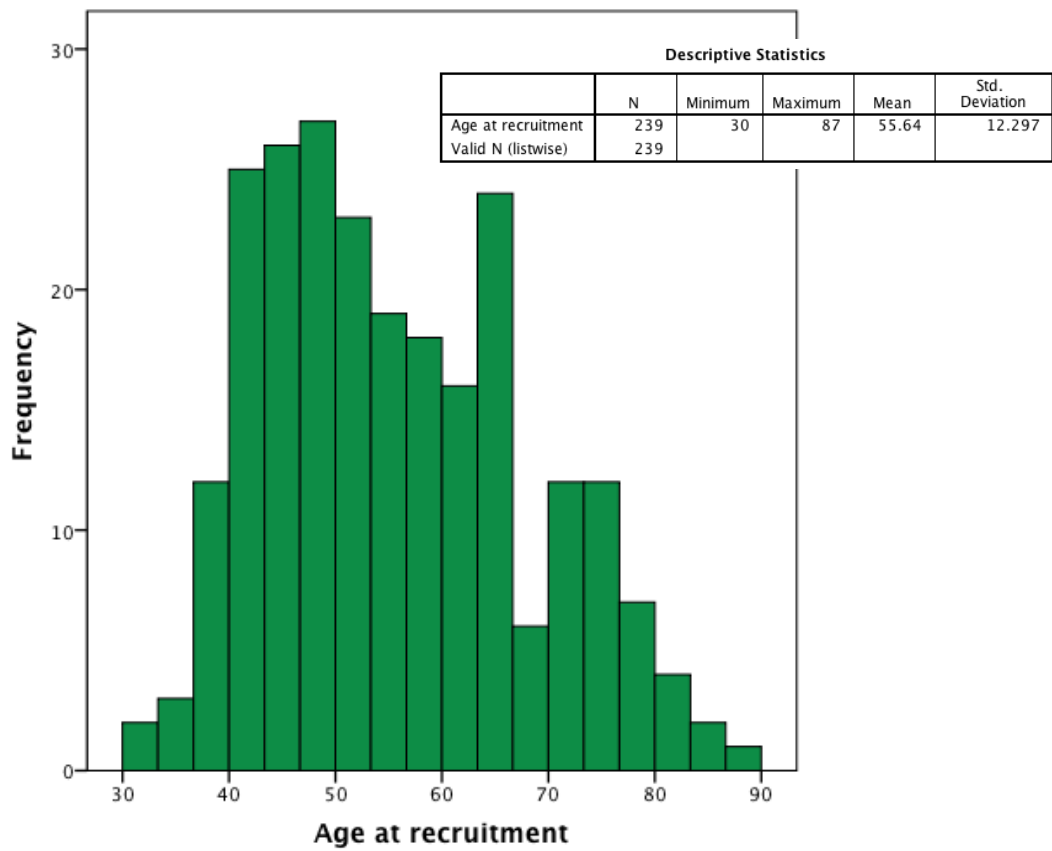


**Figure 4.6** Time from index pain to ED admission (hours).

## 4.4 Patient Demographics

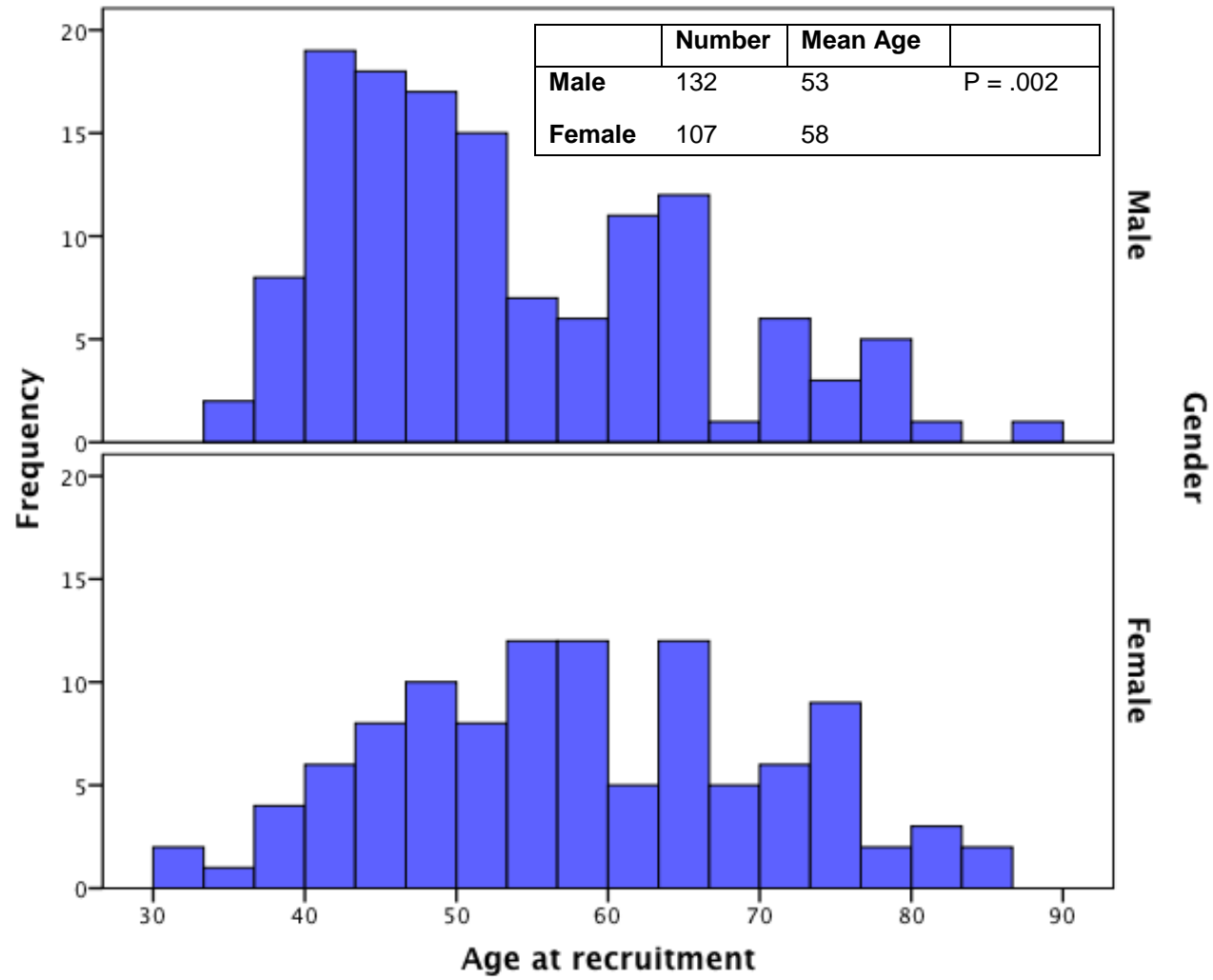
### 4.4.1 Age

A broad age range was represented in the recruited patient population, varying from 30 to 87 years, with a median age of 53 years 11 months (Figure 4.7).



**Figure 4.7** Histogram of age at recruitment (in years).

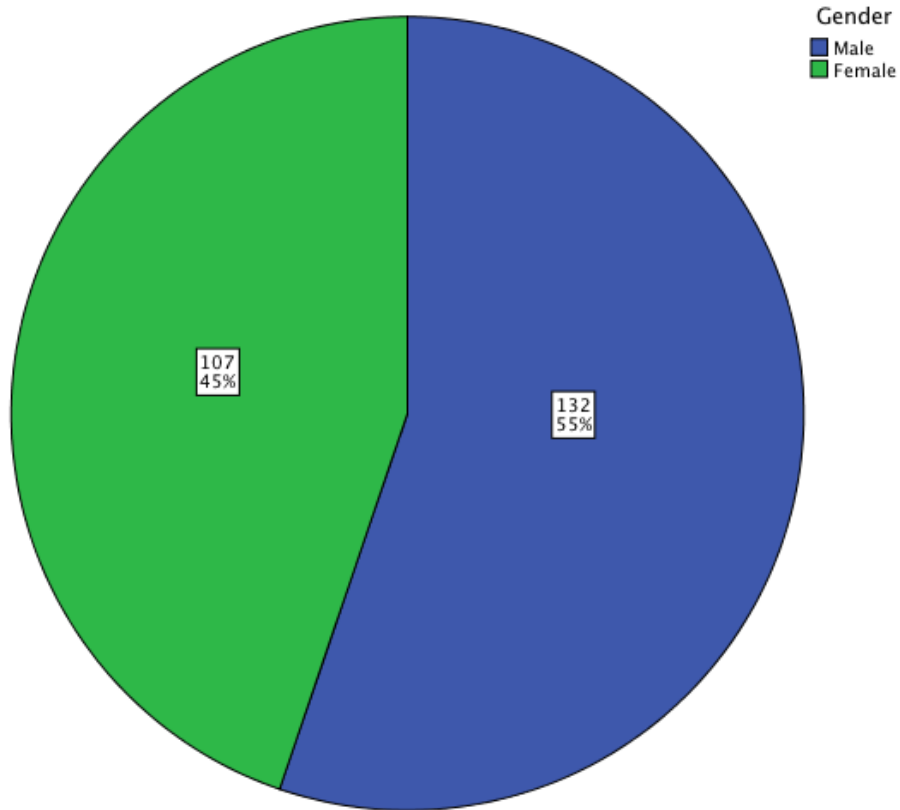
Split by sex, females patients were on average older than males by about five years (58.3 vs. 53.5 years,  $p = .002$ ). See Figure 4.8.



**Figure 4.8** Histogram of age at recruitment by sex (in years).

#### 4.4.2 Sex

55% of recruited patients were male and 45% female. This is in keeping with expected presentation patterns for chest pain patients.



**Figure 4.9** Patients by sex.

#### 4.4.3 Ethnicity

86% of patients categorised their ethnicity as 'British'. There was a wide range of ethnic groups represented as shown in Figure 4.10. The ethnicity categories were collected from standardised hospital records, and were not designed specifically for this study.

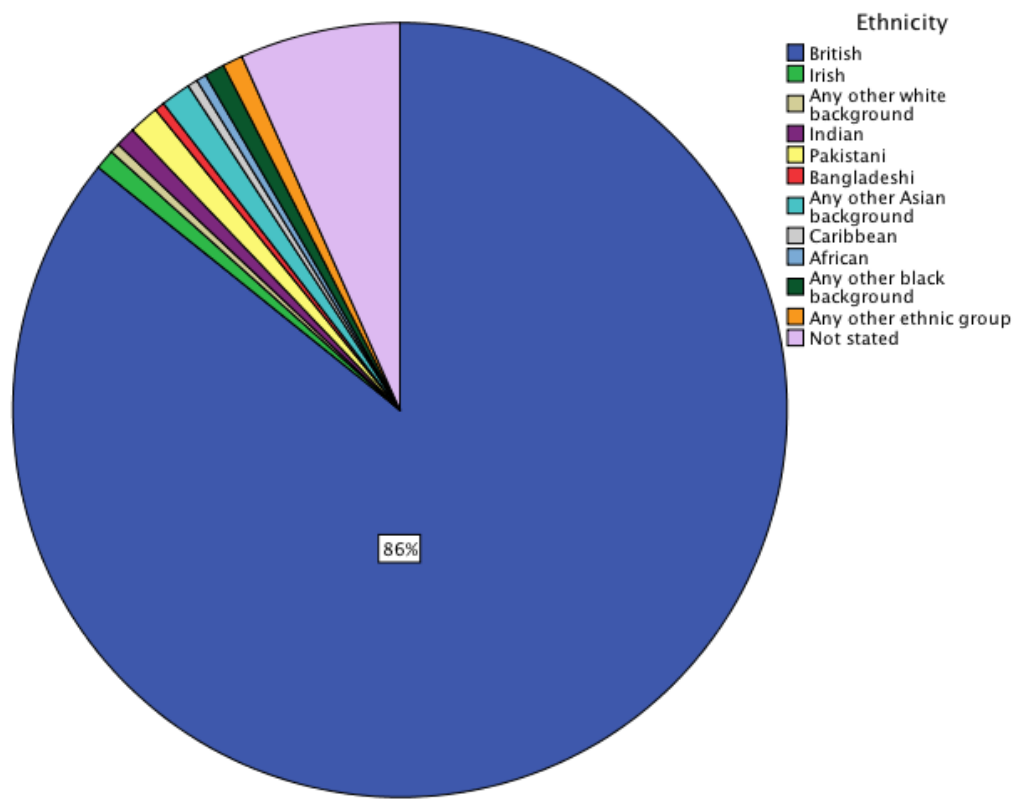
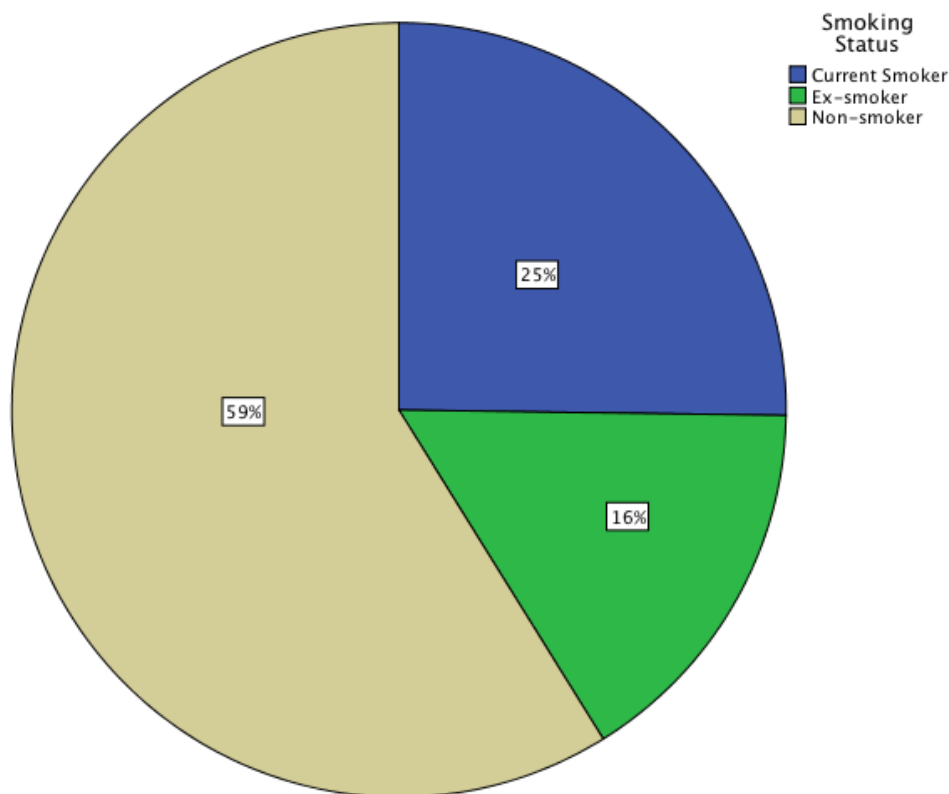


Figure 4.10 Patients by self assigned ethnic group.



## 4.5 Vascular Risk Factor Profile

25% of patients were current smokers, with a further 16% being ex-smokers (Figure 4.11).



**Figure 4.11** Smoking status.

Other risk factors are shown in Table 4.1.

- Family history of premature ischaemic heart disease (IHD) was defined as a first or second degree relative with IHD present at an age younger than 50 years. In cases where the patient could not provide a family history, for example due to being adopted, this was recorded as 'unknown'.
- Hypertension was recorded if the patient was on treatment for this, or if they reported the diagnosis.
- Hypercholesterolaemia was defined as a recorded total cholesterol  $>6.47$  mmol/litre (as per NICE Clinical Guidance 95), or if the patient was on

active treatment for this. In cases where a baseline cholesterol level had never been measured, this was marked as 'unknown'.

- Diabetes was recorded if the patient reported the diagnosis. 6% of patients had diabetes (14 patients), of which 2 had type I diabetes.
- Due to the lack of availability of routine weight and height measurements in the ED, obesity was recorded in the main based on the subjective assessment of the attending physician.
- In order to maintain high quality data collection, the study design allowed for two opportunities for this data to be collected. A record was made initially by the attending ED physician using standardised ED documentation pathways into which the research was integrated. This was cross checked and further assessed during rapid access chest pain clinic assessment. Incomplete data was limited to 3%.

Risk factor	%		
	Present	Absent	Unknown
FHx of premature IHD	26	67	7
Hypertension	36	62	3
Hypercholesterolaemia	30	60	11
Diabetes	6	91	3
Obesity	29	66	5

**Table 4.1** Risk Factor Profile.

FHx – family history,  
IHD – ischaemic heart disease.

## 4.6 Presentation Haemodynamics

The study inclusion criteria targeted low-medium risk chest pain patients. In our institution these patients are managed on a protocol driven pathway within the ED Clinical Decisions Unit (CDU). This involves observation with sequential ECGs pending the results of a 12 hour from index pain troponin result. All patients had been deemed haemodynamically stable by the assessing ED physician. The histograms below represent the first recorded observations taken in the ED. Although there are a few patients with readings outside the normal range at triage, perhaps due to on-going pain at the time, all patients were haemodynamically stable prior to admission to the CDU.

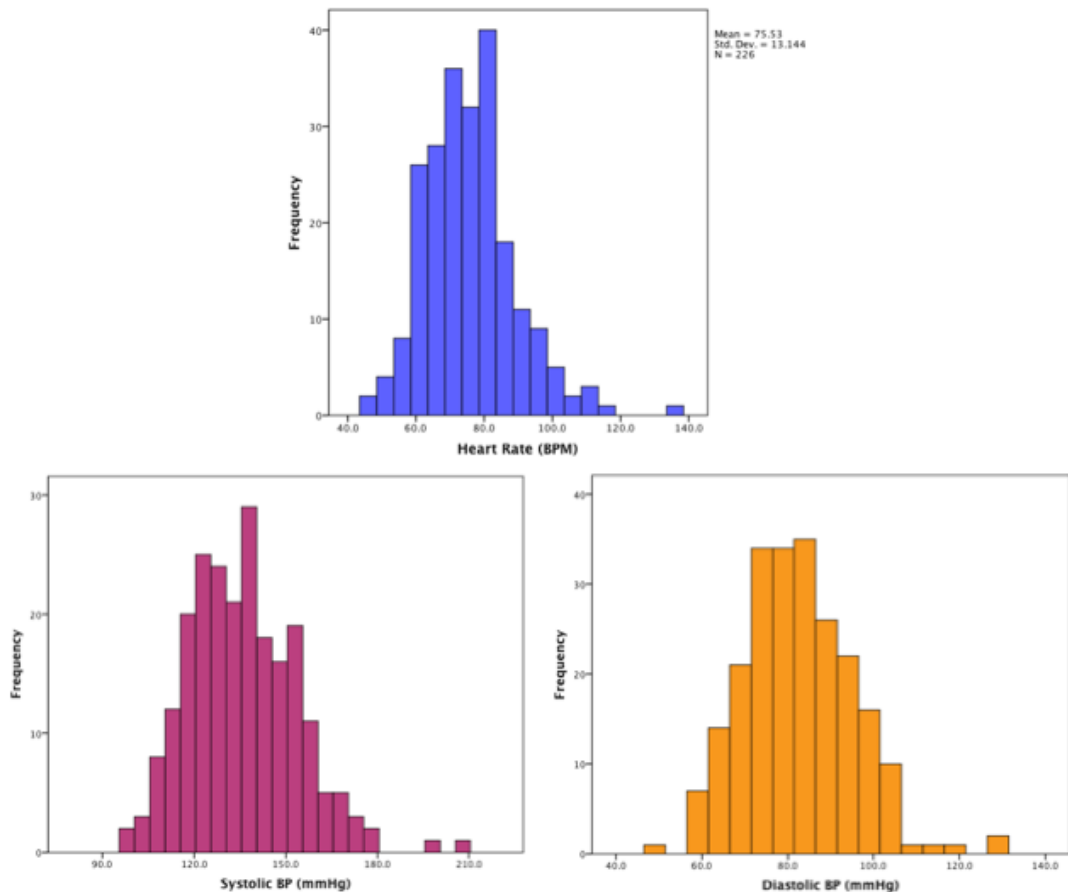
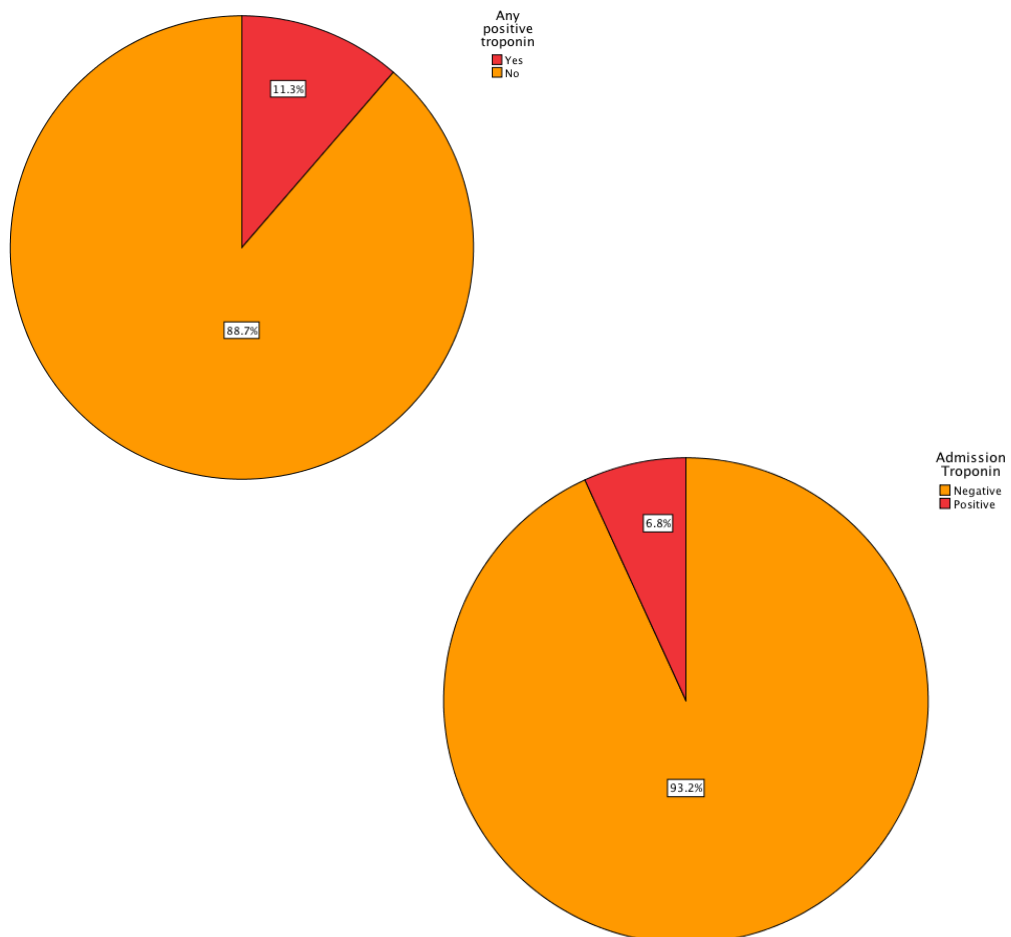


Figure 4.12 Presentation haemodynamics.

