THE ROLE OF HEART FATTY ACID BINDING PROTEIN IN THE EARLY DETECTION OF MYOCARDIAL INJURY IN PATIENTS WITH ACUTE CORONARY SYNDROMES

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by

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DECLARATION

This thesis describes research undertaken at the University of Edinburgh Cardiovascular Research Unit and the Department of Cardiology at the Royal Infirmary of Edinburgh, during the period from October 1998 to August 2001. I undertook this research in the capacity of junior research fellow. I have had invaluable help from colleagues and collaborators whom I have formally acknowledged, otherwise the work of this thesis has been my own, and the writing of the substances of the text has been entirely my own undertaking. None of the work discussed in this thesis has been published in academic journals.

Hafidh A. A. Alhadi

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ABBREVIATIONS

A&E	Accident and emergency department
ACC	American College of Cardiology
ACS	Acute coronary syndromes
AHA	American Heart Association
AMI	Acute myocardial infarction
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AUC	Area under curve
CAD	Coronary artery disease
CCU	Coronary Care Unit
CHD	Coronary heart disease
CI	Confidence interval
CK	Creatine kinase
CK-MB 1	Plasma isoform of creatine kinase muscle brain
CK-MB 2	Tissue isoform of creatine kinase muscle brain
CK-MB	Creatine kinase muscle brain
CPN	Carboxypeptidase N
CTnI	Cardiac troponin I
CTnT	Cardiac troponin T
ECG	Electrocardiogram
ESC	European Society of Cardiology
ELISA	Enzyme linked immunosorbent assay
FABP	Fatty acid binding protein
GPIIb/IIIa	Glycoprotein IIb/IIIa receptor antagonist
H-FABP	Heart fatty acid binding protein
I-FABP	Intestine fatty acid binding protein
ICU	Intensive Care Unit
IU/L	International units of activity per litre
IVUS	Intravascular ultrasound
Kda	kilodalton
LDH	Lactate dehydrogenase
LDH 1	Lactate dehydrogenase isoenzyme 1
L-FABP	Liver fatty acid binding protein
LMWH	Low molecular weight heparin
Macro CK	Macro creatine kinase (type 1 and 2)
MI	Myocardial infarction
Mit CK	Mitochondrial creatine kinase
Ng/ml	Nanogram of mass per millilitre
NPV	Negative predictive value
NSTEMI	Non- ST elevation myocardial infarction
P	Probability value
PPV	Positive predictive value
PCI	Percutaneous coronary intervention
ROC	Receiver operator characteristics curve

SBO	Side branch occlusion
SD	Standard deviation
SK	Streptokinase
STEMI	ST elevation myocardial infarction
TPA	Tissue plasminogen activator
UA	Unstable angina
Ug/l	Microgram of mass per litre

ABSTRACT

Chest pain is a non-specific complaint and is the most frequent reason for patients to seek urgent medical attention. A small group of these patients will have acute coronary syndromes (ACS). Acute coronary syndromes carry high morbidity and mortality and require rapid early identification and treatment. The current diagnostic and triage systems based on the clinical history and ECG lack both sensitivity and specificity. It may result in some of these patients being misdiagnosed and therefore admitted to the wrong units or receive inappropriate care, treatment, and investigations. In some patients the diagnosis is delayed resulting in the late administration (or no administration) of essential early treatment. A few patients with ACS may be inadvertently discharged from the accident and emergency department [A&E department] with serious health and legal consequences. These systems also result in an inappropriate admission of a large number of patients without ACS, with substantial costs. The diagnosis, triage, and management of patients with ACS can be considerably improved by implementations of serial cardiac markers testing that can identify ACS in the very early stages of presentation. The work of this thesis involves a series of studies that evaluate the role of the novel cardiac marker heart fatty acid-binding protein (H-FABP) for the early diagnosis of ACS and its comparison with standard cardiac markers creatine kinase muscle brain mass (CK-MB mass), cardiac troponin I (cTnI), cardiac troponin T (cTnT) and myoglobin.

1. The value of serial cardiac markers testing for the early diagnosis of acute myocardial infarction (AMI) was studied in 45 patients with AMI admitted to Coronary Care Unit within 6 hours after symptom onset. Heart-FABP peak concentration occurred at 8 hours and was the most sensitive early marker with 75.5% and 100% of patients with AMI identified at presentation and 2 hours after presentation respectively. The sensitivity of cardiac markers [CK-MB mass, cTnI, cTnT, and myoglobin] at presentation was < 62%. The sensitivity and negative predictive value of H-FABP was also superior to other markers within the first 2 hours of presentation. Myoglobin was the second best sensitive early marker at presentation. Peak sensitivity of cTnI, CK-MB mass, and cTnT were present at 4, 8, and 8 hours respectively. The combination of H-FABP and cTnI improves specificity. Measurement of H-FABP and cTnI at two intervals during the first 8 hours after presentation is sufficiently sensitive and specific for the early diagnosis of most patients with AMI. This serial combination testing may also be useful in; timing the onset of AMI; clarification of non-diagnostic ECG; detection of successful reperfusion and detection of re-infarction and infarct extension if it occurs early.

2. The value of serial cardiac markers testing for the diagnosis and management of patients with non-Q wave myocardial infarction [non-Q wave MI] and unstable angina [UA]) was assessed in 55 consecutive patients seen in the A&E department with acute chest pain within 7 hours after symptom onset. Heart-FABP was the most sensitive early marker with 79% of patients with non-Q wave MI showing evidence of myocardial damage at presentation [Less than 71% were detected using other markers]. Myoglobin and H-FABP had equal sensitivity (93%) at 2 hours for the

diagnosis of non-Q wave MI. Creatine kinase-MB mass and cTnI were sensitive in 93% within 4 hours of presentation. Cardiac-TnT was a late marker (> 16 hours). These serial testing also diagnosed a substantial numbers of patients with subtle ischaemic ECG changes at admission [e.g. minimal ST elevation < 1 mm, and left bundle branch block] that may qualify for early reperfusion therapy. Heart-FABP also showed high positive values in patients with UA [40%], and was low in normal coronary patients with chest pain [10%]. Cardiac-TnI, cTnT, CK-MB mass, and myoglobin were elevated in 55%, 40%, 30%, and 25% respectively of patients with UA. Implementations of serial testing policy may result in reduced costs of in-hospital care by approximately 34%. In addition, early serial testing identifies high-risk patients and can help with their management such as; prevent discharge; triage from A&E; admission to Coronary Care Unit; antiplatelet therapy; invasive investigations; and identify low-risk patients for early discharge.

3. The release characteristics, the diagnostic and prognostic value of cardiac markers were assessed in 80 consecutive patients who had elective angioplasty. Elevated concentrations of cardiac markers were correlated with demographic, angiographic and procedural variable. Patients were followed up for 20 - 26 months. Heart-FABP peaked early at 2 hours after angioplasty, and was the most useful test for the early detection of evolving AMI within 1-3 hours after angioplasty. Cardiac-TnI, myoglobin, H-FABP, CK-MB mass, and cTnT concentrations were elevated in 46.25%, 17.5%, 13.3%, 11.25%, and 7.5% respectively. Cardiac-TnI was the most sensitive marker for detecting all complications and was superior to all other markers. Elevated cTnI had positive predictive accuracy for major complications of 81%, and for all types of complications of 62%. Elevated cardiac markers were correlated with old age [$p < 0.02$], occurrence of chest pain ± ECG changes of ischaemia [$p < 0.003$], increased use of stents [$p < 0.019$] and major complications (major dissection [$p < 0.004$], transient vessel closure [$p < 0.022$] and bail out stent [$p < 0.003$], and AMI [$p < 0.042$]). Elevated cardiac markers were associated with a reduction of event-free survival [16.92 vs 20.67 months, $p < 0.03$]. However, the complications rate between patients with and without elevated cTnI was not statistically different (angina [46% vs 28%, p = NS], non-target vessel revascularisation [14% vs 9%, p = NS] and increased clinical events [73% vs 48%, p = NS]) respectively.