## 4.7 Troponin Levels

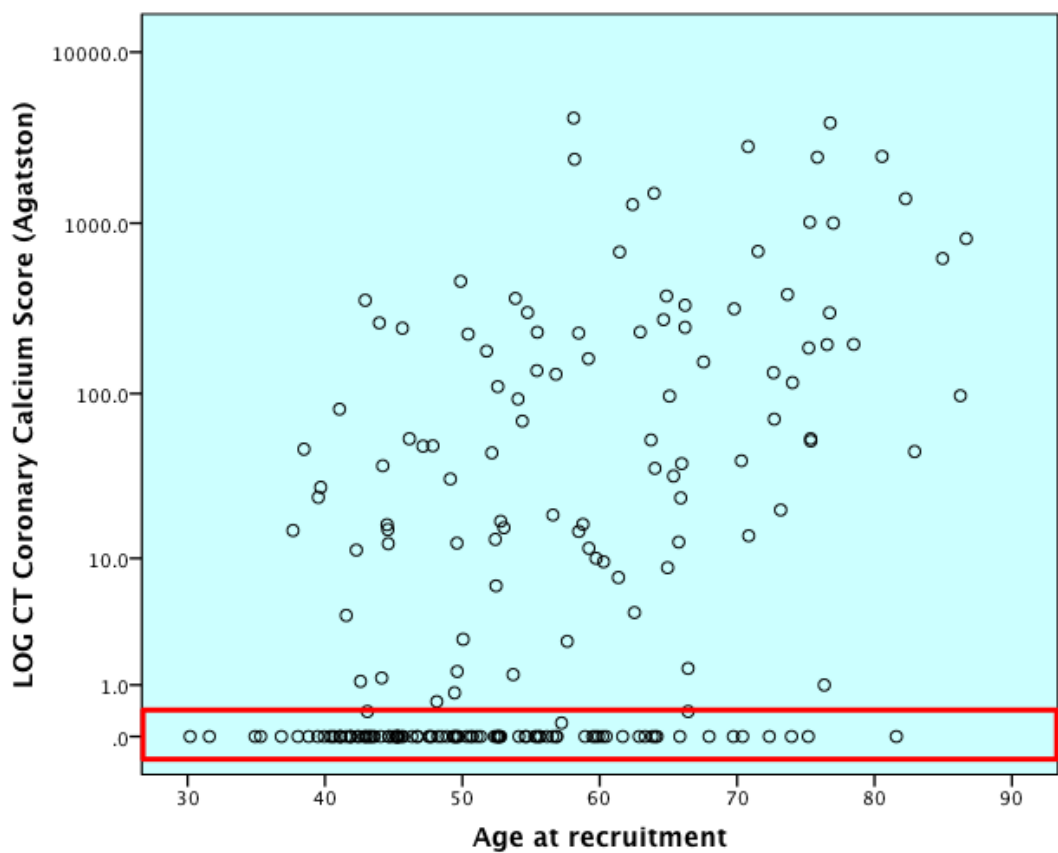
Overall 11.3% (N = 27) of recruited patients had a contemporary troponin level >99<sup>th</sup> centile reference level. In 15 of these patients the troponin level was raised on the ED admission sample, with a further 9 patients becoming troponin positive on the 90 minute sample. For clarity, where the term troponin negative or positive is used, this refers to whether the level is below or above the 99<sup>th</sup> centile reference value for the assay used.



**Figure 4.13** Troponin positivity - admission sample & overall.

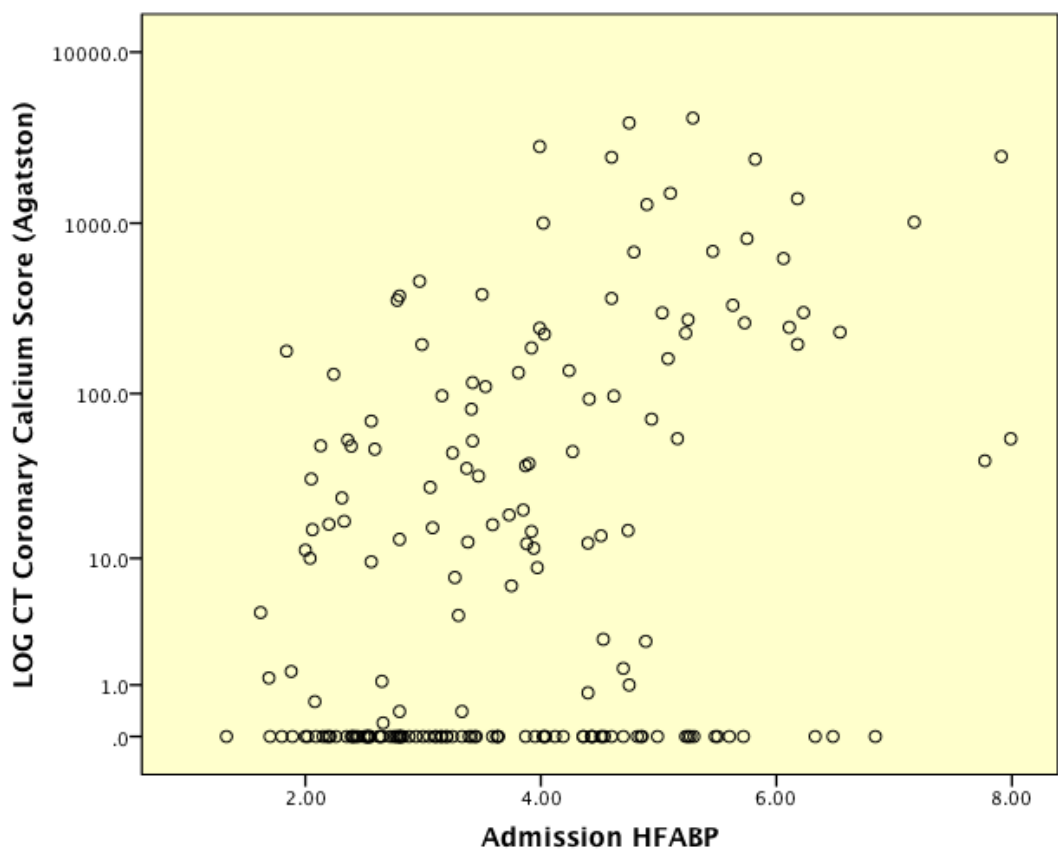
## 4.8 CT Coronary Calcium Scores

CT Coronary Calcium scans were performed on 199 patients, of which 96 had a zero Agatston score. Scores ranged from 0 to 4114. The graphs below demonstrate calcium score by age and admission H-FABP level (Figure 4.14 and Figure 4.15). Statistical correlations will be explored later in this chapter.



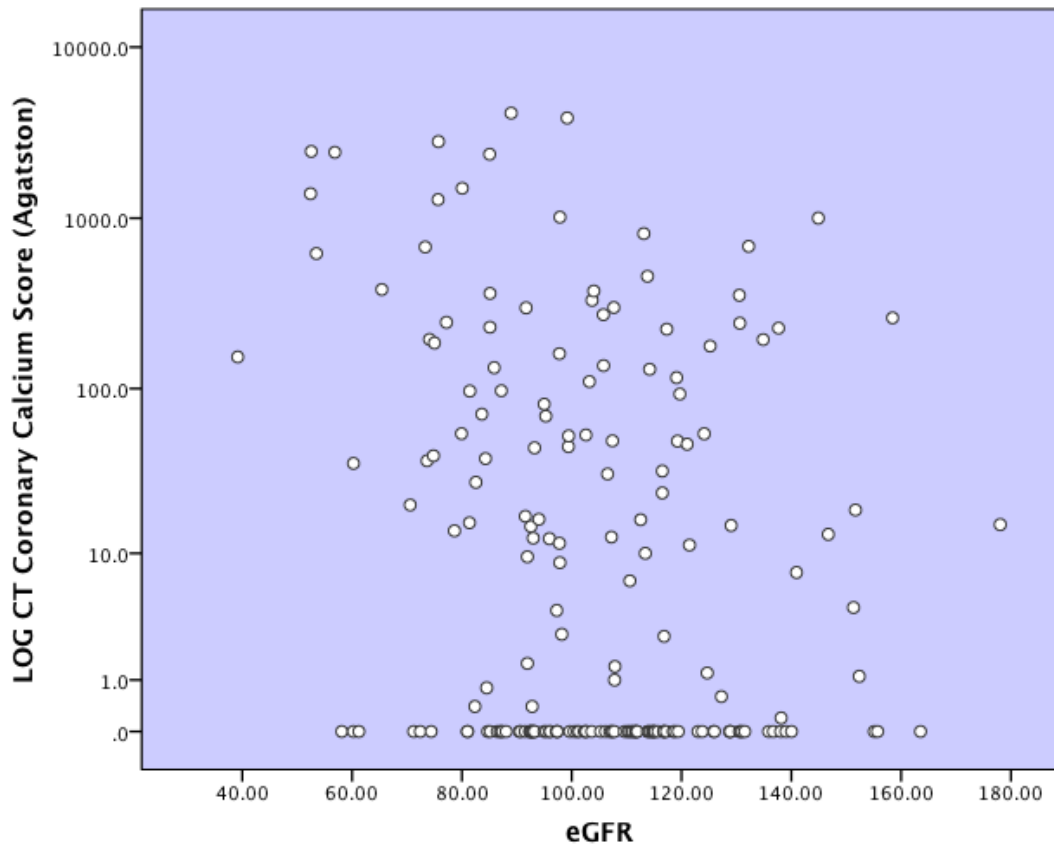
**Figure 4.14** CT Calcium Score (log Agatston score) by age (years).

Note that zero and non-zero scores occurred across the age range (red box).



**Figure 4.15** CT Calcium Score (log Agatston score) by admission H-FABP ( $\mu\text{g/l}$ ). Two H-FABP outliers removed.

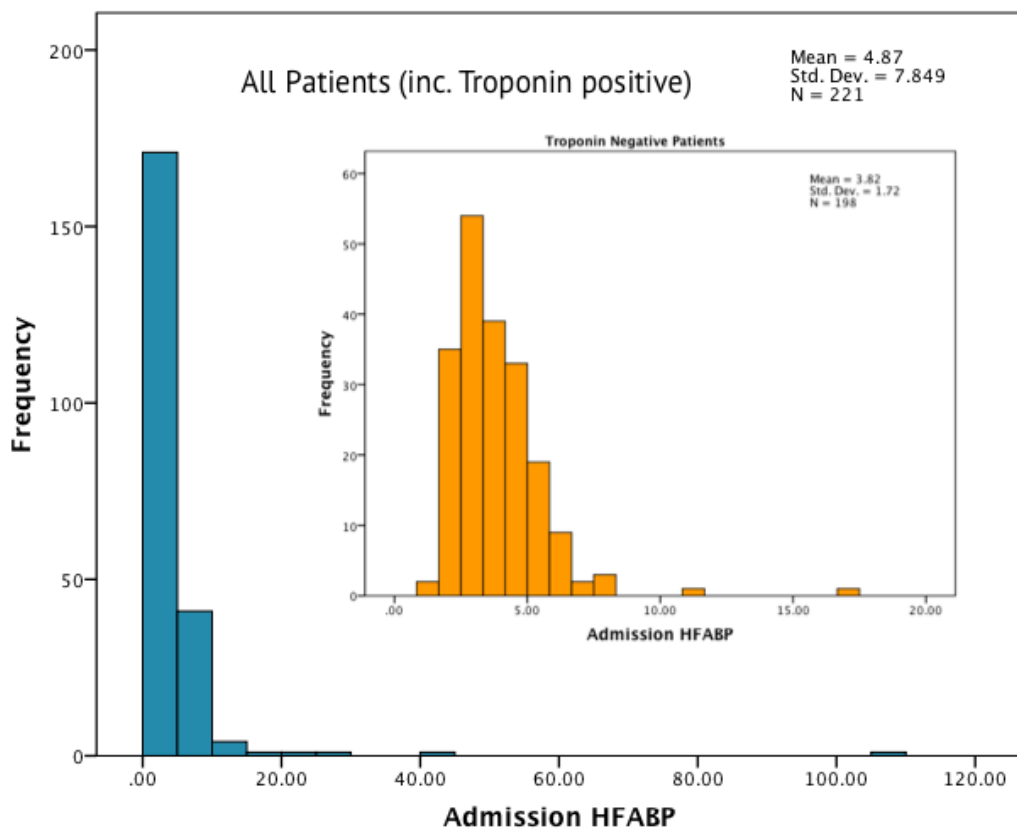
It is well documented that coronary artery disease is more common in patients with renal failure. No particular trend is seen within the data from this study - Figure 4.16 shows calcium scores plotted against estimated glomerular filtration rate (eGFR). eGFR is given in mls/min/1.73m<sup>2</sup> throughout this document.



**Figure 4.16** Log CT Calcium Score against eGFR.

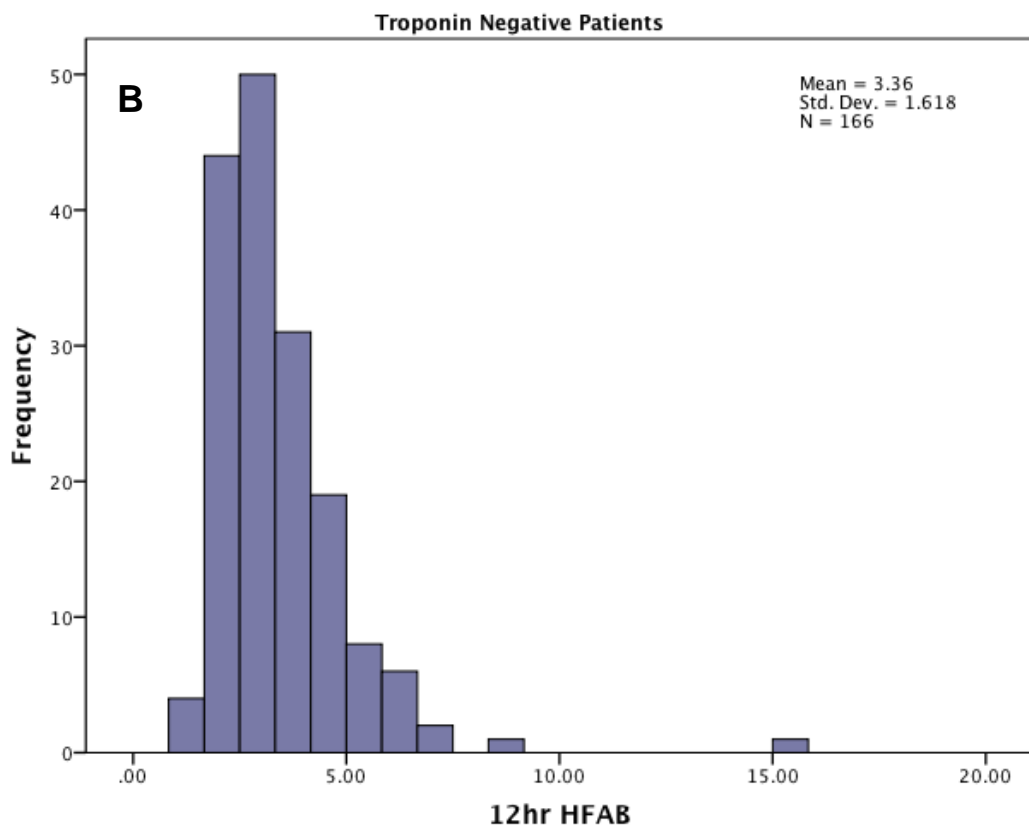
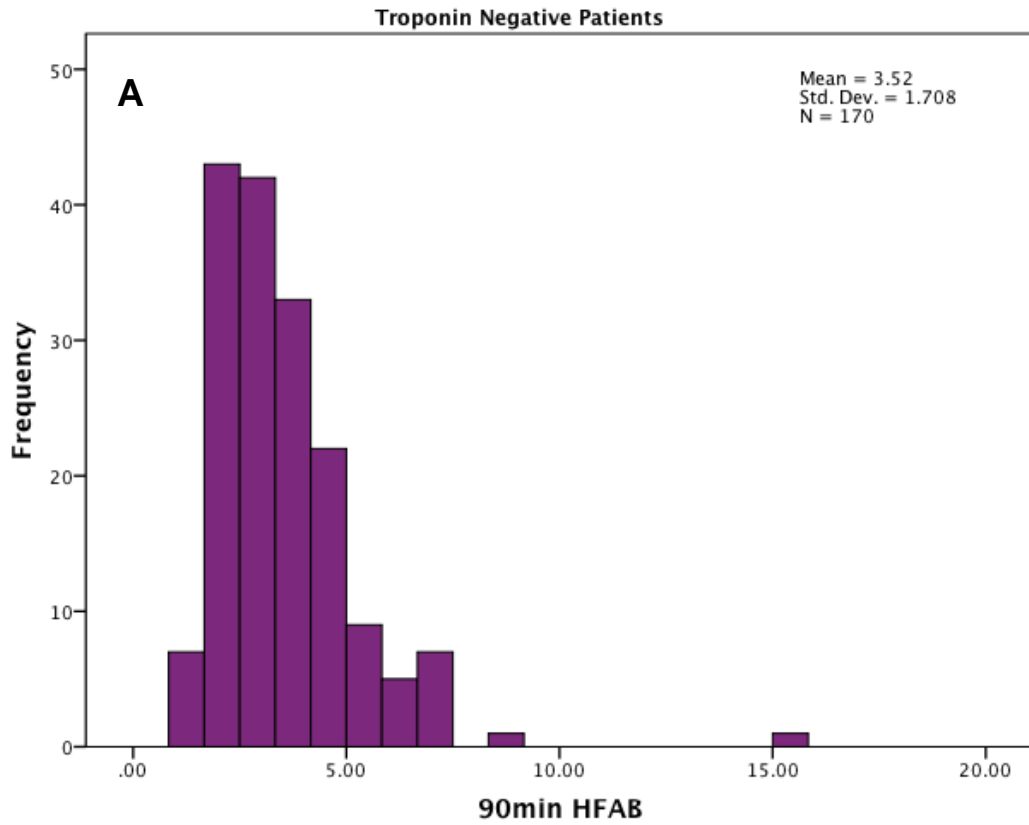
#### **4.9 H-FABP Levels**

For troponin negative patients admission H-FABP levels ranged from 1.3 to 17.1  $\mu\text{g/l}$ , with a mean of 3.8  $\mu\text{g/l}$  (N=198). For troponin positive patients the range was 2.6 to 106.4  $\mu\text{g/l}$ , with a mean of 13.9  $\mu\text{g/l}$  (N=23). Figure 4.17 and Figure 4.18 demonstrate admission, 90 minute, and 12 hour H-FABP levels.



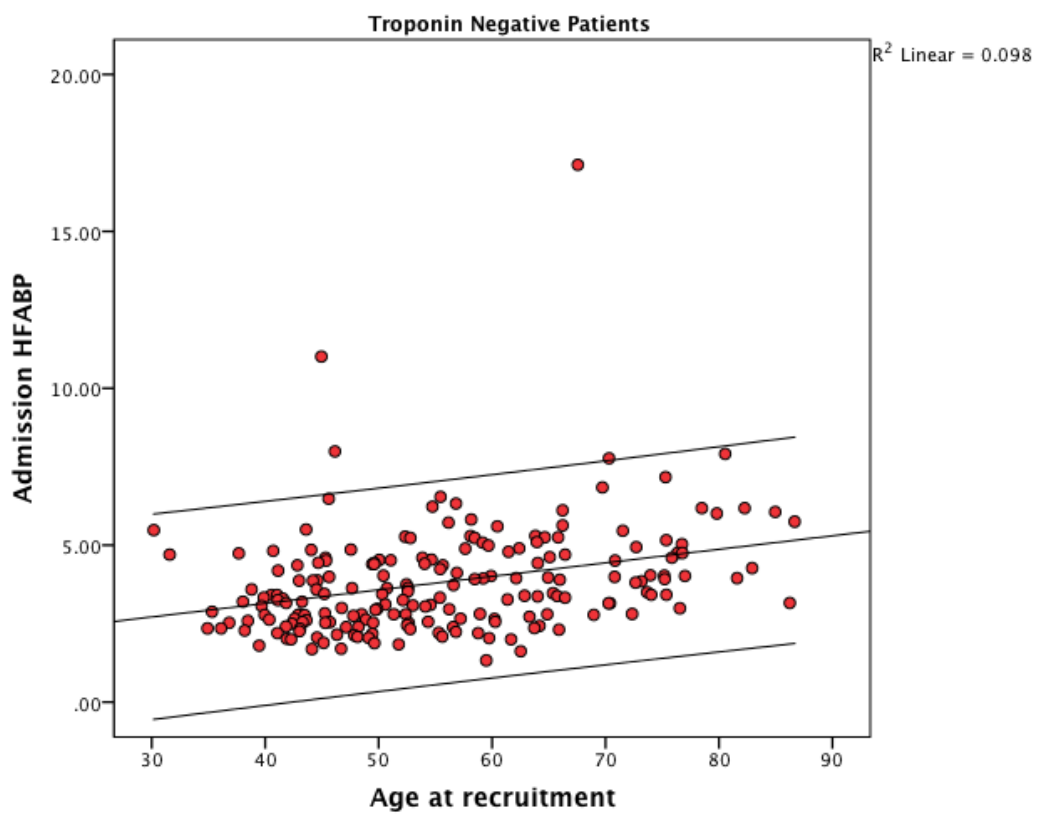
**Figure 4.17** Histogram of admission H-FABP levels for all patients ( $\mu\text{g/l}$ ).  
Inset graph - for troponin negative patients only.



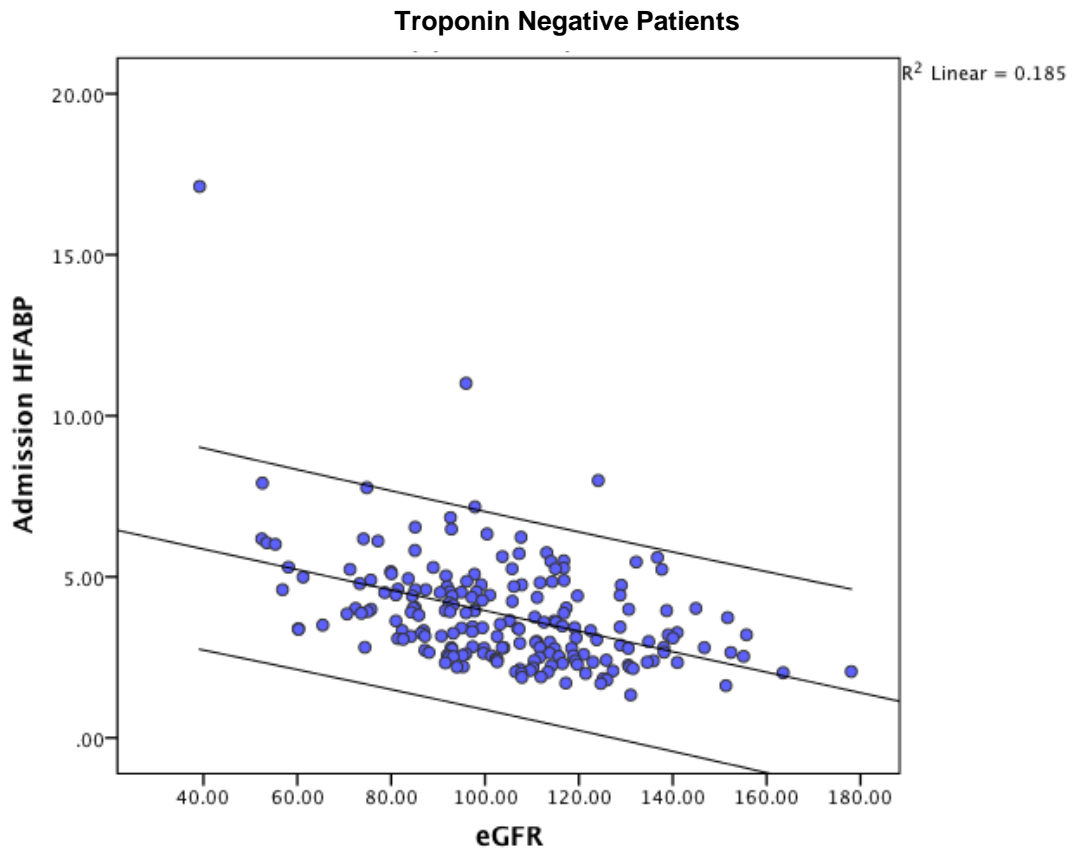


**Figure 4.18** H-FABP levels at 90 minutes (A) and 12 hours (B) - for troponin negative patients ( $\mu\text{g/l}$ ).

H-FABP levels showed an increasing trend with age, as shown in Figure 4.19. In addition there was an increasing trend with lower eGFR (Figure 4.20). These findings are in keeping with known H-FABP characteristics.