Conclusions: Heart-FABP was the most sensitive early marker for the detection of myocardial injury in patients with ACS. Measurement of H-FABP and cTnI at two intervals during the first 8 hours after presentation is sufficiently sensitive and specific for the early diagnosis of most patients with ACS. This serial combination testing may be cost effective, provides additional valuable diagnostic and prognostic informations and would potentially improve the sensitivity and specificity of the current triage systems.

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 SCALE OF THE PROBLEM

In the United Kingdom, chest pain is the most frequent reason for patients receiving emergency medical attention, and coronary heart disease (CHD) is the commonest cause of death. Although official statistics are lacking, from the data that are available it can be estimated that approximately 2.1 million people suffer from angina, 300,000 have myocardial infarction, and approximately 125,000 die from a heart attack each year.[1]

In general, the ratio of unstable angina (UA) and acute myocardial infarction (AMI) is 1.2:1 across Europe.[2] The economic burden of caring for patients with CHD is approximately £1.6 billion per year.[1] In the United States, each year 5 million patients with chest pain are admitted to hospital from accident and emergency (A&E) departments.[3] Patients presenting with chest pain form 5% of the total workload of the A&E department. It is estimated that 90% of patients presenting with chest pain do not have AMI and internationally less than 30% of patients admitted to Coronary Care Unit (CCU) or Intensive Care Unit (ICU) with suspected AMI are subsequently found to have AMI (Figure 1).[3;4-7;8]

The cost of caring for such patients in whom AMI has been ruled out is approximately $ 4 billion per year.[3;9] It is also estimated that up to 3 million patients present with silent ischaemia and between 20,000 - 40,000 patients are inappropriately discharged from A&E department with 'missed AMI' (Figure 2).[3;10] Missed AMI is the leading cause of malpractice lawsuits and settlements in A&E departments in the United States.[11;12]

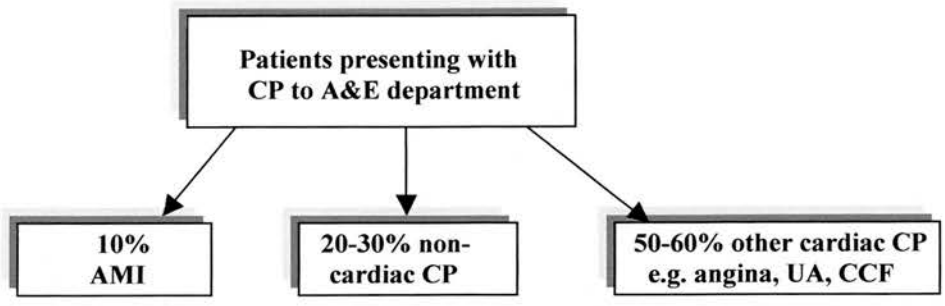

Figure 1. The schematic diagram below shows the relative percentages of patients who present to the A&E department with AMI, non-cardiac chest pain and other cardiac chest pain. Abbreviations: CP, chest pain; A&E, accident and emergency department; AMI, acute myocardial infarction; UA, unstable angina; CCF, congestive cardiac failure. The diagram is based on informations from references 4 - 7.