**Figure 4.19** Admission H-FABP ( $\mu\text{g/l}$ ) by age (years).

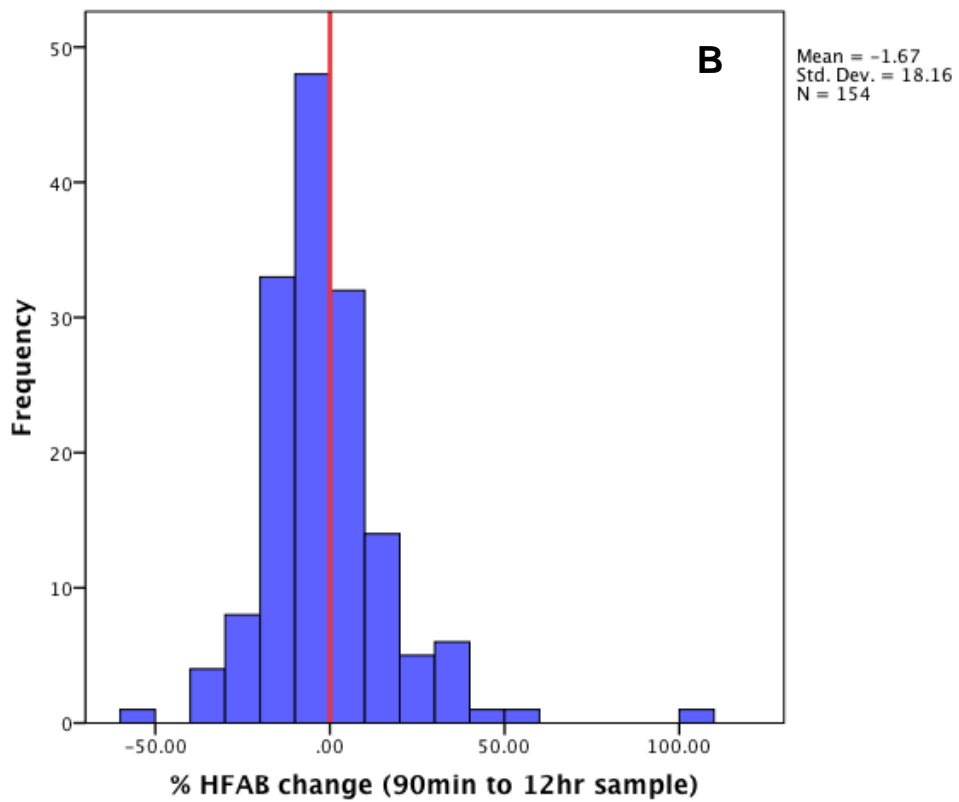
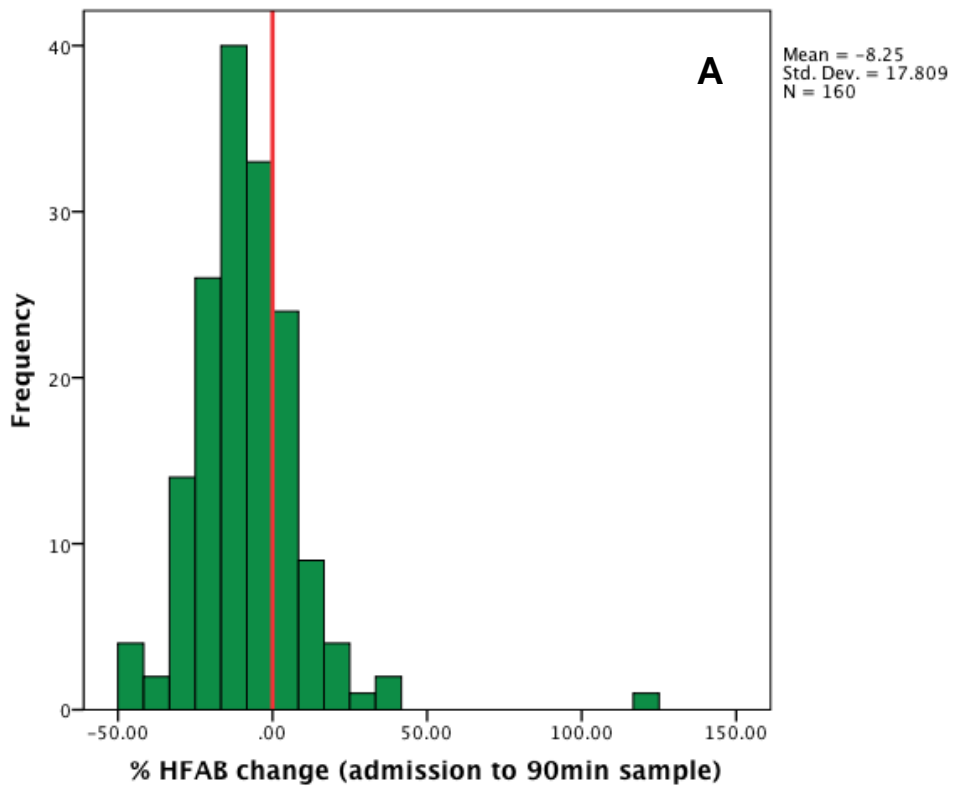


**Figure 4.20** Admission H-FABP ( $\mu\text{g/l}$ ) by eGFR .

### 4.10 H-FABP Dynamic

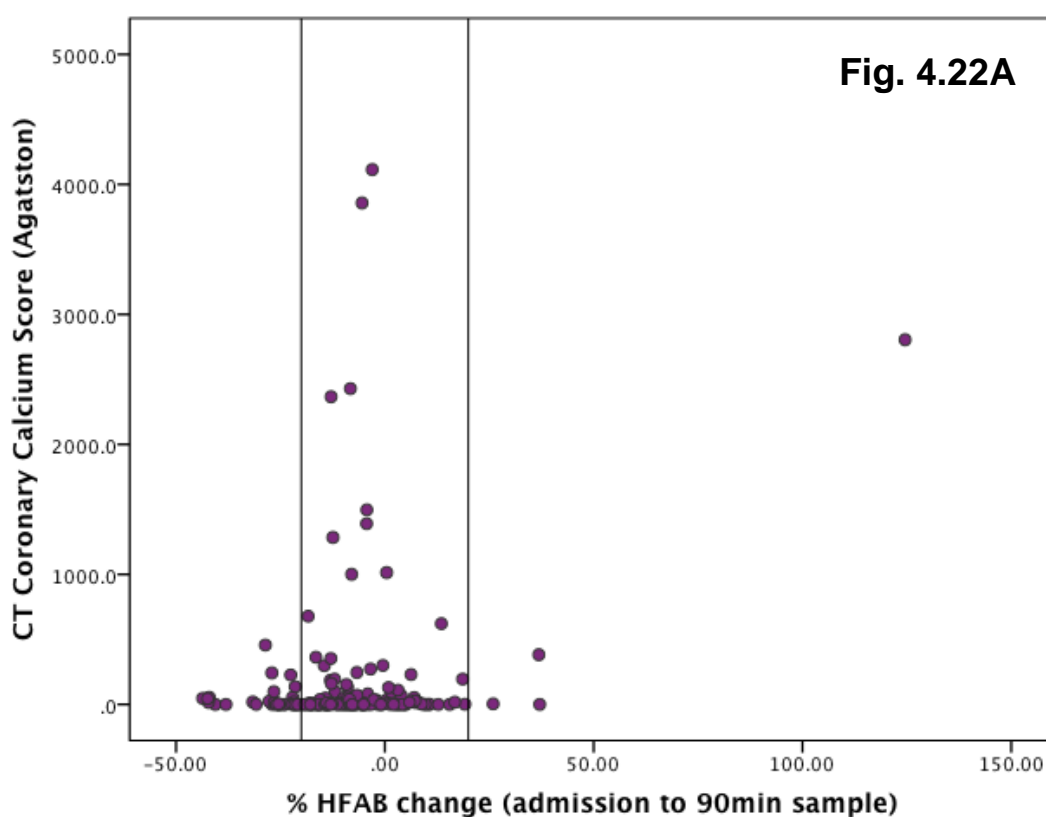
H-FABP levels were taken at three time points. As levels rise and fall quickly with cardiac ischaemia, the change from one reading to another may contain useful information.

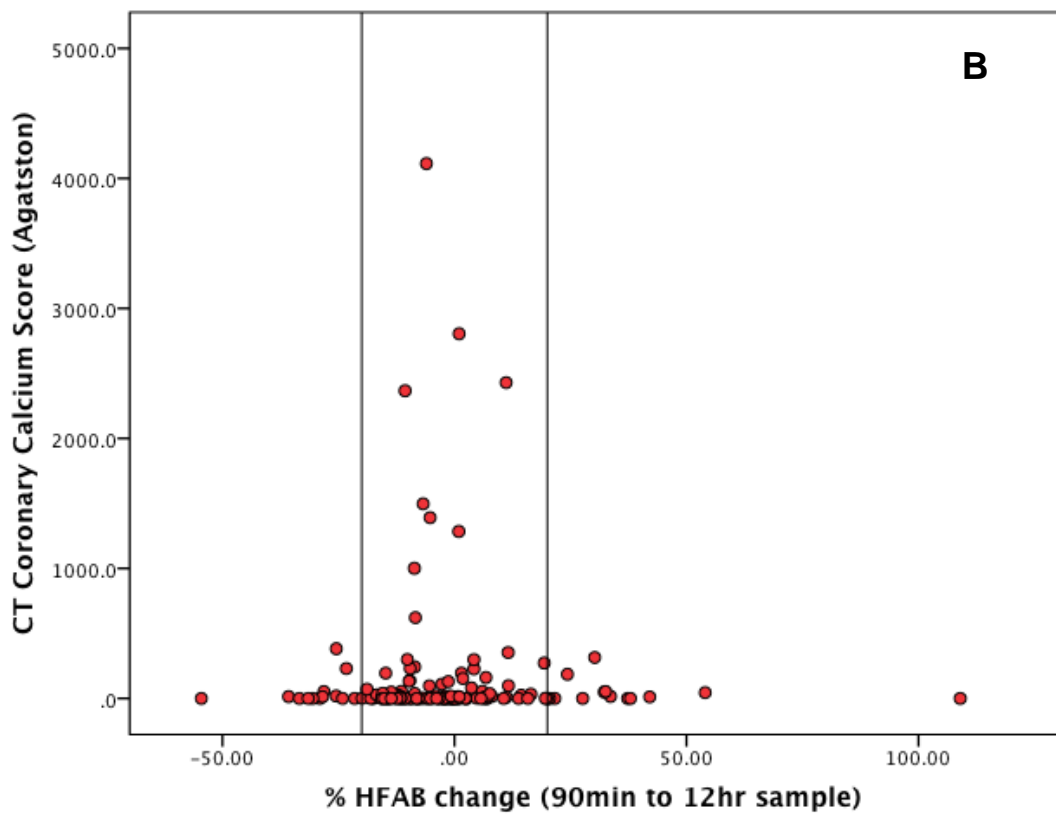
Figure 4.21 demonstrates the percentage change in H-FABP between the three sample points, for troponin negative patients. It is interesting to note that more patients had falling levels between admission and 90 minutes, than rising. The same is true comparing 90 minute to 12 hour levels, although to a lesser degree.



**Figure 4.21** Percentage H-FABP change for troponin negative patients. A - admission to 90 minutes, B - 90 minutes to 12 hour sample.

The mean CT calcium score (CTCS) was higher for patients with a less than 20% change in H-FABP level between readings as compared to those with a greater than 20% change (213 vs. 126) – Figure 4.22. These means were not however statistically significantly different from each other,  $p = .81$  (Mann-Whitney U test).





**Figure 4.22 A & B** CT Calcium Score by H-FABP dynamic (for troponin negative patients).

#### 4.11 Relating H-FABP to CT Calcium Score

In the introductory chapter I outlined the evidence behind H-FABP as a prognostic marker in patients presenting with possible ACS. The relationship between H-FABP levels and CTCS, as a marker of coronary artery atheroma burden, is therefore of major interest, and the prime aim of this mechanistic study.

As demonstrated in Table 4.2 below, there is a statistically significant positive correlation between H-FABP at all time points and CTCS, for admission H-FABP  $\tau = .22$ ,  $p$  (one-tailed)  $<.01$ . From the table you will note that age and renal function also correlate with both H-FABP and CTCS. Nonetheless, **the positive correlation between H-FABP and CTCS still holds true after accounting for age and eGFR** using second-order partial correlation (Table 4.3).

**Correlations**

			CT Coronary Calcium Score (Agatston)	Admission HFABP	90min HFAB	12hr HFAB	Age at recruitment	eGFR
Kendall's tau_b	CT Coronary Calcium Score (Agatston)	Correlation Coefficient	1.000	.223**	.208**	.237**	.339**	-.137**
		Sig. (1-tailed)	.	.000	.000	.000	.000	.004
		N	199	187	160	156	199	189
	Admission HFABP	Correlation Coefficient	.223**	1.000	.771**	.708**	.230**	-.241**
		Sig. (1-tailed)	.000	.	.000	.000	.000	.000
		N	187	219	178	168	219	219
	90min HFAB	Correlation Coefficient	.208**	.771**	1.000	.736**	.228**	-.279**
		Sig. (1-tailed)	.000	.000	.	.000	.000	.000
		N	160	178	192	170	192	179
	12hr HFAB	Correlation Coefficient	.237**	.708**	.736**	1.000	.221**	-.254**
		Sig. (1-tailed)	.000	.000	.000	.	.000	.000
		N	156	168	170	182	182	169
	Age at recruitment	Correlation Coefficient	.339**	.230**	.228**	.221**	1.000	-.317**
		Sig. (1-tailed)	.000	.000	.000	.000	.	.000
		N	199	219	192	182	239	221
	eGFR	Correlation Coefficient	-.137**	-.241**	-.279**	-.254**	-.317**	1.000
		Sig. (1-tailed)	.004	.000	.000	.000	.000	.
		N	189	219	179	169	221	221

\*\* . Correlation is significant at the 0.01 level (1-tailed).

**Table 4.2** Correlations – Kendall’s Tau.



Correlations

Control Variables			CT Coronary Calcium Score (Agatston)	Admission HFABP	90min HFAB	12hr HFAB
Age at recruitment & eGFR	CT Coronary Calcium Score (Agatston)	Correlation	1.000	.168	.281	.312
		Significance (1-tailed)	.	.026	.001	.000
		df	0	132	132	132
	Admission HFABP	Correlation	.168	1.000	.851	.799
		Significance (1-tailed)	.026	.	.000	.000
		df	132	0	132	132
	90min HFAB	Correlation	.281	.851	1.000	.927
		Significance (1-tailed)	.001	.000	.	.000
		df	132	132	0	132
	12hr HFAB	Correlation	.312	.799	.927	1.000
		Significance (1-tailed)	.000	.000	.000	.
		df	132	132	132	0

**Table 4.3** Correlating H-FABP with CTCS, controlling for age and eGFR.

#### 4.12 Patient Outcomes

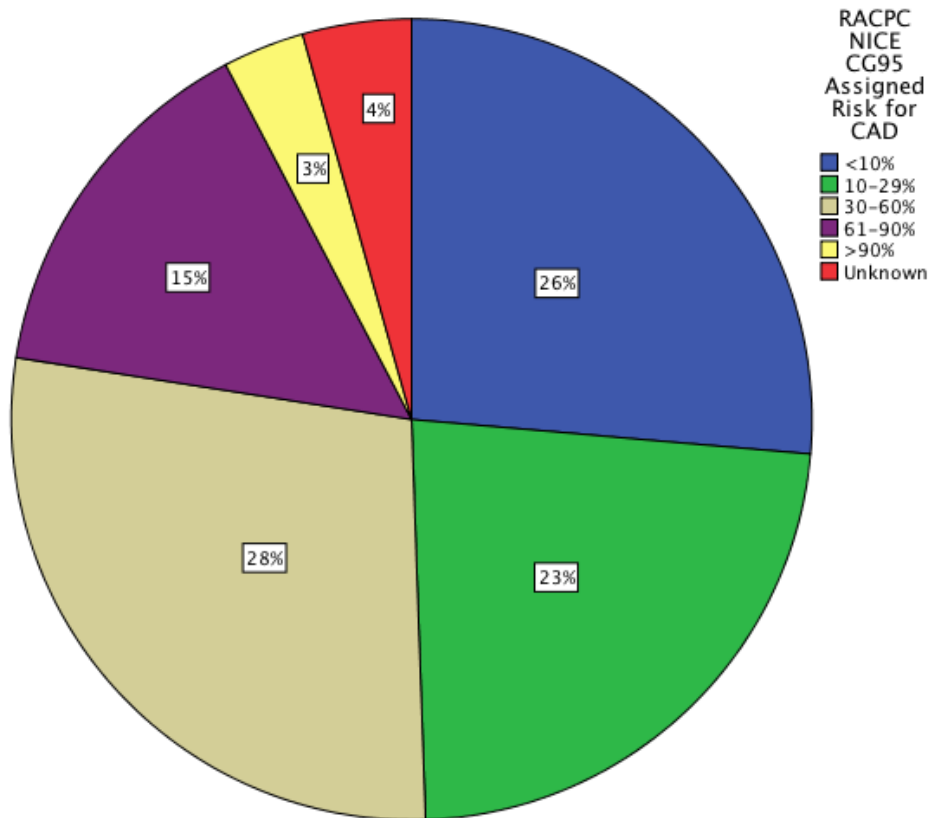
Patients participating in the study were seen in a Rapid Access Chest Pain clinic under the supervision of a Consultant Cardiologist. Appendix C contains a copy of the specifically designed clinic documentation used for this, which includes investigation guidance in keeping with current NICE recommendations (CG95). As this was an observational study, clinicians were at liberty to investigate and treat patients in accordance with their own judgment.

CG95 contains within it criteria against which patient symptoms can be matched to assign a symptom category. 9% of patients fell into the 'Typical Angina' group, 15% into the 'Atypical Angina' group, and 76% into the 'Non-Anginal' chest pain group (Table 4.4). Symptom categories were assigned to a total of 190 patients. Figure 4.23 shows the distribution of patients according to their NICE CG95 assigned risk category for coronary artery disease, which takes into account the typicality of symptoms, age, sex, and other risk factors such as smoking status, total cholesterol level and diabetic state. This was recorded in 185 cases. Some patients could not be assigned a NICE risk category due to their baseline total cholesterol level not being known.

**RACPC Assigned Symptom Category (Prior to Investigation)**

	Frequency	Percent
Typical angina	17	8.9
Atypical angina	28	14.7
Non-anginal chest pain	145	76.3
Total	190	100.0

**Table 4.4** RACPC Assigned Symptom Category (Prior to Investigation).



**Figure 4.23** RACPC Assigned NICE Risk Category for CAD.

3% of patients were commenced on treatment without further investigation. This occurred where the clinician made a clinical diagnosis of angina, decided to commence therapy for this, but took the decision at that stage not

to investigate further – this may have reflected a combination of the patient’s wishes, age and comorbidities. 21% of patients were not felt to require further investigation and were directly discharged from clinic. The rest underwent outpatient investigation: 31% undergoing stress echocardiography; 17% a nuclear myocardial perfusion scan; 6% an invasive coronary angiogram; 2% a CT coronary angiogram; with the majority of the remainder undergoing a treadmill exercise ECG. Figure 4.1 provides a summary of the investigations undertaken and their results.

Of the patients who were sent for further outpatient investigation, 13 (12%) had a positive functional or anatomical test. Of the 195 patients seen in clinic, 5 patients met the predefined study protocol secondary outcome (clinical impression of angina, supported by a positive functional test for cardiac ischaemia and a coronary stenosis of >50%). The final NICE CG95 diagnostic category given for patients after completion of investigations is shown in Table 4.5.

#### **RACPC Assigned Symptom Category (After Investigation)**

	Frequency	Percent
Typical / Atypical angina	23	12
Non-anginal chest pain	170	88
Total	193	100

**Table 4.5** RACPC Assigned Symptom Category (After Investigation).

Patients were followed up for a median of 406 days (range 207-616). 6 predefined tertiary outcomes occurred: 2 deaths from a cardiovascular cause, 1 myocardial infarct, and 3 coronary revascularisation procedures. Death certificates and / or clinical notes were obtained to substantiate cause of death. Patients will remain under surveillance for death via UK Office of National Statistic reporting.

Prevalent acute myocardial infarcts were accounted for – i.e. acute myocardial infarction diagnosed on the initial ED visit.

The third universal definition of myocardial infarctions was used. Myocardial infarction was diagnosed by a rise and/or fall of cardiac biomarker values (in this case cardiac troponin I) with at least one value above the 99<sup>th</sup> percentile upper reference limit and with at least one of the following:

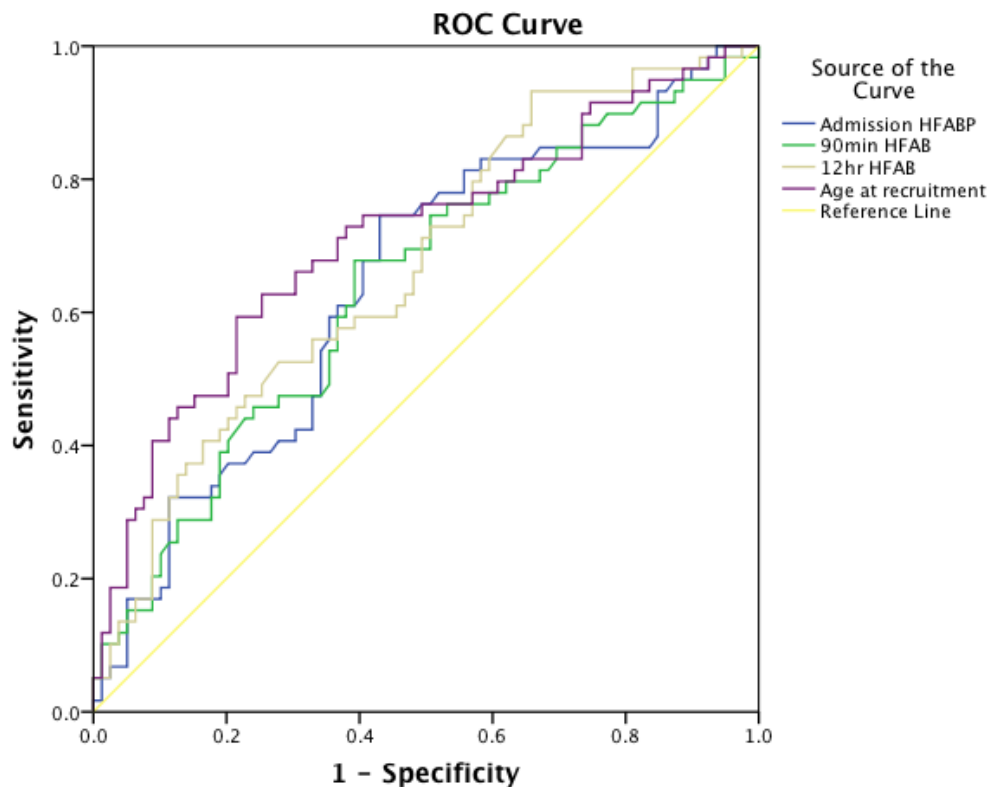
- Symptoms of ischaemia.
- New or presumed new significant ST-segment-T wave changes or new left bundle branch block.
- Development of pathological ECG Q waves.
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.
- Identification of an intracoronary thrombus by angiography or autopsy.

#### **4.13 Pre-specified Analyses**

In this section I have presented analyses pre-specified in the study protocol.

##### **4.13.1 Primary Analysis**

Comparison of the area under the H-FABP concentration curve (for admission, 90 minute and 12 hour samples) for patients with Agatston CT coronary calcium score  $\geq 10$  as compared to a score  $< 10$ .



Diagonal segments are produced by ties.

**Area Under the Curve**

Test Result Variable(s)	Area	Std. Error <sup>a</sup>	Asymptotic Sig. <sup>b</sup>	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Admission HFABP	.643	.048	.004	.549	.736
90min HFAB	.640	.048	.005	.547	.734
12hr HFAB	.669	.046	.001	.579	.759
Age at recruitment	.711	.045	.000	.623	.800

The test result variable(s): Admission HFABP, 90min HFAB, 12hr HFAB has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

- a. Under the nonparametric assumption
- b. Null hypothesis: true area = 0.5

**Figure 4.24** ROC Curve, H-FABP vs Agatston CT calcium score split (positive state  $\geq 10$ , negative state  $< 10$ ).

Comparison of the mean H-FABP level for patients with Agatston CT coronary calcium score  $\geq 10$  as compared to a score  $< 10$ . For patients with a CTCS  $< 10$  the mean admission H-FABP level was  $3.50 \mu\text{g/l}$  ( $N = 109$ ), as opposed to  $4.34 \mu\text{g/l}$  for patients with a CTCS  $\geq 10$  ( $N=80$ ),  $p = .001$ . There

were similar findings for the 90 minute and 12 hour H-FABP levels (see Table 4.6).