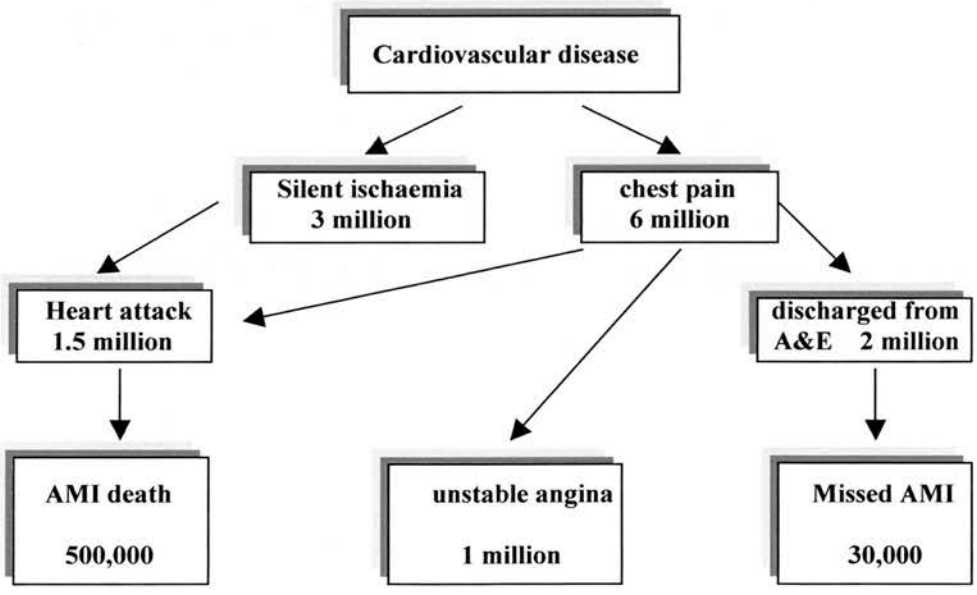

Figure 2. Shows schematic diagram of the proportions of patients with cardiovascular disease who present with chest pain and silent myocardial ischaemia, and their outcome.[10]

1.2 RECENT TRENDS OF CHEST PAIN PRESENTATION

A series of interacting developments have given rise to a marked increase in the frequency of patients presenting to A&E departments with chest pain. These include; (1) Revolutionary success with reperfusion treatment (thrombolytic treatment and PCI for AMI;[13] (2) The improved community and ambulance services; (3) Awareness of the need for early presentation of patients with AMI to hospital to achieve the maximum benefits of treatment;[14;15] and (4) The potential advantage of monitoring patients with AMI in the CCU or ICU.[16]

These factors have lead to educational programmes for the public to raise their awareness of symptoms of AMI and encourage early presentation to hospital. As a result, increasing numbers of patients present to A&E departments, and the time of presentation after symptom onset is substantially shortened. It is estimated that patients present within a mean duration of 6 hours compared to 10 - 12 hours previously.[8;16] This has increased the pressure on the A&E department physicians to make rapid but accurate diagnoses of acute coronary syndromes (ACS) and avoid inappropriate admissions and discharges.

1.3 THE DILEMMA OF ACUTE CORONARY SYNDROMES DIAGNOSIS

The accurate diagnosis of AMI and UA in the early stages of myocardial damage still represents a major challenge in clinical cardiology. Accurate early diagnosis of such patients is essential to determine whether a patient needs hospitalisation and if so, whether ischaemia and evolving infarction are present. Diagnosis of AMI relies on clinical history, the ECG, and measurement of cardiac marker proteins. The clinical

history may not be reliable as the differential diagnosis of chest pain includes many cardiac, pulmonary, gastrointestinal, and muskeloskeletal conditions that have to be differentiated from cardiac pain (Table 1).[17;18] There is insufficient time for an exhaustive history in the emergency setting. The ECG is helpful for the diagnosis of ACS when there are positive changes of ischaemia, but the early ECG can be normal or non-diagnostic in up to 50% of patients with AMI.[7;19-21] Also old ECG changes may persist in patients with new presentation of cardiac pain obscuring further interpretation. When confronted with a patient with chest pain at rest and accompanying ECG abnormalities of ischaemia, the treating physician cannot sometimes distinguish between unstable angina, non-Q wave MI and ST elevation MI in evolution until the full ECG pattern evolves or the cardiac enzymes become available.

Since the clinical history may not necessarily be reliable or specific and the early ECGs often do not show diagnostic changes, increasing reliance has been placed on biochemical cardiac markers. However, the available tests have limited ability to influence decision-making in the very early hours since they are either not detectable quickly enough or they are not sufficiently specific.

1.4 TRIAGE OF PATIENTS FROM ACCIDENT AND EMERGENCY DEPARTMENT

Due to the lack of distinguishing features of chest pain in some patients and the lack of diagnostic sensitivity of the ECG, the treating physicians tend to admit patients with uncertain diagnosis for further diagnostic work up rather than risk inappropriate

discharge with all its health and legal consequences. This strategy achieves a low rate of missed AMI but also results in the admission of many patients without AMI. As a result, increased economic pressures arise from patients consuming expensive resources and this also results in patients being subjected to investigations to rule in or rule out AMI.[22] Furthermore, the use of more invasive investigations has specific hazards. Current triage policy also results in some patients without ACS occupying intensive care beds and prevents other patients with appropriate needs gaining access to these units.

The differential diagnosis of chest pain

Cardiac causes:	***Pulmonary causes*:**
Acute myocardial infarction	**Pneumonia (pleuritis)**
Unstable angina	**Pulmonary embolism**
Stable angina	**Pneumothorax**
Aortic dissection	***Muskeloskeletal causes:***
Pericarditis	**Muscle spasm**
***Gastrointestinal causes*:**	**Muscle trauma**
Oesophageal reflux	**Costochondritis**
Oesophageal spasm/rupture	**Thoraco-cervical disc compression**
Gastric ulcer	***Dermatological causes:***
Duodenal ulcer	**Herpes zoster**
Biliary pain	
Pancreatic pain	

Table 1. This table illustrates the most frequent causes of chest pain that must be differentiated in patients who present to the A&E department.

In this era of minimisation of health costs, and the pressures for effective use of resources, there are also important legal implications of malpractice. Thus, there is a demand for a strategy to improve efficiency in managing patients presenting with ACS and to reduce unnecessary admissions and discharges and to make proper use of expensive beds, without compromising the safety of the patients. Clinical history and ECG interpretation have limited scope for further improvement. However, a potential area for improvement is in the use of cardiac markers with improved predictive accuracy to help with the early diagnosis of ACS.

A cardiac marker that is released early and is reliable would offer a simple and effective method for the identification of ACS patients and would help ensure that appropriate patients are admitted thus improving health economic efficiency. In choosing markers for this purpose some considerations need to be met. First, markers must have a high sensitivity and specificity for the early diagnosis of AMI within 6 hours after symptom onset. Second, they must have a high negative predictive value since 90% of patients will not have AMI. Third, they should have a short turn around time so that decisions regarding patients' management can be made within the time course relevant to guide early therapy.

1.5 CARDIAC MARKERS AND THE EARLY DIAGNOSIS OF ACUTE CORONARY SYNDROMES

Cardiac markers differ with respect to the time required after symptom onset in order to exceed the upper limit of normal. Current cardiac marker proteins, creatine kinase (CK), creatine kinase muscle brain (CK-MB), cardiac troponin T (cTnT), and cardiac

troponin I (cTnI) are not elevated within 6 hours of symptom onset and myoglobin lacks specificity. Creatine kinase-MB is used in many laboratories but plasma concentration of this marker are not consistently elevated until 8 - 12 hours after symptom onset, and in patients with UA and minor myocardial injury the concentration may remain normal.[9]

Cardiac-TnI and cTnT are more specific markers of myocardial damage than CK-MB, but the maximum or peak plasma concentration of these proteins can be reliably detected 12 - 16 hours after symptom onset.[23] Myoglobin is released more rapidly but it lacks cardiac specificity.[24;25] Creatine kinase-MB isoforms have recently undergone evaluation as early markers of AMI within the first 6 hours after symptom onset.[8;9;26] However, some investigators have claimed these isoforms to have similar sensitivity to CK-MB mass and myoglobin and do not offer considerable advantages over them.[27]

The low molecular weight cytosolic protein, heart fatty acid binding protein (H-FABP) is a marker of myocardial damage and has the advantage that it is released more rapidly than CK, CK-MB, cTnT, and cTnI and is more cardiac specific than myoglobin.[28-30] Recent studies using H-FABP for the diagnosis of AMI have shown promising results. The sensitivity of H-FABP for the early diagnosis of AMI within 3 hours after symptom onset was reported to be 91.4%.[31] The myoglobin and H-FABP ratio in skeletal and cardiac muscle is noticeably different. The utilisation of this ratio for the differentiation of cardiac and non-cardiac injury has also been reported to be useful.[25]