**Mean H-FABP Level by CT Calcium Score**

	Mean Admission H-FABP µg/l	Mean 90 minute H-FABP µg/l	Mean 12 hour H-FABP µg/l
CTCS <10	3.50 (N 109)	3.20 (N 89)	3.02 (N 91)
CTCS ≥10	4.34 (N 80)	4.92 (N 72)	3.92 (N 66)
	<i>P = .001</i>	<i>P = .003</i>	<i>P = .001</i>

**Table 4.6** Mean H-FABP Level by CT Calcium Score.

**4.13.2 Secondary Analysis**

Comparison of the area under the H-FABP concentration curve (admission, 90 minutes and 12 hour samples) for patients with clinical demonstration of obstructive coronary artery disease (based on clinical assessment of patients supported by a functional test of myocardial ischaemia and a stenosis of >50% in at least one artery at the time of coronary angiography).

Only 5 patients met the predefined study protocol secondary outcome. There were too few events within this category to produce a ROC curve. The study was not powered to fully examine secondary or tertiary endpoints.

**4.13.3 Tertiary Analyses**

**4.13.3.1 Quantitative relationship between actual value of H-FABP and calcium score.**

Discussed above in section 4.11 Relating H-FABP to CT Calcium Score.

#### **4.13.3.2 H-FABP as part of a multifactor model to predict primary and secondary endpoints.**

The ability of admission H-FABP to predict a combined triple outcome, in conjunction with other risk factors, was assessed using backward conditional logistic regression. The combined triple outcome included: Agatston CT calcium score >10; a positive functional assessment for cardiac ischaemia in the presence of a coronary stenosis greater than 50%; and the occurrence of myocardial infarction, coronary revascularisation, or death from a cardiovascular cause. Prevalent acute myocardial infarcts were accounted for – i.e. patients who had a negative troponin level on admission, which then became positive at a subsequent time point, were also included within the triple outcome. A rule-out strategy on admission is most useful clinically. I have therefore focussed on building a rule-out model using admission H-FABP.

Table 4.7 shows the outcome of this regression analysis. Included in the analysis were age, eGFR, and admission H-FABP as continuous variables. Included as dichotomous variables were gender, family history, smoking status, and the presence / absence of hypertension, hypercholesterolaemia and diabetes. Patients with evidence of ischaemia on their ECG were excluded from the study, but some patients included did have more minor abnormalities such as ECG criteria for left ventricular hypertrophy, or a first degree AV block. The ECG was therefore also included as a variable: completely normal versus minor abnormality.

The least powerful predictors were excluded in a stepwise fashion, as seen in Table 4.7. The four predictors remaining by the final stage were age, admission H-FABP, gender, and hypercholesterolaemia. By including these four predictors it was possible to build a regression model able to correctly assign 75.3% of cases (see Table 4.7).

Some notable observations are:

- **Admission H-FABP is a more powerful predictor than some traditional risk factors such as smoking status, diabetes, and hypertension.**

- H-FABP is also a more powerful predictor than minor ECG abnormalities and renal function.

**Table 4.7** Backward Conditional Binary Logistic Regression for Prediction of Combined Triple Outcome.

A – Step 0 Classification, B – Variables in the Equation Steps 1–7, C – Classification Steps 1-7.

**Classification Table<sup>a,b</sup>**

<b>4.7 A</b>			Predicted		Percentage Correct
			CTCS > 10, positive secondary/tertiary outcome, -ve admission trop becoming positive		
Observed		0	Outcome present		
Step 0	CTCS > 10, positive secondary/tertiary outcome, -ve admission trop becoming positive	0	91	0	100.0
	Outcome present		67	0	.0
Overall Percentage					57.6

a. Constant is included in the model.

b. The cut value is .500



**4.7 B**

**Variables in the Equation**

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 <sup>a</sup>						
Age_at_recruitment	.111	.024	21.864	1	.000	1.118
eGFR	.013	.010	1.589	1	.207	1.013
Lab_Admission_HFABP	.265	.119	4.975	1	.026	1.304
Risk1_Gender_Split(1)	1.539	.474	10.542	1	.001	4.661
Risk2_FHx_Split(1)	-.257	.471	.297	1	.586	.774
Risk3_Smoking_Split(1)	-.507	.412	1.514	1	.218	.602
Risk4_HTN_Split(1)	.007	.473	.000	1	.987	1.008
Risk5_Chol_Split(1)	-.627	.481	1.695	1	.193	.534
Risk6_DM_Split(1)	.934	1.129	.685	1	.408	2.546
Completely_normal_first_ECG(1)	-.550	.543	1.025	1	.311	.577
Constant	-9.686	2.663	13.229	1	.000	.000
Step 2 <sup>a</sup>						
Age_at_recruitment	.111	.023	22.519	1	.000	1.118
eGFR	.013	.010	1.620	1	.203	1.013
Lab_Admission_HFABP	.265	.119	4.977	1	.026	1.304
Risk1_Gender_Split(1)	1.540	.470	10.736	1	.001	4.665
Risk2_FHx_Split(1)	-.257	.470	.300	1	.584	.773
Risk3_Smoking_Split(1)	-.508	.410	1.536	1	.215	.602
Risk5_Chol_Split(1)	-.624	.451	1.916	1	.166	.536
Risk6_DM_Split(1)	.936	1.122	.696	1	.404	2.550
Completely_normal_first_ECG(1)	-.550	.542	1.033	1	.310	.577
Constant	-9.678	2.616	13.685	1	.000	.000
Step 3 <sup>a</sup>						
Age_at_recruitment	.109	.023	22.684	1	.000	1.115
eGFR	.013	.010	1.779	1	.182	1.013
Lab_Admission_HFABP	.274	.118	5.415	1	.020	1.315
Risk1_Gender_Split(1)	1.521	.467	10.587	1	.001	4.575
Risk3_Smoking_Split(1)	-.468	.403	1.353	1	.245	.626
Risk5_Chol_Split(1)	-.685	.438	2.444	1	.118	.504
Risk6_DM_Split(1)	.981	1.115	.773	1	.379	2.667
Completely_normal_first_ECG(1)	-.556	.541	1.055	1	.304	.574
Constant	-9.813	2.603	14.212	1	.000	.000
Step 4 <sup>a</sup>						
Age_at_recruitment	.108	.023	22.068	1	.000	1.114
eGFR	.011	.010	1.417	1	.234	1.012
Lab_Admission_HFABP	.263	.116	5.144	1	.023	1.301
Risk1_Gender_Split(1)	1.487	.463	10.320	1	.001	4.423
Risk3_Smoking_Split(1)	-.489	.401	1.486	1	.223	.613
Risk5_Chol_Split(1)	-.629	.431	2.126	1	.145	.533
Completely_normal_first_ECG(1)	-.522	.539	.937	1	.333	.593
Constant	-8.611	2.192	15.433	1	.000	.000
Step 5 <sup>a</sup>						
Age_at_recruitment	.107	.023	21.985	1	.000	1.113
eGFR	.012	.010	1.436	1	.231	1.012
Lab_Admission_HFABP	.262	.118	4.909	1	.027	1.300
Risk1_Gender_Split(1)	1.550	.460	11.357	1	.001	4.712
Risk3_Smoking_Split(1)	-.530	.398	1.776	1	.183	.589
Risk5_Chol_Split(1)	-.661	.426	2.403	1	.121	.517
Constant	-8.644	2.185	15.654	1	.000	.000
Step 6 <sup>a</sup>						
Age_at_recruitment	.096	.020	22.910	1	.000	1.100
Lab_Admission_HFABP	.232	.114	4.102	1	.043	1.261
Risk1_Gender_Split(1)	1.541	.455	11.467	1	.001	4.667
Risk3_Smoking_Split(1)	-.504	.396	1.620	1	.203	.604
Risk5_Chol_Split(1)	-.677	.422	2.572	1	.109	.508
Constant	-6.694	1.365	24.059	1	.000	.001
Step 7 <sup>a</sup>						
Age_at_recruitment	.094	.020	22.455	1	.000	1.098
Lab_Admission_HFABP	.229	.113	4.120	1	.042	1.257
Risk1_Gender_Split(1)	1.568	.450	12.128	1	.000	4.796
Risk5_Chol_Split(1)	-.713	.418	2.915	1	.088	.490
Constant	-6.872	1.357	25.646	1	.000	.001

a. Variable(s) entered on step 1: Age\_at\_recruitment, eGFR, Lab\_Admission\_HFABP, Risk1\_Gender\_Split, Risk2\_FHx\_Split, Risk3\_Smoking\_Split, Risk4\_HTN\_Split, Risk5\_Chol\_Split, Risk6\_DM\_Split, Completely\_normal\_first\_ECG.

Classification Table<sup>a</sup>

4.7 C			Predicted		
			CTCS > 10, positive secondary/tertiary outcome, -ve admission trop becoming positive		Percentage Correct
			0	Outcome present	
Observed					
Step 1	CTCS > 10, positive secondary/tertiary outcome, -ve admission trop becoming positive	0	77	14	84.6
		Outcome present	25	42	62.7
	Overall Percentage				75.3
Step 2	CTCS > 10, positive secondary/tertiary outcome, -ve admission trop becoming positive	0	77	14	84.6
		Outcome present	25	42	62.7
	Overall Percentage				75.3
Step 3	CTCS > 10, positive secondary/tertiary outcome, -ve admission trop becoming positive	0	77	14	84.6
		Outcome present	27	40	59.7
	Overall Percentage				74.1
Step 4	CTCS > 10, positive secondary/tertiary outcome, -ve admission trop becoming positive	0	76	15	83.5
		Outcome present	28	39	58.2
	Overall Percentage				72.8
Step 5	CTCS > 10, positive secondary/tertiary outcome, -ve admission trop becoming positive	0	74	17	81.3
		Outcome present	26	41	61.2
	Overall Percentage				72.8
Step 6	CTCS > 10, positive secondary/tertiary outcome, -ve admission trop becoming positive	0	75	16	82.4
		Outcome present	28	39	58.2
	Overall Percentage				72.2
Step 7	CTCS > 10, positive secondary/tertiary outcome, -ve admission trop becoming positive	0	78	13	85.7
		Outcome present	26	41	61.2
	Overall Percentage				75.3

a. The cut value is .500

#### 4.14 H-FABP as a Diagnostic Marker

There is limited information in the literature about the reference values for the RANDOX Laboratories immunoturbidimetric assay used in this study. Our own local published reference data was obtained using redundant serum samples from patients in primary care and those attending secondary care out-patients (excluding renal and cardiology clinics) (Carless et al., 2013). Although this is an accepted method for obtaining samples, we cannot be sure that some of these patients did not have underlying coronary artery disease. All of the patients participating in the FAB3-CT study have

been thoroughly investigated to exclude both obstructive and non-obstructive CAD, and within the recruited population therefore we can select a group who can be considered 'normal'. Looking at H-FABP levels in this population could therefore provide useful information.

From the recruited population I have selected patients with a negative troponin, normal ECG, CT Agatston score of 0, and no secondary or tertiary study outcomes (including any label of angina or atypical angina). Table 4.8 shows the H-FABP levels for this group.

Admission H-FABP

	50	3.1600
	60	3.6060
	70	4.1020
Percentiles	80	4.5520
	90	5.2620
	95	5.6530
	99	-----

**Table 4.8** Admission H-FABP Percentiles for 'Normal' Patients.

N = 73. Normal = normal ECG, CT Agatston score < 0, and no secondary or tertiary study endpoints (including any diagnostic label of angina or atypical angina).

#### 4.15 Exploring the Diagnostic Potential of H-FABP

It is worth highlighting at this stage that this study was not powered for secondary and tertiary outcomes. The exploration of ideas at this stage is useful however. In this section I would like to explore the diagnostic potential of admission H-FABP as a dichotomous variable, combined with admission ECG and troponin. We will call this a 'triple rule-out strategy', whereby patients are labelled as not having a problem if their admission ECG is normal, combined with a normal troponin level, and admission H-FABP level below the cut-off chosen. If H-FABP is going to be useful as a diagnostic tool

it is important for it to work at this time point. This has potential to save hospital and patient time currently spent waiting for 12 hour blood samples, by allowing us to select low risk patients potentially suitable for early discharge.

Naturally, whenever we choose a specific cut-off for a diagnostic blood test, there is always a trade off between sensitivity and specificity. Initially I would like to explore the potential of the triple rule-out strategy at differing H-FABP percentiles to exclude the combined endpoint of CTCS > 10, secondary and tertiary endpoints.

Table 4.9 shows the triple rule-out strategy for different admission H-FABP percentile cut-offs. The negative predictive value is better for lower H-FABP cut-offs, at the expense of fewer patients falling into the rule-out. At the 50<sup>th</sup> centile H-FABP cut-off sensitivity is 76% and specificity 37%.

**Triple Rule-out Strategy for Different H-FABP Cut-offs**

H-FABP Cut-off $\mu\text{g/l}$	Negative Predictive Value % (N/N)	Positive Predictive Value % (N/N)	Sensitivity %	Specificity %
5.65 (95 <sup>th</sup> Centile)	57.7 (75/130)	49 (25/51)	31	74
4.1 (70 <sup>th</sup> Centile)	56.5 (52/92)	44.9 (40/89)	50	51
3.16 (50 <sup>th</sup> Centile)	66.1 (37/56)	48.8 (61/125)	76	37

**Table 4.9** Triple Rule-out Strategy for different H-FABP cut-offs. For exclusion of CTCS > 10, plus any secondary / tertiary outcomes.

As most patients will have some degree of coronary calcification with age, perhaps it is more important for this triple rule-out strategy to be able to exclude secondary and tertiary endpoints alone. This is more relevant from a clinical perspective. The CT calcium score contains important prognostic information, but according to NICE CG95 cannot be used in the diagnostic pathway except in low risk patients (the 10-29% CAD risk group, representing only 22.7% of the recruited patient population). Table 4.10 shows diagnostic information for different H-FABP cut-offs for the detection of secondary and tertiary outcomes, not including calcium score.

**Triple Rule-out Strategy for Different H-FABP Cut-offs**

H-FABP Cut-off µg/l	Negative Predictive Value % (N/N)	Positive Predictive Value % (N/N)	Sensitivity %	Specificity %
5.65 (95 <sup>th</sup> Centile)	96.2 (125/130)	13.7 (7/51)	58	73.9
4.1 (70 <sup>th</sup> Centile)	96.7 (89/92)	10.1 (9/89)	75	52.7
3.16 (50 <sup>th</sup> Centile)	96.4 (54/56) <i>95% CI</i> 86.6%-99.4%	8 (10/125) <i>95% CI</i> 4.1%-14.6%	83 <i>95% CI</i> 50.9%-97.1%	31.9 <i>95% CI</i> 25.1%-39.6%

**Table 4.10** Triple Rule-out Strategy for different H-FABP cut-offs. For exclusion of secondary / tertiary outcomes (not CT calcium score).

Employing the triple rule-out strategy to detect secondary and tertiary outcomes, using a H-FABP cut-off at the 50<sup>th</sup> centile, has a 96.4% negative predictive value (95% CI 86.6%-99.4%), and sensitivity of 83% (95% CI 50.9%-97.1%). 56 out of 181 patients would have fallen into the triple rule-out (31% of the total cohort), of these 54 would have been a correct rule-out,

and 2 falsely ruled out. One of the false rule-outs was a patient who presented very early, only 33 minutes after index chest pain. This will be explored further in the discussions chapter.

## 5- Discussions

### 5.1 Key Study Findings

Chest pain presentations account for a significant healthcare burden. There are now well established pathways for dealing with the treatment and investigation of patients who have indeed had a myocardial infarct. The reality, however, is that the majority of patients presenting to our ED (79% according to the FAB2 study) do not fall into this category (Viswanathan et al., 2010). Within this larger group most patients are found not to have a cardiac problem, but some do, and a small group go undetected carrying forward significant risk of coming to harm from death and myocardial infarction. This high risk troponin negative group are very hard to detect clinically. Any prognostic aid that allows us to identify low and high risk patients would be a major step forward. H-FABP has been shown to have the potential to do this. It is important to state however, that the extent to which the risk is reversible in the high risk group is unknown. In this study 88% of recruited patients were not felt to have had a cardiac aetiology to their presenting symptoms, after review / investigation in the RACPC. If H-FABP could help us avoid having to follow-up some of these patients, this would have major implications in reducing patient anxiety, time spent attending clinic and investigations, and would conserve resources.

In the FAB3-CT study we targeted our attention onto the lower risk end of the spectrum of patients presenting to the ED. We did this by excluding patients with a prior history of coronary artery disease, haemodynamic instability, an ischaemic ECG, or troponin level >99<sup>th</sup> centile. Nonetheless, we have seen that there is still a high prevalence of traditional cardiovascular risk factors within this group: with 25% being current smokers; 16% ex-smokers; 36% having hypertension and 30% hypercholesterolaemia. Similarly, when matched to current NICE CG95 risk categories for coronary artery disease, only 26% of these patients were in the <10% risk category. A further 15% fell into the 61-90%, and 3% into the >90% risk category.

One of the primary aims of this study was to provide information about how H-FABP may be predicting risk. We used coronary calcification as a marker of coronary artery atheroma burden. **There was a statistically significant positive correlation between H-FABP and CT calcium score, independent of age and renal function.**

We assessed the diagnostic ability of H-FABP as a continuous variable to diagnose CTCS >10, and the clinical demonstration of obstructive coronary disease. Used on its own H-FABP was poor at doing this, with areas under the ROC curve around 0.6. When used as part of a multifactorial model to predict these endpoints **H-FABP was, however, a more powerful predictor than some traditional risk factors such as smoking status, diabetes, and hypertension, as well as renal function.**

H-FABP showed the greatest promise when used as a bivariate within what we termed a 'triple rule-out' strategy. Here, H-FABP was used alongside the ECG and troponin to help identify patients at low risk of obstructive coronary artery disease and tertiary events, over the median follow-up period of 406 days (range 207-616) (death from a cardiovascular cause, myocardial infarction, coronary revascularisation). Using a H-FABP cut-off at the 50<sup>th</sup> centile (3.16 µg/l for the Randox Immunoturbidometric Immunoassay), the triple rule-out strategy had a 96.4% negative predictive value (95% CI 86.6%-99.3%), and sensitivity of 83% (95% CI 50.8%-97.1%). The positive predictive value was low at 8%, so this model could not be used to rule-in patients likely to have a problem, just to rule out patients who were at low risk. 31% of the recruited population fell into the rule-out group (56 out of 181 patients). Of the 56 patients ruled out, 54 were a correct rule-out. Only 2/56 were a false rule-out. It is useful to have a look at these two patients to see if improvements to the rule-out model can be made.

- Patient 1: A 43 year old man who had presented to the ED only 33 minutes after his index chest pain. Both ECG and troponin were normal (including 12hr troponin). H-FABP was not raised beyond the 50<sup>th</sup> centile at any time point. He was allowed home after a period of observation and sequential ECGs. CTCS was 0. He was seen in the RACPC and given a diagnosis of non-anginal chest pain with a 30-



60% NICE CG95 risk score for CAD. No further investigations were deemed necessary. 317 days after his index ED attendance he presented with an MI and received coronary intervention. Although not picked up by the triple rule-out strategy, he was also not picked up by current practice.

- Patient 2: A 70 year old man who presented 5:23 hours after index chest pain. ECG and troponin were normal, including H-FABP < 50<sup>th</sup> centile at all time-points. After clinical review he was felt to require in-patient investigation with a coronary angiogram and received coronary intervention based on the finding of a coronary stenosis. It is possible that his presenting symptoms were nonetheless non-cardiac.

If the triple rule-out was to be used clinically, all patients would obviously still require clinical review by a senior physician prior to discharge, to help establish a diagnosis and exclude serious non-cardiac caused such as pulmonary embolus and aortic dissection. In addition, given the known release characteristics of H-FABP, patients presenting earlier than one hour should have sampling delayed until at least one hour from index symptoms.

There are some important limitations to these secondary analyses, which will be considered below.

## **5.2 Study Conduct**

Overall, the study was well received by patients, who felt that they had benefitted from taking part. As a reflection of this, over 99% provided consent at a second stage to allow long term follow-up and use of their samples for further research. Few patients refused initial consent, and there was a low rate for investigation and clinic non-attendance.

Investigation in the RACPC seems to have been thorough. I had hoped that this would have followed current NICE CG95 guidance more rigidly to allow relevant information to be collected about how this was working in practice: this would have produced additional interesting material for publication. CG95 does not advocate the use of treadmill ETT, which was used in some patients. It must be remembered however, that this was an observational

study. The investigating Cardiologist was at liberty to investigate as they saw fit, and this was in-line with standard local practice. NICE CG95 has not yet been fully adopted in most areas of the UK due to its reliance on cardiac CT, for which there are on-going capacity issues.

Blood samples were overall taken within good time, allowing for patient movements through a busy ED, with many potential delaying factors.

### **5.3 Study Limitations**

All patients were considered eligible if their presenting symptoms were felt to be suggestive of possible ACS by the assessing ED physician in conjunction with the cardiology research physician, or specialist research nurse. When in doubt, the final judgment regarding appropriateness of inclusion into the study was made following discussion of the case with the principal investigator (STA). It is accepted that there are potential differences of having chest pain assessed by different healthcare professional groups. This is however reflective of real life practice for the management of this group of patients with heterogeneous presentations. All recruited patients were managed according to a standardised pathway, which should have minimised variability in practice.

The availability of troponin results at presentation and 90 minutes later was not standard practice within our ED at the time of starting this study. This will have impacted on patient management. It will have facilitated the earlier initiation of ACS therapy, and the availability of more than one troponin reading allowed the utilisation of the international definition for the diagnosis of MI. The measurement of admission troponin for study patients brought practice in line with NICE guidelines (CG95). The release of 90 minute troponin results was felt necessary from an ethical perspective and is part of practice in some UK centres. As recruited patients who developed a troponin >99<sup>th</sup> centile at any stage were excluded from subsequent parts of the study, apart from monitoring, this is not expected to impact on the study findings.

The results of a CT calcium score may have influenced management in the RACPC. The use of CTCS is part of NICE guidelines for the assessment of patients with estimated pre-test likelihood of CAD of 10-29% (CG95). The

availability of the result for patients within other pre-test likelihood categories may have influenced secondary prevention measures and investigation decisions, but was felt to be an ethical requirement in this case. A high score may have resulted in a lower threshold for further cardiac investigation, or the initiation of secondary prevention medications. From the study perspective the RACPC assessment was intended to allow the detection of clinically significant CAD, so this is not expected to have impacted adversely. The initiation of risk modifying medications is unlikely to have affected event rates within the relatively short follow-up period of this study.

The predominant day-time recruitment of patients may have impacted on the type of patient recruited, or even the ACS rate. Within the limits of the study it is not possible to quantify this.

There are some important limitations to the secondary analyses that should be considered. This study was not powered for secondary and tertiary events, so the analysis of these will be underpowered. This is reflected in the wide confidence intervals. It is also not ideal to draw main conclusions based on secondary analyses. Furthermore, deriving a H-FABP cut-off then applying it to the same population may have unanticipated consequences. There are also limitations of drawing conclusions about a triple rule-out strategy involving ECG ischaemia and troponin elevations when the study excluded patients with both of these parameters. The proposed strategy therefore requires validation on an independent patient cohort.

#### **5.4 Applying FAB3-CT Findings to Previously Recruited Patient Populations**

In order to gain confidence with the potential for H-FABP to be used as a diagnostic tool within a rule-out strategy, I would like to take advantage of the availability of a previously recruited patient population to test how it would have worked if applied to them. The FAB2 study, from which some analyses were published in 2010 using 12 hour blood samples (Viswanathan et al., 2010), included almost 500 patients who also had admission H-FABP and troponin levels taken. The study recruited patients with possible or definite ACS between May 2006 and April 2007. All individuals aged 18

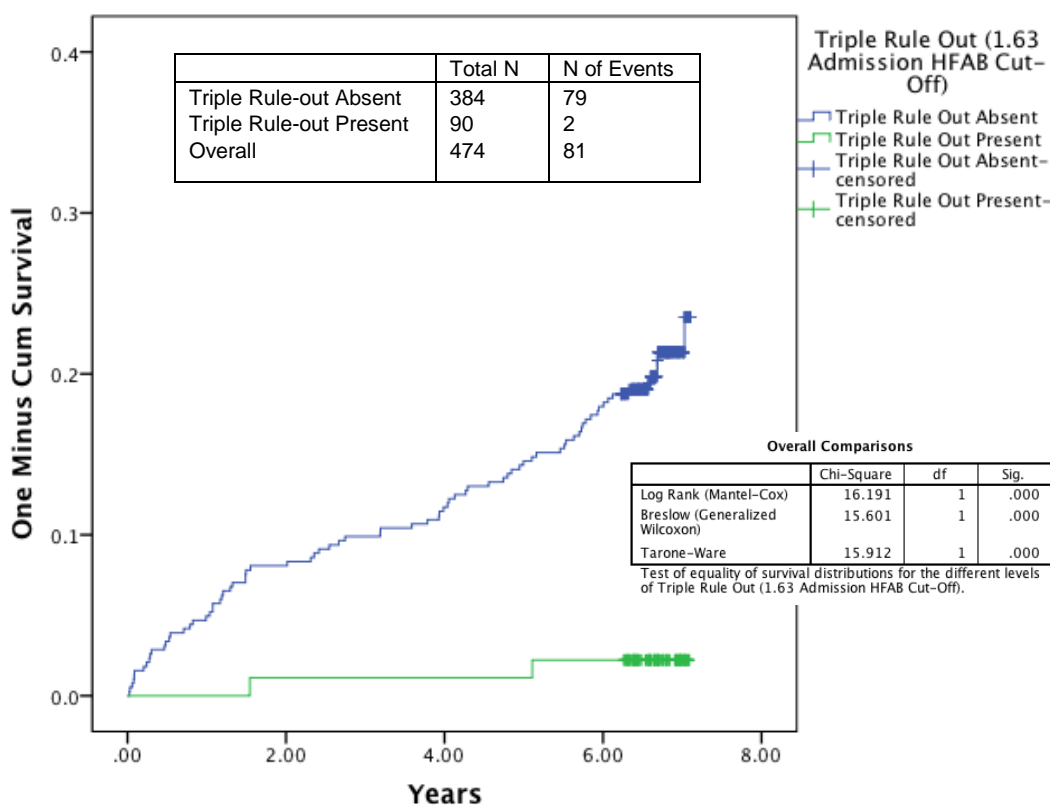
years or above with chest pain of possible or definite cardiac aetiology were deemed eligible for recruitment, irrespective of ECG changes. Of those patients recruited, 79% had a negative troponin results  $\geq 12$  hours from pain. We have obtained updated survival status through the UK Office of National Statistics (censorship date 18 June 2013), and now have over six years of follow-up data for these patients. In this section I would like to present some de-novo analyses from this patient population, applying the triple rule-out strategy discussed above in order to help establish the long term prognostic abilities of this rule-out model.

Incorporating a 50<sup>th</sup> percentile H-FABP cut-off into the triple rule-out strategy was associated with the highest negative predictive value and sensitivity combination for secondary and tertiary outcomes in the current FAB3-CT study (Table 4.10). FAB3-CT used the Randox Laboratories Immunoturbidimetric Immunoassay, whereas the FAB2 study used the Randox Laboratories Biochip Array Assay. Our own Chemical Pathology Department has previously set reference values for both assays using reference populations (Carless et al., 2013). The H-FABP 50<sup>th</sup> centile for the Biochip Array assay was 1.63  $\mu\text{g/l}$ .

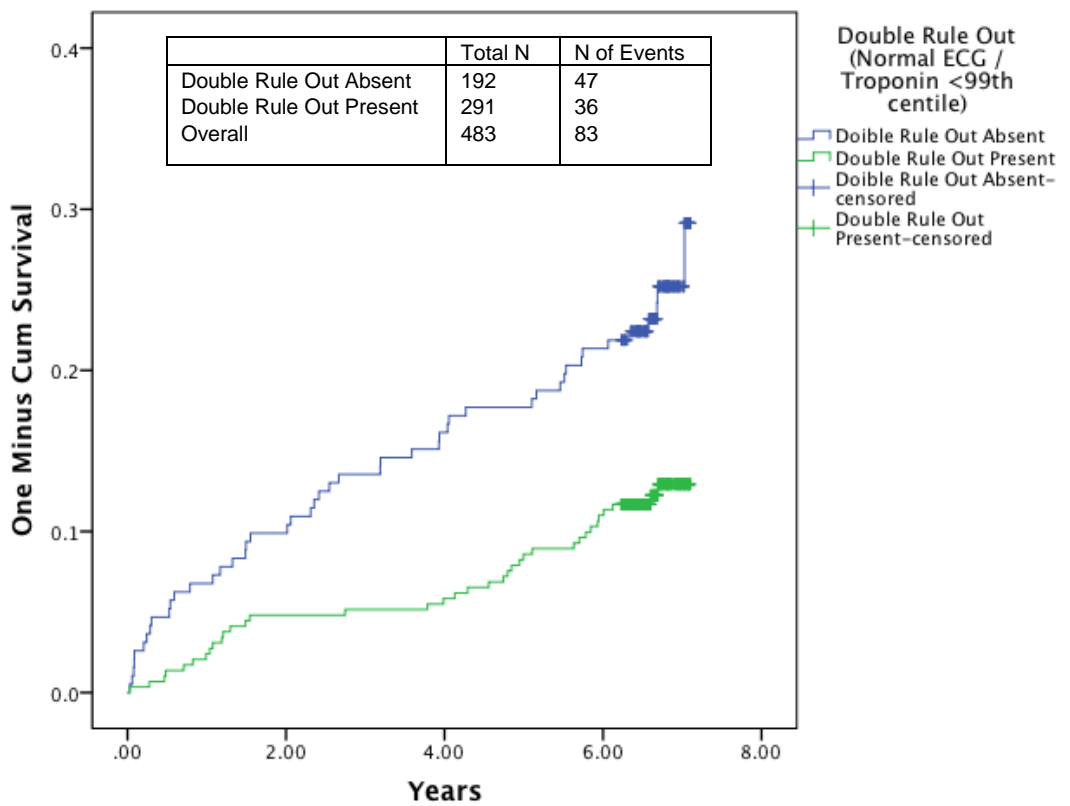
Figure 5.1 shows a Kaplan Meier curve for death event-free survival, split by the 50<sup>th</sup> centile H-FABP triple rule-out strategy (admission H-FABP <50<sup>th</sup> centile, normal ECG, and admission troponin <99<sup>th</sup> centile). You will note from the inset table that 19% of patients fell into the rule-out category, with a marked difference in the number of deaths seen between the two groups. There were only 2 deaths in the rule-out group over the six year follow-up period (2.2% all-cause six year mortality), as compared to 79 in the group that did not meet triple rule-out criteria (20.6% all-cause six year mortality). This equates to an all-cause six year mortality sensitivity of 97.5% (95% CI 90.5%-99.6%); specificity of 22.4% (95% CI 18.4%-26.9%); negative predictive value of 97.8% (95% CI 91.4%-99.6%) and positive predicative value of 20.6% (95% CI 16.7%-25.0%). Clearly H-FABP correlates with survival but H-FAB also correlates with age. The mean age for patients in the rule-out group is 49.93 years, as apposed to 62.23 years in the non rule-out group ( $p = .001$ ). UK Office for National Statistics data released in October 2014 records a mortality rate of 0.31% for people aged 50-54 years,

and 0.79% for people aged 60-64 years. The differing mortality seen between the rule-out and non rule-out groups is not therefore a factor of age alone.

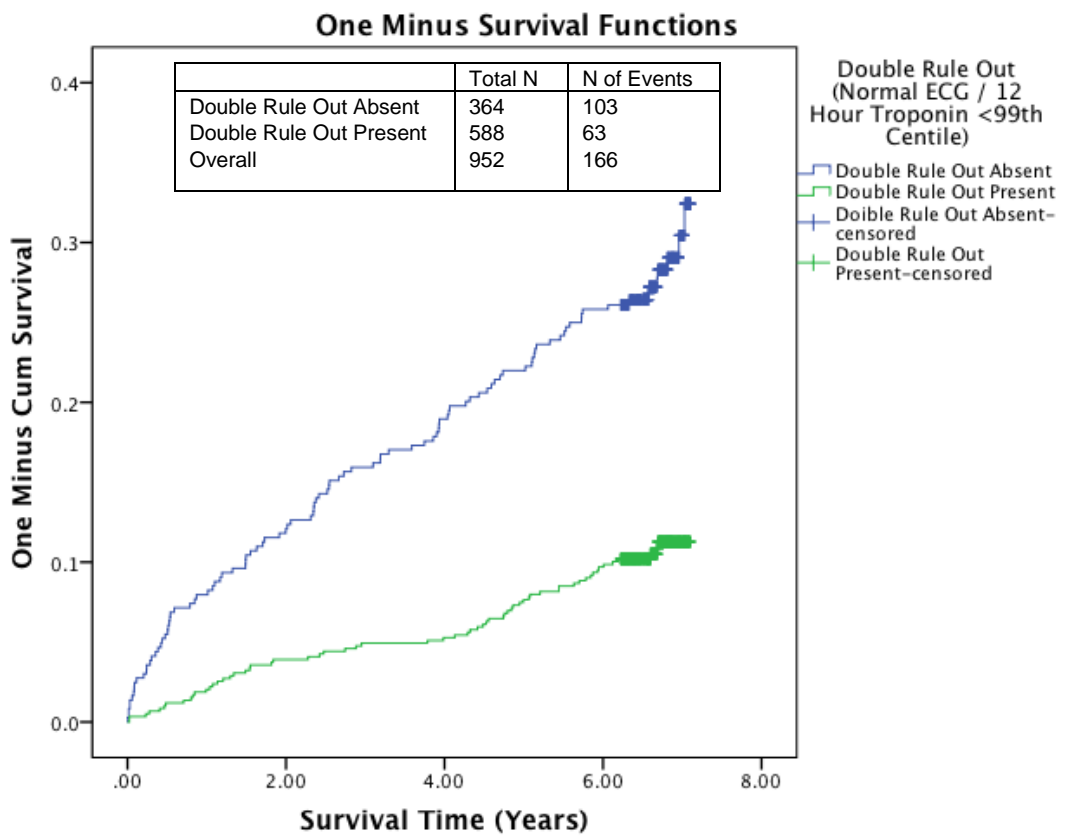
Figure 5.2 demonstrates the same Kaplan Meier curve with H-FABP removed from the model, resulting in poorer event separation between the two groups. This helps to clearly demonstrate the significant contribution of H-FABP to the accuracy of the rule-out model. The mean age of patients in this rule-out group is 57.09 years, versus 64.31 in the non rule-out group ( $p = .001$ ). For comparison, Figure 5.3 is a Kaplan Meier curve for death event free survival, split by ECG and 12-hour troponin <99<sup>th</sup> centile alone, equivalent to our current practice – patients with a normal ECG and 12-hour troponin had a six year all-cause mortality of 10.7%.



**Figure 5.1** Kaplan Meier curve for death event free survival, split by 50th centile H-FABP triple rule-out strategy.



**Figure 5.2** Kaplan Meier curve for death event free survival, split by double rule-out strategy alone (ECG and admission Troponin <99<sup>th</sup> centile alone).



**Figure 5.3** Kaplan Meier curve for death event free survival, split by double rule-out strategy alone (ECG and 12-hour Troponin <99<sup>th</sup> centile alone).

## 5.5 Conclusion & Future Direction

The analyses demonstrate the great potential for H-FABP to be used as part of a triple rule-out strategy, guided by clinical assessment, alongside the ECG and troponin to identify low risk patients as they present to the ED. H-FABP provides additional prognostic information on top of current standard practice at an earlier time point, and could allow earlier discharge of patients from hospital in a safe manner, potentially avoiding unnecessary out-patient investigation.

Validation of the triple rule-out strategy on the FAB2 study population has been presented for mortality only. Work is currently on-going to update the follow-up of the large FAB2 cohort for other tertiary events. This will allow the triple rule-out strategy to be validated further. The strategy would also benefit from external validation.

Modelling the triple rule-out strategy on the FAB2 study population, demonstrates that patients meeting the rule-out only had a 2.2% six year mortality, as compared to a six year mortality of 10.7% if discharged on the basis of current practice ECG / 12-hour troponin alone. This echoes findings of the FAB1 study which demonstrated zero mortality in the troponin / H-FABP negative group at six months (Kilcullen et al., 2007). It is worth noting that FAB3-CT and FAB2 used different H-FABP assays, which may limit the external validity of the finding. There is nonetheless growing evidence for the utility of H-FABP measurement to identify patients at very low risk as they present to the ED.

There are certainly a number of exciting research avenues that could be explored. This study used CTCS in the investigation strategy in part to reduce radiation exposure to this low risk population, but CT technology has moved on at such a quick pace that it is now possible to do a full contrast CT coronary angiogram for similar radiation doses. CT angiography could be used for future research projects. Another avenue that could be explored is H-FABP measurement before and after stress echocardiography, where stress echo has already been requested on clinical grounds. The biomarker response to cardiac stress may carry additional prognostic information. A



randomised study utilising H-FABP clinically in one arm would also be useful, although such a study was not deemed necessary for the introduction of other tests into clinical practice, such as troponin.

## **6- Introducing H-FABP into Clinical Practice**

A working group was convened to discuss potential modifications in the Leeds Teaching Hospitals chest pain pathways. Amongst other options, the possibility of introducing H-FABP as part of a triple rule-out strategy was explored. On the basis of these discussions, a Business Case was formulated exploring potential modifications in current practice. This is included below, and has formed the basis for on-going discussions.

As part of this service development process, in October 2014 admission troponin measurement was made available for all patients presenting to the ED with suspected cardiac chest pain.

## Business Case

### Introduction of Chest Pain Accelerated Discharge Protocol

Abdel-Rahman ST, Webster A, Barth JH, Hassan T, Hall AS

#### Executive Summary

Around 14,000 patients with chest pain attend the LTHT Emergency Department (ED) each year. The purpose of this business case is to identify modifications in the LTHT chest pain pathway that could utilise advances in biomarker technology to facilitate early safe patient discharge, as part of an accelerated discharge protocol (ADP).

#### Objectives / Benefit

- To bring LTHT practice in-line with current NICE guidance.
- To allow the safe discharge of selected low-risk patients within the 4-hour ED window.
- To reduce Clinical Decision Unit (CDU) admissions for chest pain.
- To reduce CDU Rapid Access Chest Pain Clinic (RACPC) referrals.

#### Options Considered

- 1- Introduction of ED admission troponin (alone).
- 2- Introduction of ED admission troponin, combined with H-FAB measurement.
- 3- An alternative biomarker strategy.
- 4- No change.

#### Preferred Option & Cost

- Introduction of ED admission troponin, combined with H-FAB measurement
- Cost – £16,800 plus £11,250 for the respective components.

#### Benefit Appraisal

- ED admission troponins would be available for patient benefit, bringing LTHT practice in-line with current NICE guidance.
- 20% of chest pain patients could be discharged within the ED 4-hour window. Equating to an anticipated accumulated 550 bed day saving.
- Most patients discharged on the ADP would not require RACPC referral.

#### Risk Analysis

We would be the first centre worldwide to introduce this method of risk assessment. The proposal is based on extensive research performed within LTHT. The new pathway will be subject to audit at one month, and again at six months.

#### Timetable for Implementation

Implementation to be completed by September 2014.

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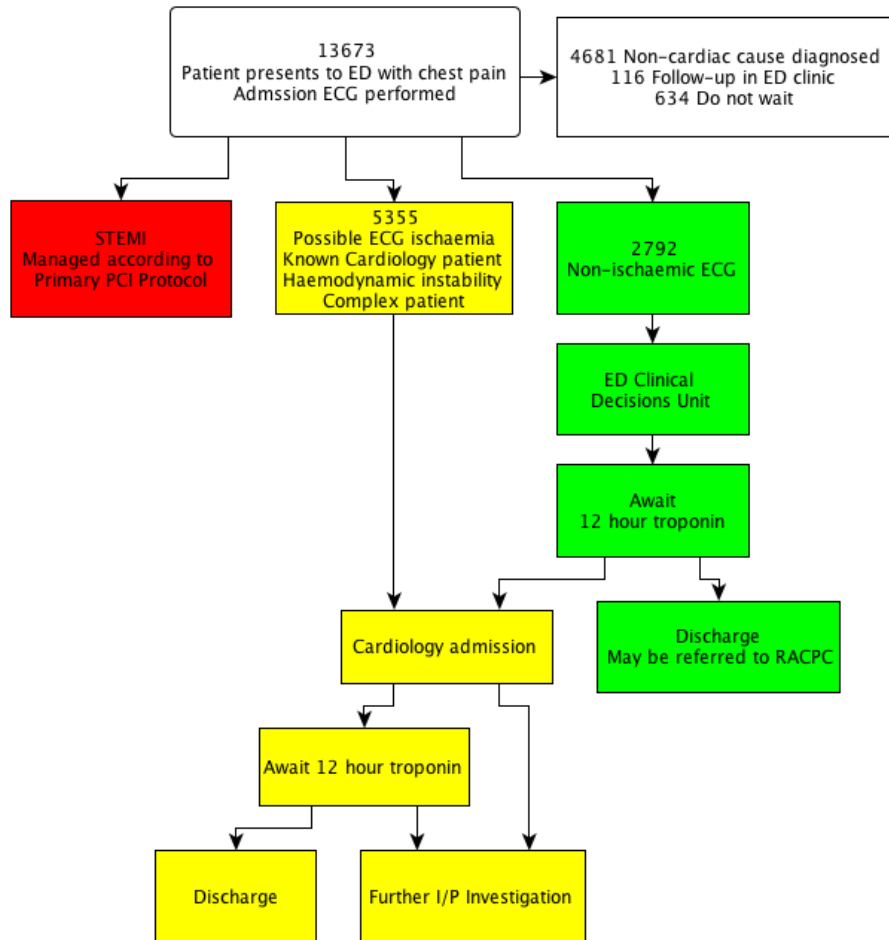
**Setting the Scene.** Around 14,000 patients with chest pain attend the LTHT Emergency Department (ED) each year. Although the majority (>80%) do not have a final diagnosis of acute coronary syndrome (ACS), they often undergo lengthy assessment to exclude it. This is because our current practice is based on risk stratification utilising a troponin taken 12 hours after pain onset. The mean length of stay for a patient admitted to the Clinical Decisions Unit (CDU) on the Chest Pain Protocol is just under 12 hours.

There is a growing interest in 'early' cardiac biomarkers that could allow the risk stratification of patients sooner. The purpose of this business case is to identify modifications in the LTHT chest pain pathway that could utilise advances in biomarker technology to facilitate early safe patient discharge, as part of an accelerated discharge protocol (ADP).

**Why are changes needed?**

- Patients presenting with chest pain account for a significant proportion of our ED and acute medical inpatient workload.
- Our current chest pain pathway does not fully comply with NICE guidance.
- Changes would allow us to identify and treat ACS at an early stage – this is prognostically important for our patients.
- An accelerated discharge protocol will speed up the diagnostic journey for patients, save 550 bed days per annum and relieve increasing pressure on the ED, CDU, and Cardiology admission ward.

**Current LTHT Practice.** At present, patients presenting to the ED are triaged according to ECG findings, haemodynamic state, and prior cardiac history. The current pathway is summarised in Figure 1.



**Figure 1. Current LTHT Practice. Numbers represent patients between August 2012 and September 2013. RACPC = Rapid Access Chest Pain Clinic.**

### NICE Compliance

Current NICE Guidance ('CG 95, Chest Pain of Recent Onset') states:

- 1.2.4.1 Take a resting 12-lead ECG and a blood sample for troponin I or T measurement **ON ARRIVAL** in hospital.
- 1.2.5.2 Take a second blood sample for troponin I or T measurement 10– 12 hours after the onset of symptoms.

Early troponin measurement (i.e. on ED admission) is not currently available within LTHT.

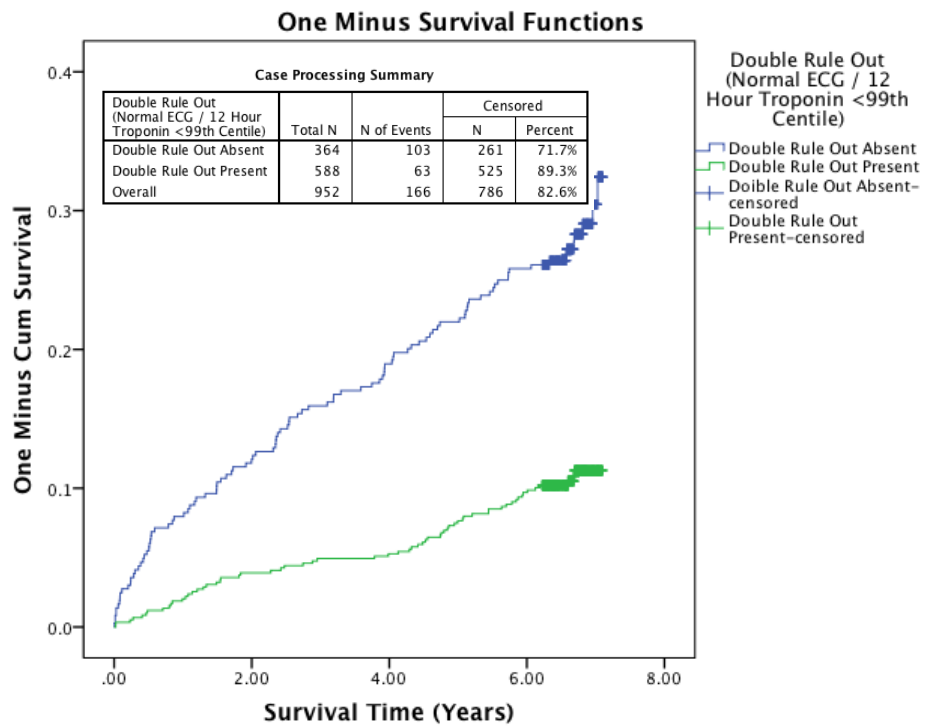
Data from the FAB3-CT Study completed at LTHT in May 2013 using a translational research grant from Heart Research UK, supports the clinical utility of early troponins (Abdel-Rahman et al. Awaiting publication).

- 239 CDU patients were recruited. Blood samples were drawn on admission, 90 minutes later and at 12 hours from chest pain onset.
- Of the 26 patients who were troponin positive, 68% were positive on their ED admission sample. Identifying ACS sooner ensures that appropriate therapies are given at the earliest opportunity.

**Current Service Performance.** At present, treatment and investigation decisions are made on the basis of clinical assessment combined with the use of an ECG and 12 hour troponin. Examining the performance of current practice is essential as a comparator to assess the potential benefits / pitfalls of any proposed service changes.

The FAB2 study, performed at LTHT, collected information on 1080 consecutive patients with chest pain ?ACS as they presented to the ED (Viswanathan K, 2010). 79% of these patients had negative troponin results  $\geq$  12 hours from pain. We have obtained updated survival status for these patients through the UK Office of National Statistics (censorship date 18 June 2013), and now have over six years of follow-up data.

The Kaplan-Meier plot shown in Figure 2, illustrates survival from recruitment out to just over six years. As this was an observational study, these patients were managed in line with current LTHT practice. The patients have been split into two groups based on ECG and troponin findings. The green line represents patients with a normal ECG and 12 hour troponin: patients who meet the current discharge criteria subject to clinical assessment (which is in line with national standards). The mortality in this group for the six-year period was 10.7%. Patients discharged using an ADP should not fair worse.



**Figure 2. Kaplan-Meier Curve for Patients Managed According to Current LTHT Practice. Patients are split by ECG and 12 hour troponin findings.**

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**Relevant constraints in current service provision:** At present, the Chemical Pathology laboratory does not process troponin assays overnight.

**Management arrangements for delivery of plan:** The Department of Emergency Medicine will oversee delivery of service changes arising from this business case.

- Dr Andrew Webster, ED Clinical Lead for CDU, will be the clinical lead for implementation in the ED.
- Dr Julian Barth, will be clinical lead for implementation in the Department of Blood Sciences.

- Prof Alistair Hall, will be the lead contact for liaison with the Department of Cardiology.