1.6 ACUTE CORONARY SYNDROMES

Acute coronary syndromes describe a clinical spectrum of conditions including AMI and UA, which occur following rupture or erosion of an atheromatous plaque with subsequent platelet aggregation and thrombus formation. These events lead to some degree of coronary artery occlusion, and thus reduce blood flow. This produces myocardial ischaemia, which leads to UA and may progress to myocardial infarction, depending on the severity and duration of occlusion. Proteins are released from the infarcted myocardium and these can be measured in plasma.

Heart-FABP is a recently characterised protein, which appears to be an early and sensitive marker of myocardial injury.[28] Measurement of this protein would enable rapid assessment of patients with chest pain, allowing physicians to institute early treatment of patients with ACS, and to differentiate cardiac from non-cardiac chest pain, and may prevent unnecessary or prolonged admissions.

In this thesis studies are described to examine the use of this biochemical marker protein in patients with ACS in comparison with other currently available markers of myocardial injury like CK-MB mass, cTnI, cTnT, and myoglobin. The aim is to determine whether H-FABP is a reliable, early and sensitive marker of ACS.

1.7 ORIGINAL HYPOTHESES

Pilot phase findings were obtained prior to the work of this thesis using serum H-FABP in patients with ACS and suggested that further and more detailed studies

should be carried out in order to evaluate the role of this marker protein, thus the hypotheses are:

1. Serum/plasma H-FABP is the most useful (optimal sensitivity and specificity) early marker for AMI, whilst the cardiac specific markers cTnI (or cTnT), and CK-MB mass are more useful indicators of myocardial damage at later time points.

2. Serum/plasma H-FABP can identify myocardial injury in unstable angina and non-Q wave MI prior to the release of cTnI (or cTnT), or CK-MB mass.

3. Serum/plasma H-FABP is a sensitive and specific marker of injury induced by brief episodes of myocardial ischaemia during coronary angioplasty.

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CHAPTER 2

THE ROLE OF CARDIAC MARKERS IN THE EARLY DIAGNOSIS OF ACUTE CORONARY SYNDROMES

2.1 INTRODUCTION

Acute myocardial infarction is the leading cause of death in the United Kingdom and other developed countries.[1] The in-hospital mortality from this cause has been declining over the last three decades.[2;3] This reduction in mortality coincides with improvement in health and living standards and with new treatments like thrombolysis and the introduction of new interventions like percutaneous coronary intervention and coronary artery bypass grafting (CABG). Secondary and primary prevention strategies have contributed significantly and the age-adjusted mortality is expected to continue to decline with further improvements in treatments, better uptake of primary and secondary prevention strategies and also with further improvement in our ability to recognise this challenging disease very early in its course. The success of treatment rests on two elements; (1) The identification of patients in the very early stages of AMI and implementation of treatment to recanalise the occluded artery; (2) Access to early defibrillation and admission to properly monitored units (CCU or ICU) for the detection and treatment of complications.

The diagnosis of AMI is based on the criteria set by the World Health Organisation (WHO) and must include two of the following; (1) Typical history of prolonged ischaemic chest pain; (2) The presence of typical acute ischaemic changes on the admission ECG; (3) Typical rise and fall of cardiac enzymes in blood.[4] This old definition is likely to change with the recent publication of a new definition of myocardial infarction by The Joint European Society of Cardiology/American College of Cardiology (ESC/ACC) Committee, which redefines myocardial

infarction according to cardiac markers as; (1) An increase in cardiac markers cTnI, cTnT exceeding the decision limit (99th percentile of the value for a reference control group) on at least one occasions; (2) an increase in CK-MB (preferably CK-MB mass) exceeding the decision limit (99th percentile of the value for a reference control group) on at least two occasions with a rise and fall pattern, or greater than twice the upper limit of normal on one occasion.[20] Within this definition ACS are also classified into ST elevation and non-ST elevation (Figure 1).