### **Key Timescales**

Preferred option to be decided by February 2014

Implementation to be completed by September 2014

### **Fit with Trust / National Strategy**

- To ensure LTHT chest pain pathways are in line with current national guidelines, in keeping with the trust's medium term strategic objective of 'continuing to improve standards of care and patient safety'.
- To update the pathways in line with the current evidence base, in keeping with:
  - The Trust's vision 'to be a locally, nationally, and internationally renowned centre of excellence for patient care'.
  - The Trust's goal 'to achieve the best possible clinical outcome for every patient, every time', and to 'expand the boundaries of healthcare'.

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### **Options Considered**

#### **1 – Introduction of ED admission troponin (alone)**

As specified within NICE CG95 'Chest Pain of Recent Onset', but not yet implemented at LTHT.

Our own local data suggests that this would allow up to 68% of patients who have a positive 12-hour troponin to be detected early.



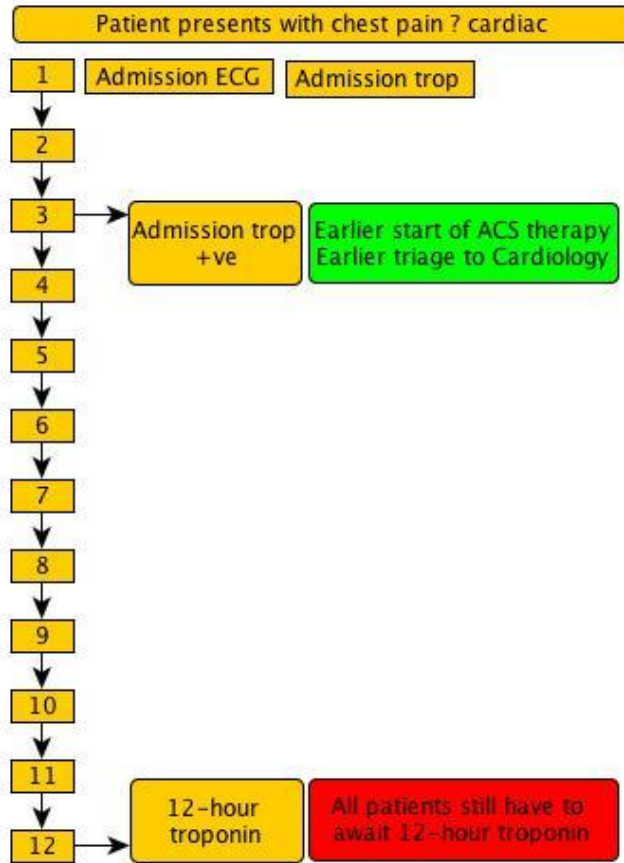


Figure 3. Option 1 - Introduction of ED admission troponin (alone).

**Pros**

- Brings LTHT practice in line with current NICE guidance.
- Allows earlier treatment of ACS.

**Cons**

- Patients will still have to await a 12-hour troponin for discharge.

### **Risk Analysis**

- Patients with a negative admission troponin will still have to wait for a negative 12-hour troponin prior to being considered for discharge. Measures will need to be put in place to ensure that a patient cannot be discharged on the basis of a negative admission troponin alone.
- A trial of making early troponins accessible has already been undertaken successfully within ED, as part of the FAB3-CT study, which recruited patients from the LGI ED between October 2011 and November 2012. ED admission and 90 minute troponins were released for recruited patients live onto the results server. Staff training was introduced at the ED induction sessions, combined with clear labels as to the timing of the troponin sample on the results server. No adverse events were seen.

### **Cost**

- Additional 8000 troponin assays (8000 x £2.10 = £16,800).
- Chemical Pathology advise that there is no additional service costs for the extension of troponin measurement to a 24-hour service.

2- Introduction of ED admission troponin, combined with H-FAB measurement

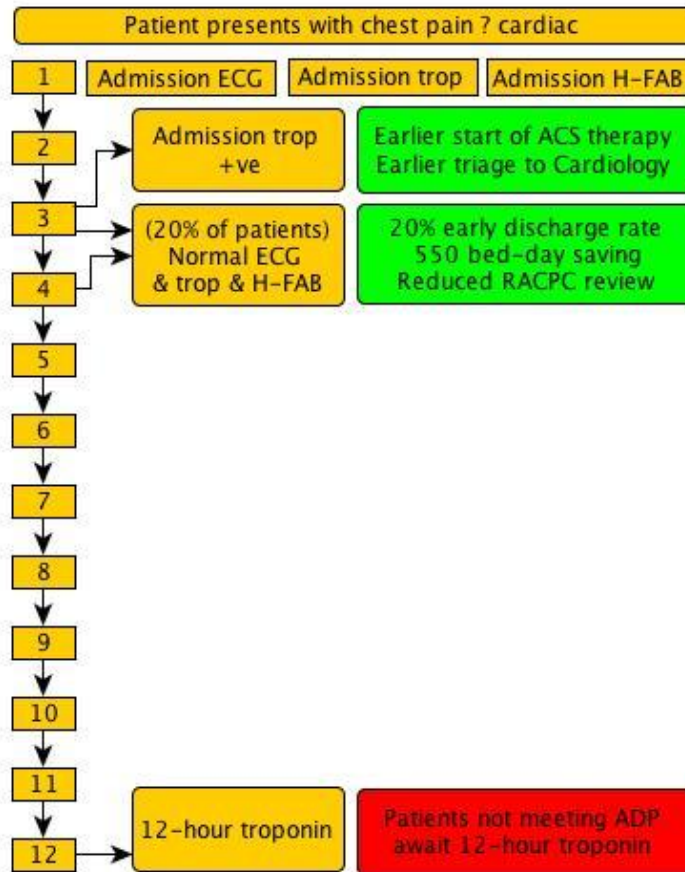


Figure 4. Option 2 - Introduction of ED admission troponin, combined with H-FAB measurement.

Heart-type fatty acid binding protein (H-FABP) is a cytoplasmic protein found abundantly within cardiac myocytes. Its small size allows it to be readily released into the circulation after both myocardial ischaemia and infarction.

There is a growing evidence base supporting the high potential of H-FABP levels in the early diagnosis of ACS, the risk assessment of patients presenting with chest pain, and emerging evidence suggesting that H-FABP levels are closely related to angiographic findings in patients presenting with ACS. Appendix A contains a

summary of the current evidence base for the diagnostic and prognostic potential of H-FABP.

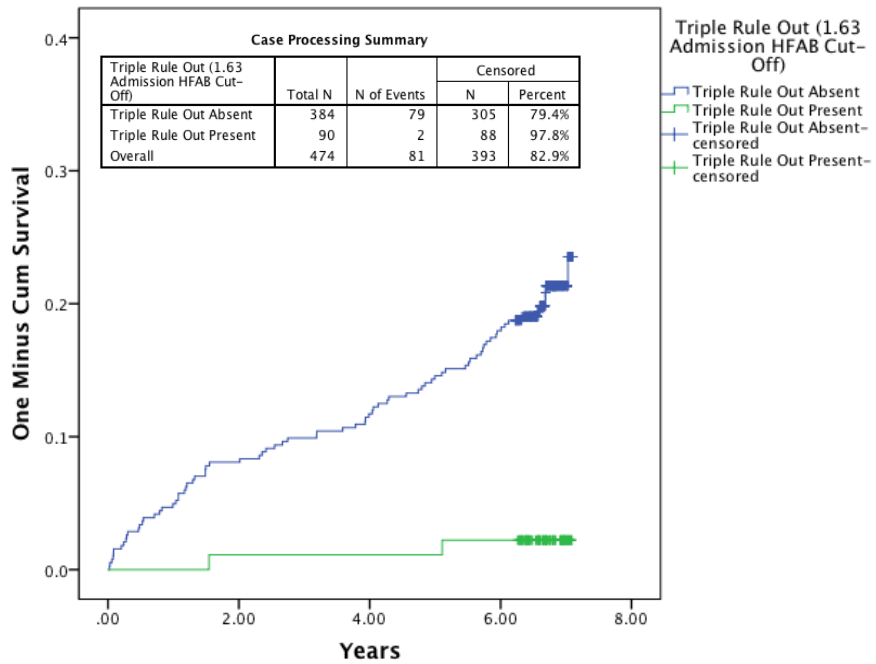
Three studies have now been performed within LTHT examining the clinical potential of H-FABP. Overall these studies have included in excess of 2700 patients. Details of the first two (published in the Journal of the American College of Cardiology) are given in appendix A. The FAB3-study was completed in May 2013, and is awaiting publication pending the results of two patent applications.

We have identified a risk stratification strategy that could be applied to patients on admission to the ED, which is able to identify an exceptionally low risk group suitable for early discharge. This utilises a 'Triple-Rule-Out' strategy, whereby patients fall into the low risk group if they have 1- a normal presenting ECG, 2- an admission troponin I < 99<sup>th</sup> centile, and 3- an admission H-FAB <50<sup>th</sup> centile. This EDP was devised using FAB3-CT study data, and has been validated by applying it to the FAB2 study population for which we now have over six years of follow-up data. Modelling performed on the FAB2 study population estimated that 20% of presenting patients would fall into the low risk group, with only a 2.2% observed six-year mortality (Figure 5) – the first death occurred almost two years into follow-up. None of the patients identified as low risk on admission went on to have a 12-hour troponin above the clinical decision cut off. You will recall for comparison that patients discharged according to current practice have a 10.7% six-year mortality.

All patients would obviously still require clinical review by a senior doctor prior to discharge from the ED, to help establish a diagnosis and exclude serious non-cardiac causes such as pulmonary embolus and aortic dissection. Given the known release characteristics of H-FAB, patients presenting earlier than 1 hour from index chest pain, should wait one hour before initial blood tests are taken. Patients not meeting the Triple-Rule-Out will be managed in accordance with current practice, and await a 12-hour troponin on CDU.

Patients falling into the Triple-Rule-Out have such a low mortality risk, that they could be discharged directly from the ED within the four-hour ED wait window. Data from the FAB3-CT study demonstrates that the Triple-Rule-Out strategy has a 96.4% negative predictive value for the detection of clinically significant coronary artery disease (awaiting publication). Clinically significant coronary artery disease was

defined as 1- the clinical demonstration of obstructive coronary artery disease based on clinical assessment of patients supported by a functional test of myocardial ischemia and a stenosis of >50% in at least one artery at the time of coronary angiography, or 2- the occurrence of a major adverse cardiovascular event in the first six months after index assessment (defined as death from a cardiovascular cause, myocardial infarction, or coronary revascularisation). For patients without a prior history of coronary artery disease, It would not be considered routine, therefore, for these patients to be assessed in the RACPC upon discharge, although this could be arranged when the assessing ED Physician feels it is appropriate. If symptoms continue these patients can be referred for further review by their GP.



**Figure 5. Kaplan-Meier curve showing mortality for FAB2 study patients split by Triple-Rule-Out strategy on ED admission.**

The green line represents the low risk group identified by the Triple-Rule-Out strategy on ED admission, proposed for early discharge.

### Pros

- ED admission troponins would be available for clinical benefit as with option 1.
- 20% of patients could be discharged early without having to wait for a 12-hour troponin result. Equating to an anticipated accumulated 550 bed day saving (1629 patients anticipated to be discharged on ADP, with reduced length of stay from 12 to 4 hours).
- Reduced admission pressure on CDU & Cardiology Admission Ward.
- Reduced referrals to the RACPC.
- Quicker pathway for patients.

### Cons

- Cost of introducing admission H-FAB and troponin.
- There are no anticipated negative consequences for the Department of Cardiology. The EDP is relevant in the main for patients who are currently managed on CDU. Patients not falling into the EDP will be managed in-line with current practice.

### Risk Analysis

- We would be the first centre worldwide to introduce this method of risk assessment. The proposal is based on extensive research performed within LTHT.
- The pathway will be audited at one month and again at six months. This will record patient flow through the pathway, combined with mortality and ACS rates on patients discharged on the ADP.

### Cost

- An additional 8000 troponin assays (8000 x £2.10 = £16,800), as in option one.

- Plus 2500 H-FAB assays (2500 x £4.50 = £11,250).
- Total cost £28,050.

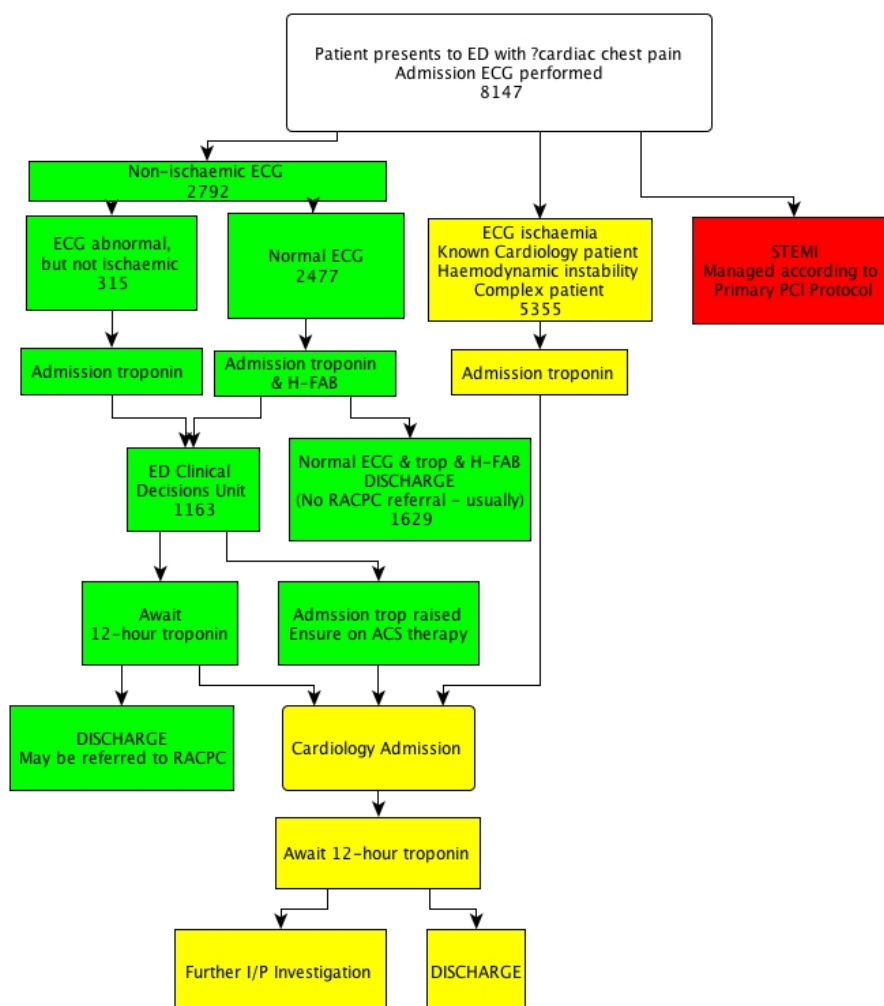


Figure 6. Proposed pathway according to option 2 - the 'Triple-Rule-Out' strategy. Numbers represent expected patient flow.

### 3- An alternative biomarker strategy

A literature search has been performed to identify any other accelerated discharge protocols (ADP) that could be a viable alternative. Although this is not intended to be

an exhaustive summary of the literature base, the most relevant papers are discussed below.

Greenslade et al has examined the use of high sensitivity troponin in combination with the Vancouver chest pain rule. Of the 1635 patients recruited, 33% were assigned as low risk within 2 hours, although 5.6% of these went on to have ACS. They conclude that the sensitivity is insufficient to enable safe early discharge (Greenslade JH, 2013).

In 2012 the ADAPT Trial investigators reported a 2-hour accelerated diagnostic protocol tested on a cohort of 1,975 patients (Than M, 2012). This used a combination of ECG, TIMI risk score, zero and 2 hour contemporary troponin measurement. 20% of patients were identified as low risk. Only 1 (0.25%) patient had a MACE event within the one month follow-up period, but there was a relatively high therapeutic (18.3%) and procedural (2%) intervention rate amongst the ADP negative group. Using this strategy could therefore allow early discharge, but all the patients would need to be seen in the RACPC. No long term follow-up data is provided for the early discharge group.

Another published study incorporating 1,285 consecutive presenting patients (Ng SM, 2001) used point of care testing for a combination of CK-MB, myoglobin, and troponin I. Combined with ECG findings and clinical assessment 40% of presenting patients could be allowed home using 90 minute from ED biomarker analysis. There was however, a 1% re-admission rate within the first month with unstable angina (0.8%) or myocardial infraction (0.2%), and no long-term mortality follow-up is available for the discharged patients.

Lastly, following discussions at the 2013 Cardiology Consultant 'Time-Out' meeting, Prof Hall was asked to review a recent BMJ Commentary (Shah AS, 2013) (Siu) (Ng SM, 2001), which explored the use of high-sensitivity troponin at zero and 3-hours. It was noted that the proposed pathway did not cover all types of patient outcomes (e.g. Troponin >99<sup>th</sup> centiles at time zero and 3 hours without >20% change; Troponin <99<sup>th</sup> centile at time zero and >99<sup>th</sup> centile at 3 hours but <50% change). The proposed pathway was also based on theory rather than clinical data showing that it worked.

The literature search has not identified any appealing alternate ADPs.



#### **4- Do nothing**

This was not considered a viable option, given the fact that our current chest pain pathway does not fully comply with NICE guidance.

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#### **Preferred Option**

2- Introduction of ED admission troponin, combined with H-FAB measurement

#### **Benefits Appraisal**

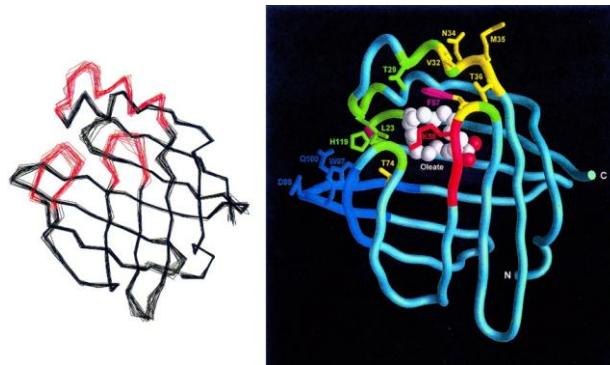
- ED admission troponins would be available for patient benefit, bringing LTHT practice in-line with current NICE guidance.
- Allow up to 20% of ?-cardiac chest pain patients to be discharged within the ED 4-hour wait time.
- Reduced CDU admissions and length of stay for patients on the chest pain protocol.
- Reduced CDU referrals to the RACPC.

The pathway will be audited at one month and again at six months. This will record patient numbers, combined with mortality and ACS rates on patients discharged on the ADP. Data will be obtained from the Department of Informatics on CDU length of stay. CDU RACPC referral numbers will also be monitored, combined with admission Troponin & H-FAB assay requests.

## Appendix A

### Summary of Current H-FABP Evidence Base

Heart-type fatty acid binding protein (H-FABP) is a cytoplasmic protein found abundantly within cardiac myocytes (Glatz et al. 1997). Its small size (15 Kd) allows it to be readily released into the circulation after both myocardial ischaemia and infarction. Following myocardial infarction serum levels peak after around 6-8 hours, and return to normal within about 24 hours (Glatz, van der Voort and Hermens 2002; Glatz et al. 1994). Although highly cardiac-specific, small concentrations are found in tissues outside the heart. Other limitations include its renal excretion (like troponin) potentially making it less useful in patients with renal impairment, and the relatively narrow diagnostic time window. Elevated H-FABP levels are reported in patients with atrial fibrillation, atrial flutter, and after electrical cardioversion, and care should be used therefore if being used as a biomarker in these patient groups (Mazovets et al. 2006).



**Figure 7 Three dimensional structure of human H-FABP.**  
Biochemical Journal (2001) 354, 259-266.

There is a growing evidence base supporting the high potential of H-FABP levels in the early diagnosis of ACS, the risk assessment of patients presenting with chest pain, and emerging evidence suggesting that H-FABP levels are closely related to angiographic findings in patients presenting with ACS.

### A.1 H-FABP as a Diagnostic Marker

There are now a good number of studies published in this area, although many include fewer than one hundred patients, and used differing H-FABP assays. Nonetheless the overall evidence base supports the utility of H-FABP for the early diagnosis of ACS.

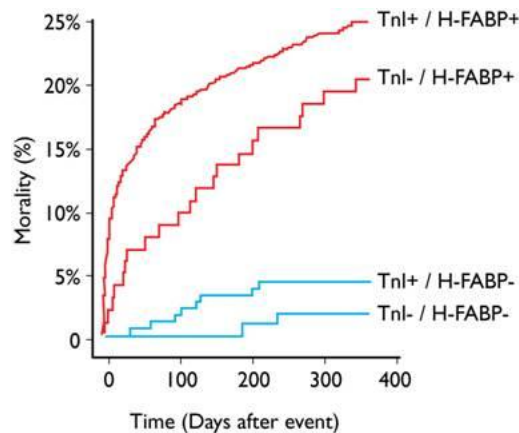
In a study of 705 patients presenting with suspected cardiac chest pain, in blood drawn at presentation, H-FABP was demonstrated to be superior to CK-MB, myoglobin and troponin for the detection of MI (area under ROC curve 0.86 (95 % CI 0.82-0.90) (Body et al. 2011). Although in this study no single marker could enable clinically acceptable exclusion of MI on its own, the combination of troponin I and H-FABP was found to be the optimal biomarker combination. Similarly, in 485 patients Gururajan *et al*/ demonstrated H-FABP, measured 3-6 hours after the onset of pain, to be a good discriminator between patients with ACS as compared with those felt to have non-cardiac symptoms and normal controls (area under ROC 0.97, 95% CI 0.95-0.98)(Gururajan et al. 2010).

The absolute reported sensitivity and specificity of H-FABP for the early diagnosis of ACS varies in the literature, probably reflective of the differing assays used, combined with variations in the way ACS is categorised and the time blood samples were drawn. In general the sensitivity averages around 80%, and the specificity around 75%. Perhaps more important is that the evidence base shows H-FABP to be superior in many instances to troponin for the diagnosis of ACS early following symptom onset. In the study by Charpentier *et al*/ using samples from 677 patients with suspected ACS on admission to the ED, H-FABP was predictive of the diagnosis of ACS with an OR of 4.65 (95 % CI = 2.39 – 9.04), specificity of 96.8%, but low sensitivity of 13.5% (Charpentier et al. 2010). In this study H-FABP was not felt to add significant additional information on top of standard diagnostic tools. Figiel *et al*/ demonstrated a similar specificity for diagnosing non-ST segment elevation MI in 100 patients, using blood drawn at presentation, although sensitivity in this study was found to be much higher at 94.7% (Figiel et al. 2008). Cavus *et al*/ reported a sensitivity of 97.6% on samples taken during the first hour from presentation (Cavus et al. 2006). **H-FABP has a high negative predictive value for MI, 93% at 0 to 3 hours from pain onset, increasing to 97% at 3 to 6 hours. Combining H-FABP with troponin increases this to 94 and 98% respectively (McMahon et al. 2012).**

Numerous other studies support the use of H-FABP for the early diagnosis of MI (Nakata et al. 2003; Okamoto et al. 2000; Orak et al. 2010; Ruzgar et al. 2006; Valle et al. 2008; Kim et al. 2011; Kim et al. 2010).

### A.2 H-FABP as a Prognostic Marker

One of the largest published studies on H-FABP, conducted by our Department, examined the ability of H-FABP levels to predict long-term mortality in patients with confirmed ACS. This used samples taken at 12-24 hours after chest pain from 1448 patients. H-FABP was strongly predictive of all-cause mortality at one year after index hospital admission, independent of traditional clinical risk factors, and across the whole spectrum of ACS. Of great interest, was the ability of H-FABP levels to separate troponin negative patients into a low and high-risk group. Unstable angina patients with a low H-FABP level (<5.8 µg/l) had a 1-year all-cause mortality of 2.1%, whereas patients with levels above this cut off had a mortality of 22.9%. **The occurrence of a negative test for both H-FABP and troponin was associated with zero mortality prior to six months (Kilcullen et al. 2007).**



**Figure 8 All-cause mortality by Troponin and H-FABP clinical cut-offs.** Note the ability of H-FABP levels to separate patients with negative troponin into low and high risk groups. Tnl = Troponin I, - = below clinical cut-off, + = above clinical cut-off. JACC (2007) 50, 2061-7.

A follow on study further examined the role of H-FABP in the low to medium risk population by using 1080 consecutive presenting patient with suspected rather than confirmed ACS (Viswanathan et al. 2010). 79% of these patients had negative troponin results  $\geq 12$  hours from pain. The risk of death or MI increased with increasing H-FABP levels, confirming the ability to H-FABP to predict events independent of other clinical risk factors, including troponin assessed by an ultra sensitive assay. The findings from these two studies are in keeping with those reported by O'Donoghue *et al* using samples taken from patients recruited into the OPUS-TIMI 16 trial (O'Donoghue et al. 2006).

Garcia-Valdecasas *et al* demonstrated that H-FABP, measured within the first 3-6 hours after the onset of chest pain, was not only more sensitive than troponin I in the early diagnosis of MI, but was an independent predictor of events within a 6-month follow-up (Garcia-Valdecasas et al. 2011).

Numerous other studies confirm the prognostic potential of H-FABP (Suzuki et al. 2005; Erlikh et al. 2005)

### **A.3 Relating raised H-FABP to Stenotic Atherosclerosis**

To my knowledge there is only one study examining the correlation between H-FABP levels and coronary stenoses. In a study of 93 patients with ACS presenting within two hours of onset of chest pain, H-FABP was measured at 2, 4, and 6 hours from symptom onset. All patients underwent coronary angiography. Peak H-FABP levels were seen at 4 hours. Measured at 2 hours the sensitivity of H-FABP for at least 50 % stenosis was 70%, rising to 85% at four hours for  $\geq 50\%$  stenosis, and 88% at four hours for  $\geq 70\%$  stenosis. The sensitivity and positive predictive value for revascularisation was 89 and 80% respectively at four hours (Kalay et al. 2010). There are no published studies examining the correlation between H-FABP and total atherosclerosis burden (i.e. a non-obstructive and obstructive coronary artery disease end-point).

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## List of Abbreviations

ACS	Acute coronary syndrome
ADP	Accelerated discharge protocol
AMI	Acute myocardial infarction
BNP	B-type natriuretic protein
CAD	Coronary artery disease
CG	Clinical guideline
CI	Confidence interval
CKMB	Creatine Kinase MB
CT	Computerised axial tomography
CTCS	CT Calcium Score
ECG	Electrocardiograph
ED	Emergency Department
eGFR	Estimated glomerular filtration rate
FHx	Family history
H-FABP	Heart-Type Fatty Acid Binding Protein
Hr	Hour
hsCRP	High sensitivity c-reactive protein
hsTn	High sensitivity troponin
hsTnT	High sensitivity troponin T
IHD	Ischaemic heart disease
MACE	Major adverse cardiac event
MI	Myocardial infarction
MMP	Multi-biomarker panel
NICE	National Institute of Clinical Excellence
N	Number
NPV	Negative predictive value
OR	Odds ratio

POC	Point of care
PPV	Positive predictive value
ROC	Receiver operating characteristic
STE	ST elevation
TIMI	Thrombolysis in myocardial infraction
Tn	Troponin
Tnl	Troponin I

## **Appendix A Power Calculations**

Extract taken from study protocol.

### **A.1 Sources of Variability**

(i) RANDOX H-FABP assay – Coefficient of Variation at clinical decision point <5%

(ii) 64 Slice Cardiac CT – Sensitivity and Specificity for presence of significant CAD >99%.

(iii) Within observer variation in identifying coronary calcium score  $\leq 1\%$ .

(iv) Between observer variation in identifying coronary calcium score  $\leq 1\%$ .

### **A.2 Prevalence of Primary Endpoint**

A recently published study of 44,052 subjects with no known CAD that were assessed by CT calcium scoring and followed for an average of 5.6 yrs (Blaha et al., 2009). A total of 19,989 (45%) subjects had a calcium score of 0 while an additional 5,388 (12%) had a score of between 0 and 10. After correction for other known risk factors, the risk of death was 2x higher in the second group than the first. A further 18,766 (43%) had a score of >10 and an adjusted relative risk of death that was 5x higher than for those with a score of 0.

### **A.3 Prevalence of Secondary Endpoint**

Studies that have performed coronary angiography in unselected patients with suspected acute coronary syndrome that were found to be '*troponin negative*' report the presence of significant coronary artery disease to be 23% (deFilippi et al., 2000).

#### **A.4 Measured Differences in H-FABP in FAB-2 Study (Viswanathan et al., 2010)**

(i) H-FABP concentration at 12 hours after onset of symptoms for patients found to have a normal troponin I concentration and alive vs. dead at 12 months: 2.56 µg/L (SD 1.91; n=733) vs. 4.93 µg/L (SD 3.05; n=26). Difference in H-FABP 2.37 µg/L or 92.6% (p<0.0001).

(ii) H-FABP concentration at 12 hours after onset of symptoms for patients found to have a normal troponin I concentration and “*alive with no MI*” vs. “*dead or with MI*” prior to 12 months: 2.54 µg/L (SD 1.86; n=719) vs. 4.56 µg/L (SD 3.27; n=40). Difference in H-FABP 2.02 µg/L or 79.5% (p<0.0001).

#### **A.5 Power Calculation**

The proportion of ‘*troponin negative*’ patients with significant CAC is assumed conservatively to be 43% (Blaha et al., 2009) and those with stenotic coronary artery disease as 23% (deFilippi et al., 2000). We estimate that recruitment of at least 86 patients that are subsequently shown to have a CAC score >10 and at least 86 patients with a CAC score ≤10 is required to reliably detect a difference in H-FABP of 0.5 µg/L (>90% power at the 5% significance level). However, we are opting to recruit a total of 250 patients (estimate 107 CAC>10 & 143 with CAC ≤10) and will ensure that a total of >200 (estimate 86 CAC>10 & 114 with CAC ≤10) complete the study as far as the Rapid Access Chest Pain Assessment Clinical assessment. This will also ensure that any patients excluded due to study withdrawal or inter-current events are substituted and that the target numbers are attained. **This will allow reliable detection of a difference in H-FABP as small as 0.3 µg/L (>90% power at the 5% significance level).**



## Appendix B

### Emergency Department Clinical Decisions Unit Protocol – relevant pages (includes Patient Information Sheet & Consent Form)

## Clinical Decision Unit Protocol

### SUSPECTED CARDIAC CHEST PAIN

#### LGI

Patient Details or Addressograph label		Date & Time of arrival in ED		
		Date & Time of arrival in CDU		
		Time of onset of chest pain		
		Date & Time '90 Mins from ED Presentation' FAB3-CT research samples to be taken		
Included in FAB3-CT	Yes	No	Date & Time 12 hour samples to be taken	
<b>INCLUSION CRITERIA</b>				
<ul style="list-style-type: none"> <li>• Chest pain which is possibly cardiac in origin with non-diagnostic ECG</li> </ul>				
<b>EXCLUSION CRITERIA</b>				
<ul style="list-style-type: none"> <li>• <b>Medium risk (refer to cardiology)</b> <ul style="list-style-type: none"> <li>○ Definite unstable angina (with ECG changes)</li> <li>○ Possible cardiac pain with ECG changes (which may be old)</li> </ul> </li> <li>• <b>High risk / AMI (refer to cardiology)</b> <ul style="list-style-type: none"> <li>○ prolonged cardiac pain with ECG changes</li> </ul> </li> <li>• Haemodynamic instability (dysrhythmia or hypotension), (<b>refer to cardiology</b>)</li> <li>• Known history of IHD on anti-anginal treatment <b>with active cardiology follow-up</b></li> <li>• History suggestive of alternative diagnosis                             <ul style="list-style-type: none"> <li>○ E.g. pneumothorax, PE, dissection of thoracic aorta (treat appropriately)</li> </ul> </li> </ul>				

Document admitting doctor and senior doctor case discussed with:

ADMITTING DOCTOR	SIGNATURE	SENIOR DOCTOR	SIGNATURE

For patients entered into the FAB3-CT Study - it is **ESSENTIAL** that you :

- Complete the patient consent forms in this protocol
- Inform the CDU nurse of the time of the FAB3-CT sample '90 minutes from ED presentation' and 12 hours post last severe episode of pain (document in boxes above)
- A CSW or nurse may take the sample - but it is **ESSENTIAL** it is taken

## Management pathway for Suspected Cardiac Chest Pain

### LOW RISK PATIENT WITH SUSPECTED CARDIAC CHEST PAIN

**ED Management**

- Complete patient assessment in this book
- Consider recruitment to FAB3-CT & complete paperwork including consent
- Complete medication chart and relevant risk assessments

**Investigations**

- ECG
- Consider chest x-ray to identify other non-traumatic causes of chest pain
- Take bloods including
  - FBC, UE, glucose, cholesterol and initial troponin
  - Samples for FAB3-CT study STEP 1 (includes troponin when sent)
  - D-dimer only if PE can not be excluded



**ENSURE '90 mins from ED presentation' (STEP 2) samples are taken for patients entered into FAB3-CT Study**



**CDU Management**

- ECG at 4 and 12 hours after onset of chest pain
- Troponin at 12 hours after onset of chest pain
- FAB3-CT study samples at 12 hours (includes troponin when sent) STEP 3



**If further episodes of chest pain but no new ECG changes, keep for 12 hrs and repeat troponin**

**Discharge Criteria**

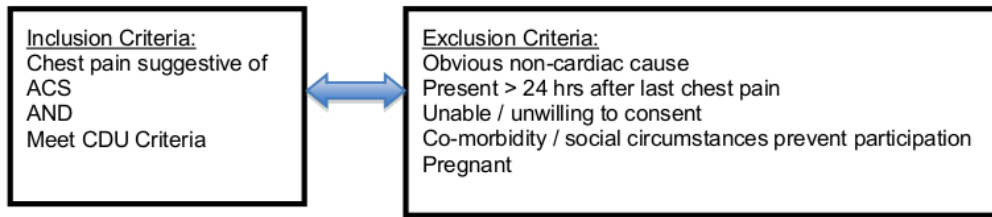
- No ECG changes
- Normal **12 hour** troponin
- No further pain

Discharge to primary care. All FAB3-CT patients need 1- Cardiac CT Calcium Score requesting & 2- Referral to Rapid Access Chest Pain Clinic (STEP 4)

**Admission Criteria at any time:**

- Positive troponin at any stage
- Recurrent pain suggestive of ACS
- ECG changes suggestive of ACS
- Alternative diagnosis or further tests required
- Social circumstances

## CDU FAB3-CT



### STEP 1

At ED presentation

- 1- Rapid Verbal consent
- 2- Routine admission bloods  
PLUS  
**4ML GOLD TOPS (x2) STUDY SAMPLES**

### STEP 2

90 mins from ED presentation

- 1- Written consent
- 2- **4ML GOLD TOPS (x2) STUDY SAMPLES 'MIN 90'**
- 3- **DNA Blood (PINK TOP) & RNA Saliva Sample**

### STEP 3

12 hours after worst / last pain

- 1- **4ML GOLD TOPS (x2) STUDY SAMPLES 'HR 12'**  
**Routine 12 hour clinical troponin will be measured off these samples**

### STEP 4

#### 12 hour troponin negative

- 1- **Request Cardiac CT Calcium Score (FAB3-CT STUDY REQUEST CARD (LILAC) completed and faxed to x 25620)**
- 2- **Refer to FAB3-CT RACPC**

#### 12 hour troponin positive

- Admit to Cardiology
- Research team will withdraw patient from study

<b>Name:</b>		<b>DOB:</b>		<b>Unit No:</b>	
ED CLINICAL ASSESSMENT (Circle appropriate fields)					
ONSET TIME	DURATION		hours	mins	
<b>ONSET</b> (to peak severity)	Sudden (over seconds)	Gradual (over minutes)	Gradual (over hours)	Comment:	
<b>SEVERITY</b>	Mild	Moderate	Severe	Unbearable	
<b>LOCATION</b>	Central	Left sided	Right sided	Generalised	
<b>CHARACTER</b>	Crushing	Burning	Sharp	Tight	
	Heavy	Aching	Tearing	Other:	
<b>RADIATION</b>	Left arm	Right arm	Both arms	Neck/Jaw	
	Back	Abdomen	Comment:		
<b>ASSOCIATED SYMPTOMS</b>	Nausea	Vomiting	Sweating	Belching	
	SOB	Feeling cold	Dizziness	Other:	
<b>AGGRAVATING FACTORS</b>	None	Exertion	Breathing	Coughing	
	Movement	Pressure	Position	Other:	
<b>RELIEVING FACTORS</b>	None	GTN	Antacids	Position	
	Rest	Other:			
Other relevant history to the presenting complaint:					
<b>PAST MEDICAL HISTORY</b>					
	Yes	No	Comment		
Previous angina					
Previous MI / PCI / CABG					
Previous heart failure					
Previous stroke / TIA					
Previous PVD					
Previous admission with chest pain					
Previous admissions to CDU					
Previous admissions to cardiology					
Previous echocardiogram			Details if known:		
Previous angiogram			Details if known:		
<b>OTHER RELEVANT PMH</b>			<b>SOCIAL HISTORY</b>		
<b>RISK FACTORS</b>					
	Yes	No	Comment		
Family history (age<50)					
Smoking			If no ?ex-smoker:		
Hypertension					
High cholesterol					
Diabetes			Treatment type:		
Overweight					

**TRUST MEDICATION REQUISITION LIST**

**On this page**

Name: _____
DOB: _____ Unit Number: _____

**Physical examination:**

**GENERAL APPEARANCE:**

**VITAL SIGNS**

Heart rate			GCS Total	
Blood pressure	Right		Left	EMEWS Score
Resp rate				Blood sugar
SpO2 (%)				Weight (KG)
Temperature				

**CVS**

JVP		Oedema	<input type="checkbox"/> None	<input type="checkbox"/> Legs
HS			<input type="checkbox"/> Ankles	<input type="checkbox"/> Sacrum
Murmurs		Chest wall tenderness?		

**Respiratory System**      **Abdominal System**

Chest expansion:

PN:

Auscultation:




**Other physical findings:**

Name: _____
DOB: _____ Unit Number: _____

CLINICAL SUMMARY AND MANAGEMENT PLAN			
<p><b>S</b>ituation</p> <ul style="list-style-type: none"> <li>What are the likely diagnoses?</li> </ul>			
<p><b>B</b>ackground</p> <p>Provide pertinent background information</p>			
<p><b>A</b>ssessment</p> <p>What have you found...</p> <ul style="list-style-type: none"> <li>Vital Signs</li> <li>Results</li> </ul> <p>What do you think the problem is?</p>	General comments		
	Investigations		
		Done / Sign	Result / Sign / Time
ECG	Admission		
	4 hrs		
	8-12 hrs		
Troponin	Admission		
	90 mins (FAB3-CT)		
	12 hour		
D-dimer (if done)			
CXRAY			
Other			
<p><b>R</b>ecommendation</p> <p>What needs done... (see discharge algorithm p8)</p> <ul style="list-style-type: none"> <li>Review by medical staff</li> <li>Advice and guidance of what to do next and when</li> </ul>			

DISCHARGE/REFERRAL ALGORITHM

**Bayer Ultra TROPONIN POSITIVE (equal to or > 50 ng/l)**

Always refer to the in-patient team.  
Discuss the case with the Cardiology SpR prior to referral to the CCU SHO.  
Commence appropriate therapy

**TROPONIN NEGATIVE < 50 ng/l**

**Referrals to in-patient Cardiology team**

- Further **cardiac-type pain** while on **CDU** if suspicious of ACS
- Pain not settling after nitrates and oxygen in A&E/CDU (unless clear musculoskeletal pain has been diagnosed)
- ECG changes suggestive of ischaemia while on CDU
- Recurrent attenders on CDU, for advice

**Referrals to Rapid Access Chest Pain Clinic (RACPC)**

- Troponin I level <50 ng/l and
- Patients with **cardiac type pain** and possible **risk factors for IHD** (risk factors not essential)
- Start aspirin 75mg OD, GTN spray pm and atenolol 50mg OD **or** diltiazem 90mg BD if contraindications to beta blockade

**If uncertain discuss with the cardiology registrar**

All FAB3-CT Patients should be 1 - referred for Cardiac CT Calcium Score, & 2- referred to RACPC

**Referrals to GP**

- Troponin I level <50 ng/l
- Patients with **non-specific chest pain**.
- All patients should have a GP letter and discharge advice

All FAB3-CT Patients should be 1 - referred for Cardiac CT Calcium Score, & 2- referred to RACPC





**CLINICAL DECISION UNIT MEDICAL PATIENT DISCHARGE SUMMARY  
NON TRAUMATIC CHEST PAIN**

<b>HOSPITAL</b>	LGI	SJUH
Patient Name/Address	GP	
Date of birth	Date admitted	
Consultant	Date discharged	

Dear Dr \_\_\_\_\_  
Your patient attended the Clinical Decision Unit at the above hospital with chest pain.  
Your patient was admitted and investigated on the unit and discharged with the diagnosis and treatment indicated below.

Diagnosis	Circle	Further information
<b>Non-specific chest pain</b>	Yes	ECG
		Troponin
		FBC
		U&E
<b>Possible cardiac pain but MI ruled out</b>	Yes	Glucose
		Chest x-ray
		D-dimer
		LFTS

Discharge medication (see flow chart on page 8)	Dose	Directions	Length of supply	Reason – if not prescribed	Continue by GP? (Y/N)

Additional information	Circle	Date and time
Referred to Rapid Access Chest Pain Clinic. Referral letter faxed (Fax 25442)	Yes	
Referred for CT Calcium Score as part of FAB3-CT Study (Fax LILAC request to 25620)	Yes	
To attend GP or ED if any further problems	Yes	

Discharge signature and name	Date

Leeds Teaching Hospitals NHS Trust  
LGI

CDU Discharge Instructions

**Discharge instructions for patients admitted with  
chest pain**

You have been investigated on the Clinical Decision Unit for the cause of the pain in your chest.

We have specifically looked for signs of a problem with your heart.

\* We have NOT found any signs of a problem with your heart today. However we have made plans for you to return to the Rapid Access Chest Pain Clinic for further assessment on:

\_\_\_\_\_

(If you have taken part in the FAB3-CT Study you will be contacted with an appointment for your Cardiac CT Calcium Score in due course)

\* We have NOT found any signs of a problem with your heart today and have made a diagnosis of:

\_\_\_\_\_

Please continue to take any medications that you have been prescribed. A letter will be sent to your GP regarding your investigation and care on the CDU.

**You should return to the Emergency Department if you develop further chest pain or breathlessness.**

**You can telephone for advice if you are unsure:**

**CDU: 0113 3928381? Or 0113 3927101**

**ED: 01132432799**

**NHS Direct: 0845 4647**

**or contact your G.P.**

**The FAB3-CT Study  
Patient Information Sheet (PIS)**

**THE FAB-3CT STUDY – IDENTIFICATION OF SIGNIFICANT CORONARY ARTERY DISEASE BY THE MEASUREMENT OF H-FABP (HEART –TYPE FATTY ACID BINDING PROTEIN) FOR PATIENTS WITH NORMAL TROPONIN CONCENTRATIONS. THE FAB3-CT STUDY**

*'You are being invited to take part in a research study. Before you decide, it is important for you to understand why this study is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if anything is not clear or if you would like more information. Thank you for reading this.'*

**What is the purpose of the study?**

The term acute coronary syndrome or ACS includes patients with 'a *threatened or confirmed heart attack*'. Doctors currently use a blood test, called "*troponin*", to help them diagnose patients who are admitted to hospital with suspected heart attacks. This troponin blood test measures heart muscle damage and is usually taken at least 12 hours from the onset of pain, a time when it is most accurate. The results of this test not only help doctors to make the correct diagnosis but also to decide on the best course of treatment.

**What additional blood tests will be performed in the study?**

Our aim in this study is to assess the value of a new marker in the blood which also measures heart muscle damage. This new marker is called H-FABP (heart-fatty acid binding protein), H-FABP is released into the blood much earlier than troponin, so it can be measured sooner than troponin. Our research has already shown that H-FABP is useful in assessing the risk in patients who have had actual or threatened heart attacks. We now wish to find out whether measuring H-FABP in patients *suspected of having a heart attack* will provide useful information to the doctors regarding the presence of underlying heart artery disease, so that patients can be treated earlier and more effectively in the future. In conjunction with H-FABP, we will measure two other markers called hs-CRP (Highly Sensitive C-Reactive Protein) and BNP (Brain-type Natriuretic Peptide) which have also been shown to be useful in assessing the best treatment for patients with suspected heart attacks. In addition we are interested to test the value of two promising markers (metalloproteinase-2 and copeptin) which have not yet been fully established as having clinical value. **As this is a research study, the results of these blood tests will not affect your current care and they will not be made available to the doctors who are caring for you.**

**What additional X-ray tests will be performed in the study?**

To assess the presence or absence of coronary artery disease we will be performing a CT heart scan to look for evidence of the mineral calcium (present in bones and teeth) – within the heart arteries. The test is quick and convenient (takes only minutes to perform), and is non-invasive and painless. The scan uses a low amount of X-Rays and has been shown to be an effective method for early detection of disease. As with the performance of a chest X-ray the dose administered is kept to a minimum but may be greater for larger patients.

It is equivalent to the amount of radiation exposure - and hence also additional lifetime risk of cancer (1 in 6600) - that a person might be exposed to during 4 routine flights across the Atlantic Ocean or the amount of background radiation we are all exposed to in one year. Patients who are pregnant will be excluded from this study. A high calcium score may sometimes be followed by other diagnostic tests for heart disease, which may not otherwise be performed, and which might cause side-effects. To the contrary, especially in patients less than 50yrs, coronary artery disease can be present without calcium – and so may not be detected by this particular additional test. **Because of its established value, the results of this heart scan will be made available to the doctors who are caring for you and so may influence your future care. This will be in addition to the otherwise standard tests currently in use.**

FAB3-CT Patient information Sheet Version 1.4 (22/12/2010)

#### What genetic tests will be performed in the study?

Heart artery disease is known to run in families and to have some underlying genetic causes. In particular 30 gene variations, that are very common, have been shown to have weak effects on their own – but increasing effects when present together. Most people will have between 10 and 16 of these and an average risk while a minority will have either less or more than this. We wish to investigate whether this sort of test can help doctors when assessing patients such as you. **As this is a research study, the results of these DNA blood tests will not affect your current care, nor your insurance status and they will not be made routinely available to you or the doctors who are caring for you.**

#### What samples for genetic study will be taken?

We are interested to obtain an additional small (approximately 5mls. / 1 teaspoon) sample of blood and also saliva (spit) in order to isolate and store (for up to 15 years) a DNA (gene) and RNA (gene copy) sample. Should you ever wish to request it, you will be able to have your samples withdrawn from the study and destroyed at any time prior to the intended 15 year time point. There have been major advances in identifying the genes that associate with heart artery disease and so we would like to see if testing for these would allow more accurate prediction of best care. Such a study will be confined only to genes relevant to the occurrence of heart disease and its underlying causes (e.g. blood fats / cholesterol).

#### Why have I been chosen?

You have been identified as a possible study participant by the doctors who saw you earlier in the Emergency Department. The majority of patients identified for this study will have been admitted to hospital with a possible heart attack. Others may be identified because one of the doctors who saw you may have wanted to perform a test to exclude a heart attack, along with other tests. The total number of participants needed for this study is 250.

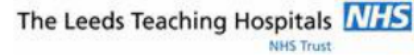
#### Do I have to take part?

It is your choice whether or not to take part. If you do decide to take part, and later change your mind, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or not to take part, will not affect the standard of care you receive.

#### What does taking part involve?

- A research nurse (or doctor) will contact you while you are in hospital, show you this information sheet and discuss it with you. If you agree to help with the study by taking part, you will NOT be asked to take any extra drugs or other treatments.
- **BLOOD SAMPLE-1** We would like to perform some additional tests on the blood sample taken routinely at hospital admission. *(This sample is one that the doctors or nurses asked if they might take for research purposes when they were taking other blood samples on your arrival at hospital. The sample will have been temporarily stored and will only be used if you now give us your full written consent after having read this information sheet and spoken to a doctor or nurse about this study. Please note that if you are unwilling to participate in this study, the blood sample collected already will be destroyed.)*
- **BLOOD SAMPLE-2** We would like to sample and test your blood at one additional time 90 minutes after arrival at hospital. This is the only "EXTRA" blood sampling that we will perform.
- **BLOOD-SAMPLE-3** We would like to perform some additional tests on the blood sample taken routinely 12 to 24 hours after start of any symptoms. *(A 9ml (2 teaspoons) blood sample collected from you after you have agreed to take part and signed the consent form. The third set of samples will be collected with your permission at the same time as taking blood*
- **GENE SAMPLES** will be taken at the earliest time convenient to you and in parallel with the other tests.
- **SAMPLE-STORAGE** With your permission we will freeze, store and use these samples for our research for up to 15 years after which they will be destroyed.

FAB3-CT Patient information Sheet Version 1.4 (22/12/2010)



- **SIX MONTHS** after your stay in hospital, the research nurse (or doctor) will contact you again, by letter (or by telephone, if no response to letter), to ask about your current health, any subsequent hospital admissions or special tests and your current medications. This will also give you the opportunity to ask any questions about your condition. If you wish, an additional hospital clinic visit may also be arranged.
- If you agree to take part, you will be asked to sign a consent form; you will be given a copy of the signed consent form and this information sheet to keep.

#### Out Patient Visits

- **CT Scan** – The heart scan which we wish to perform may take place during your initial short hospital stay, but more usually will take place shortly afterwards. This will involve an extra visit to the hospital for less than half a day.
- **Cardiology Clinic** – It is usual for patients such as yourself to be seen (after discharge from the Emergency Department) in one of the heart specialist (cardiology) outpatient clinic. At this time an exercise test is usually performed and advice given by the specialist. This will involve an expected visit to the hospital for less than half a day.

#### How else will I be followed up?

With your permission some of the information held by the NHS and records maintained by The NHS Information Centre and the NHS Central Register may be used to help contact you and provide information about your health status. Other sources of information for following up your health status would be from local patient administration systems and the Yorkshire Heart Centre registers. We will also obtain similar information by contacting your GP. All the information gathered will allow us to evaluate your long-term health and well-being. Specifically, we will be seeking to document any future visits to hospital, the use of heart drugs plus other treatments, the need for medical tests and also the occurrence of other important medical events such as a future heart attack – over the next 12 months. We will also be able to monitor your survival long-term (up to 15 years).

#### What are the possible benefits of taking part?

We are continuously trying to improve the quality of care we give to our patients. The information we get from this study may help us to treat future patients with suspected heart attacks better. By participating in this study, you will help us in this endeavour. The additional information gained from the CT heart scan would also be expected to be helpful to the doctors involved in deciding how to give you the best possible future care.

#### Will my taking part in the study be kept confidential?

Yes. All the information, which is collected, about you during the course of the research will be kept strictly confidential. Personal information collected and used by the NHS is controlled by the Data Protection Act 1998. At any time we will delete your data at your request, but otherwise we would wish to keep your data until the end of the study or for 15 years. To allow this to take place we will continue to be able to identify your data by your name and NHS number. With your consent we will inform your GP of your participation in the study and to inform them of all clinical information relevant to your care during the follow up period.

#### What will happen to the results of the research?

The staff who have been directly involved in your care will receive regular feedback. This will enable them to make changes to and improve the quality of care received by patients with suspected heart attacks. The final results are expected after the completion of the study and will be disseminated among the medical community via presentations and publications, locally, nationally and internationally. In addition the research will be the subject of a higher educational degree (thesis) for a senior Cardiology Specialist Trainee doctor working on the project. You will not be identified in any report / publication.

FAB3-CT Patient information Sheet Version 1.4 (22/12/2010)

**Who is organising and funding the research?**

This work is being organised by the Leeds General Infirmary together with Leeds University Medical School. The study is being funded by the charity Heart Research UK. Your doctor will not be paid for including you in this study.

**What if there is a problem?**

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally you can do this by contacting, The Patient Relations Manager, Trust Headquarters, St James's University Hospital, Beckett Street, Leeds, LS9 7TF, Tel. 0113 206 6261

**Harm**

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence, then you may have grounds for a legal action for compensation against Leeds Teaching Hospitals Trust, but you may have to pay for your legal costs.

**Who has reviewed this study?**

All research in the NHS is looked at by an independent group of people called a Research Ethics Committee, to protect your interests. **This study has been reviewed and approved by the East Yorkshire & North Lincolnshire Research Ethics Committee.**

**Investigators: Professor Alistair S Hall (Cardiology), Dr Julian Barth (Clinical Biochemistry), Dr Taj Hassan (Emergency Department), Leeds General Infirmary. Tel (0113) 22735 / Fax (0113) 39 28613**

FAB3-CT Patient information Sheet Version 1.4 (22/12/2010)

**Note : for clinicians recruiting patients into the FAB3-CT study:**

- Please ensure that all entries on pages 1, 4, 5, and 12 of the CDU protocol are completed fully.
- Please note that patients presenting to ED more than 24 hours from pain onset / worst pain should not be recruited. Patients presenting more than 12 hours from pain onset / worst pain are also not ideal candidates.
- Please ensure that patients agreeing to participate in the study are given a copy of the patient information sheet and a copy of the signed consent form to keep.
- The following documents are required by the research team for each patient enrolled into the study:
  - 1) A copy of the signed consent form
  - 2) Photocopy of pages 1, 4, 5, 6, 7, and 12 of the completed CDU protocol
  - 3) Photocopies of initial ECG and any subsequent ECGs (if changed from the initial one)

These may be stapled together and left on the designated rack for the FAB3-CT study on CDU.

For any queries, please do not hesitate to contact us (contact details available on CDU)

Many thanks,

The FAB3-CT study research team



'A collaborative project between the Departments of Emergency Medicine & Cardiology'  
Dr Saif-El-Dean Abdel-Rahman, Prof Alistair Hall, Dr Taj Hasan, Dr Andy Webster, Christine Morrell, Nicola Stockbum, Nichola Myers, Helen Ockleton, Dr Julian Barth, Dr Anthony Balmforth, Prof L Bun Tan, Dr Christopher Gale, Dr Wazir Baig, Prof Mohan Sivanathan, Dr M Darby





**Appendix C**  
**Rapid Access Chest Pain Clinic Documentation Pack**  
**(includes FAB3-CTx Patient Information Sheet & Consent Form)**

RAPID ACCESS CHEST PAIN CLINIC		FAB3-CT
Name: DOB: Unit Number:	(or ID sticker)	Date attended CDU: Date today:
<b>History:</b>		
<b>Risk Factors:</b> FHx <input type="checkbox"/> HTN <input type="checkbox"/> Cholesterol <input type="checkbox"/> Smoker <input type="checkbox"/> Obesity (BMI > 30) <input type="checkbox"/> DM <input type="checkbox"/>		
<b>Past Medical History:</b>	<b>Allergies:</b> NKDA <input type="checkbox"/> <b>Medications:</b>	
<b>Examination:</b>	BP:                      HR:	<b>ECG:</b>
		<b>CT Calcium Score:</b>

## STEP 1

### Impression:

- 1- Typical angina
- 2- Atypical angina
- 3- Non-anginal chest pain

'Anginal pain is:

- constricting discomfort in the front of the chest, or in the neck, shoulders, jaw, or arms
- precipitated by physical exertion
- relieved by rest or GTN within about 5 minutes

Three features = typical angina. Two features = atypical angina. One or none = non-anginal chest pain'.

## STEP 2

### Probability of CAD according to NICE risk table

- 1- 10 – 29%
- 2- 30 – 60 %
- 3- 61 – 90 %
- 4- > 90 %

5 – Established prior diagnosis of CAD

How was the diagnosis made \_\_\_\_\_

(Refer to NICE management pathway for 'Established prior diagnosis of CAD' – Page 5)

Percentage of people estimated to have coronary artery disease according to typicality of symptoms, age, sex and risk factors

	Non-anginal chest pain				Atypical angina				Typical angina			
	Men		Women		Men		Women		Men		Women	
	Lo	Hi	Lo	Hi	Lo	Hi	Lo	Hi	Lo	Hi	Lo	Hi
Age (years)												
35	3	35	1	19	8	59	2	39	30	88	10	78
45	9	47	2	22	21	70	5	43	51	92	20	79
55	23	59	4	25	45	79	10	47	80	95	38	82
65	49	69	9	29	71	86	20	51	93	97	56	84

For men older than 70 with atypical or typical symptoms, assume an estimate > 90%.

For women older than 70, assume an estimate of 61–90% EXCEPT women at high risk AND with typical symptoms where a risk of > 90% should be assumed.

Values are per cent of people at each mid-decade age with significant coronary artery disease (CAD).

Hi = High risk = diabetes, smoking and hyperlipidaemia (total cholesterol > 6.47 mmol/litre).

Lo = Low risk = none of these three.

The shaded area represents people with symptoms of non-anginal chest pain, who would not be investigated for stable angina routinely.

Note:

These results are likely to overestimate CAD in primary care populations.

If there are resting ECG ST-T changes or Q waves, the likelihood of CAD is higher in each cell of the table.

Name: \_\_\_\_\_ (or ID sticker)  
DOB: \_\_\_\_\_  
Unit Number: \_\_\_\_\_

### STEP 3 (refer to NICE flow pathways overleaf)

#### Plan

- 1- Treat without further investigation
- 2- CT Coronary angiogram
- 3- Stress echocardiogram
- 4- Myocardial perfusion scan
- 5- Stress / perfusion MRI
- 6- Invasive angiogram

#### Other (CXR, bloods)

\_\_\_\_\_

#### Medications started

Aspirin \_\_\_\_\_  
Statin \_\_\_\_\_  
Antianginal \_\_\_\_\_  
GTN spray \_\_\_\_\_  
Other \_\_\_\_\_

#### Advice given

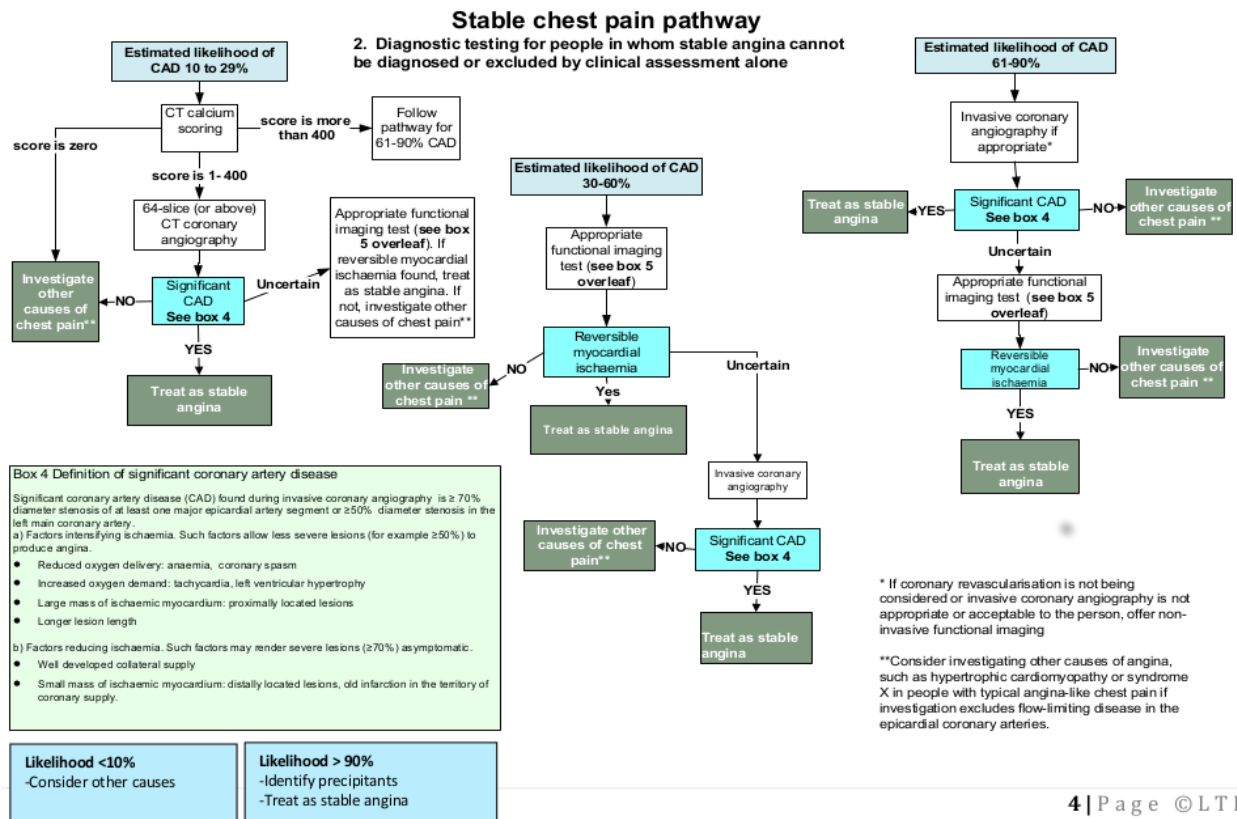
- When to seek help
- How to use GTN
- Smoking cessation
- Diet
- Exercise
- Driving

#### Other

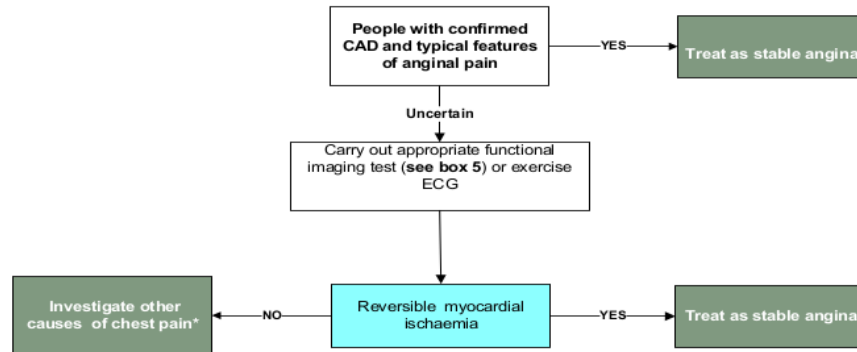
\_\_\_\_\_

STEP 3

Appendix - NICE CG 95 - 'Chest Pain of Recent Onset'



**Stable chest pain pathway**  
3. Established prior diagnosis of coronary artery disease



**Box 5**

When offering non-invasive functional imaging for myocardial ischaemia use:

- myocardial perfusion scintigraphy with single photon emission computed tomography (MPS with SPECT) or
- stress echocardiography or
- first-pass contrast-enhanced magnetic resonance (MR) perfusion or
- MR imaging for stress-induced wall motion abnormalities.

Take account of locally available technology and expertise, the person and their preferences, and any contraindications, when deciding on the imaging method.

Note: This recommendation updates and replaces recommendation 1.1 of NICE technology appraisal guidance 73.

\* Consider investigating other causes of angina, such as hypertrophic cardiomyopathy or syndrome X in people with typical angina-like chest pain if investigation excludes flow-limiting disease in the epicardial coronary arteries.

RAPID ACCESS CHEST PAIN CLINIC		FAB3-CT
Name: DOB: Unit Number:	(or ID sticker)	<b>Follow-up</b> Date today:
<b>Results</b>		
<u>CT Coronary angiogram</u>	Positive <input type="checkbox"/>	Negative <input type="checkbox"/> Equivocal <input type="checkbox"/> _____
<u>Stress echocardiogram</u>	Positive <input type="checkbox"/>	Negative <input type="checkbox"/> Equivocal <input type="checkbox"/> _____
<u>Myocardial perfusion scan</u>	Positive <input type="checkbox"/>	Negative <input type="checkbox"/> Equivocal <input type="checkbox"/> _____
<u>Stress / perfusion MRI</u>	Positive <input type="checkbox"/>	Negative <input type="checkbox"/> Equivocal <input type="checkbox"/> _____
<u>Invasive angiogram</u>	Positive <input type="checkbox"/>	Negative <input type="checkbox"/> Equivocal <input type="checkbox"/> _____
<u>Other:</u>	Any stenoses > 50 % Yes <input type="checkbox"/> No <input type="checkbox"/>	
<b>Clinical Progress</b>		
<b>Diagnosis</b>		
<b>Plan</b>		

RAPID ACCESS CHEST PAIN CLINIC

FAB3-CT

Name: (or ID sticker)  
DOB:  
Unit Number:

**Follow-up**  
Date today:

**Note NICE CG 126 'Management of stable angina'**

- Offer a short-acting nitrate
- Offer optimal drug treatment (one or two anti-anginal drugs as necessary plus drugs for secondary prevention)
- Offer either a beta blocker or calcium channel blocker as first-line treatment, based on comorbidities, contraindications and the person's preference
- Consider revascularisation if symptoms not satisfactorily controlled with optimal drug treatment

**RAPID ACCESS CHEST PAIN CLINIC**

**FAB3-CT**

Name: \_\_\_\_\_  
DOB: \_\_\_\_\_  
Unit Number: \_\_\_\_\_

(or ID sticker)

**Follow-up**

Date today: \_\_\_\_\_

--	--



**Patient Information Sheet (PIS)**  
**THE FAB-3CT EXTENSION STUDY (FAB3-CTx)**

*'You are being invited to take part in an extension to the FAB-3CT research study. Before you decide, it is important for you to understand why this study extension is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if anything is not clear or if you would like more information. Thank you for reading this.'*

**What is the purpose of this extension to the FAB-3CT study?**

The aim of the main FAB-3CT study that you have kindly helped with, is to assess the value of a new marker in the blood which also measures heart muscle damage. This new marker is called H-FABP (heart-fatty acid binding protein), H-FABP is released into the blood much earlier than troponin, so it can be measured sooner than troponin. In the future other blood tests that may also perform this task (in a similar, worse or better way) are likely to emerge. **The FAB-3CTx study will assess these additional future markers – using samples and data that have already been collected as part of the FAB-3CT study.**

**What additional blood tests will be performed in the study?**

No additional blood or other samples will be collected. The tests that will be performed will be of markers (proteins or genes) that have not been identified at this time. The ability to study the value of additional tests on samples and data already collected provides a useful opportunity to extend medical knowledge without the need to recruit new patients. Should you ever wish to request it, you will be able to have your samples withdrawn from the study and destroyed at any time prior to the intended 15 year time point. **As this will continue to be a research study, the results of any gene or blood tests will not influence you current or future care and they will not be made available to the doctors who are caring for you.**

**What genetic tests will be performed in the study?**

Heart artery disease is known to run in families and to have some underlying genetic causes. Currently, 30 gene variations, that are very common, have been shown to have weak effects on their own – but increasing effects when present together. Most people will have between 10 and 16 of these and an average risk while a minority will have either less or more than this. In the future we wish to investigate whether any gene abnormalities that have not yet been discovered might help doctors when assessing patients such as you. **As this will continue to be a research study, the results of these DNA blood tests will not affect your current or future care, nor your insurance status and they will not be made routinely available to you or the doctors who are caring for you.**

**What samples will undergo future genetic study?**

With your consent we previously obtained and have stored a sample of DNA (gene) and RNA (gene copy). There will continue to be major advances in identifying the genes that associate with heart artery disease and so in the future we would like to see if testing for these would allow more accurate prediction of best care. Such a study would be confined only to genes relevant to the occurrence of heart disease and its underlying causes (e.g. blood fats / cholesterol).

**Why have I been chosen?**

You have previously been part of the FAB-3CT study and so are also eligible to provide consent for this study extension.

**Do I have to take part?**

It is your choice whether or not to take part. If you do decide to take part, and later change your mind, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or not to take part, will not affect the standard of care you receive.

**What does taking part involve?**

If you agree for the samples and data already collected to be reused in future studies, you will be asked to sign a consent form; you will be given a copy of the signed consent form and this information sheet to keep.

**What are the possible benefits of taking part?**

We are continuously trying to improve the quality of care we give to our patients. The information we get from this study extension may help us to treat future patients with suspected heart attacks better. By participating you will help us in this endeavour.

**Will my taking part in the study extension be kept confidential?**

Yes. All the information, which is collected, about you during the course of the research will be kept strictly confidential. Personal information collected and used by the NHS is controlled by the Data Protection Act 1998. At any time we will delete your data at your request, but otherwise we would wish to keep your data until the end of the study or for 15 years. To allow this to take place we will continue to be able to identify your data by your name and NHS number.

**What will happen to the results of the research?**

Any future results obtained from this study extension will be disseminated among the medical community via presentations and publications, locally, nationally and internationally. You will not be identified in any report / publication.

**Who is organising and funding the research?**

This work is being organised by the Leeds General Infirmary together with Leeds University Medical School. The main FAB-3CT study is being funded by the charity Heart Research UK. Your doctor will not be paid for including you in this study extension.

**What if there is a problem?**

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally you can do this by contacting, The Patient Relations Manager, Trust Headquarters, St James's University Hospital, Beckett Street, Leeds, LS9 7TF, Tel. 0113 206 6261

**Harm**

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence, then you may have grounds for a legal action for compensation against Leeds Teaching Hospitals Trust, but you may have to pay for your legal costs.

**Who has reviewed this study?**

All research in the NHS is looked at by an independent group of people called a Research Ethics Committee, to protect your interests. **This study has been reviewed and approved by the East Yorkshire & North Lincolnshire Research Ethics Committee.**

**Investigators: Professor Alistair S Hall (Cardiology), Dr Julian Barth (Clinical Biochemistry), Dr Taj Hassan (Emergency Department), Leeds General Infirmary. Tel (0113) 39 22735 / Fax (0113) 39 28613**

FAB3-CTx Patient information Sheet Version 1.0 (04/10/2010)



# Appendix D Academic Integrity Form

[www.leeds.ac.uk/rcs/assets/word/Forms/ac\\_integrity\\_transfer.doc](http://www.leeds.ac.uk/rcs/assets/word/Forms/ac_integrity_transfer.doc)

## Research Degree Transfer Assessment: Academic Integrity, Safeguarding Data and Ethical Requirements

To be completed by the candidate and submitted with the transfer report

Candidate Name:	Dr Saif-El-Dean Abdel-Rahman	Student ID Number	2006698393
Title of Report	'Identification of significant coronary artery disease by the measurement of H-FABP (Heart-type Fatty Acid Binding Protein) for patients with normal troponin concentrations'		

### Ethical Considerations of the Project

Before completing this section of the form, please read the guidance notes published at [http://researchsupport.leeds.ac.uk/index.php/academic\\_staff/good\\_practice/university\\_ethics\\_policies/](http://researchsupport.leeds.ac.uk/index.php/academic_staff/good_practice/university_ethics_policies/)

(i) Is ethical review required?	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/>	If Yes please go to section (ii) or If No please go to section (v)
(ii) Has ethical approval been granted?	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/>	If Yes please go to sections (iv) or If No please go to section (iii)

(iii) If you have answered No to question (ii) please provide additional information here:

(iv) If you have answered Yes to question (ii) please state from which body approval was sought (eg Research Ethics Committee (for research with animals), University Faculty Research Ethics Committee for research that should be reviewed), NHS or other lead institution AND give reference number for approval (if appropriate)

East Yorkshire & North Lincolnshire Research Ethics Committee (REC Ref. 10/H1304/36)

(v) I confirm that I am aware of and comply with the University's procedures for the review of ethical issues arising from research involving animals; human participants, their data or their tissue; or the potential for significant environmental impact

[http://researchsupport.leeds.ac.uk/index.php/academic\\_staff/good\\_practice/university\\_ethics\\_policies/](http://researchsupport.leeds.ac.uk/index.php/academic_staff/good_practice/university_ethics_policies/)

Signature of Candidate		Date:	31/01/13
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### Statement of Academic Integrity & Safeguarding the University's Data

I confirm that the attached transfer report is my own work and I have not presented anyone else's work as my own and that full and appropriate acknowledgement has been given where reference has been made to the work of others

I have read and understood the University's published rules on plagiarism and also any rules specified at School/Faculty level. I understand that if I commit plagiarism I can be expelled from the University and that it is my responsibility to be aware of the University's regulations on plagiarism and their importance.

[http://www.leeds.ac.uk/rcs/assets/word/policies/guidance\\_plagiarism\\_proced.res.doc](http://www.leeds.ac.uk/rcs/assets/word/policies/guidance_plagiarism_proced.res.doc)

I consent to the University making available to third parties (who may be based outside the EEA) any of my work in any form for standards and monitoring purposes including verifying the absence of plagiarised material. I agree that third parties may retain copies of my work for these purposes on the understanding that the third party will not disclose my identity

I confirm that I am aware of and comply with the University's policy for "Safeguarding Data - Storage, Backup and Encryption" <http://campus.leeds.ac.uk/isms/policies/safeguarding/>

Signature of Candidate		Date:	31/01/13
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