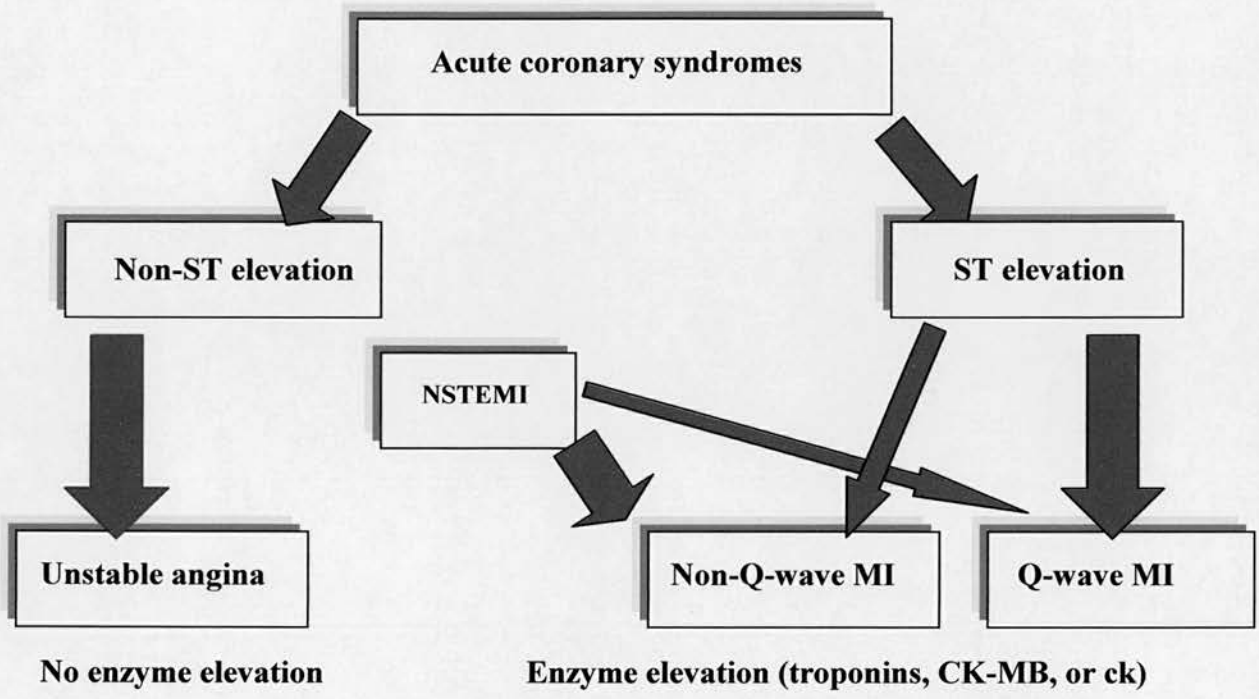

Figure 1. The new ESC/ACC clinical classification of ACS. Acute coronary syndromes are classified into ST and Non-ST elevation. ST elevation ACS is further classified into Q-wave and non Q-wave MI. Non-ST elevation MI (with cardiac markers elevation) is also classified into Q-wave and non Q-wave MI. Non-ST elevation ACS without cardiac markers elevations is called unstable angina.[20] Abbreviations: ST, Q, are ST segment and Q wave of the ECG; NSTEMI, non-ST elevation myocardial infarction; MI, myocardial infarction.

The very early diagnosis of AMI can be a challenging task to many physicians in the A&E department. When typical history is present it helps to orientate the clinician to

the diagnosis, but its absence by no means rules it out. This is often the case in a significant proportion of patients in whom history is either atypical or absent. Diabetic, hypertensive, and elderly patients often have silent AMI. In these cases AMI may go unnoticed or may produce atypical symptoms such as hypotension, breathlessness, or arrhythmias.[5] The ECG is an important tool to rule in AMI, but it lacks sensitivity and as many as 30 - 50% of patients may initially present with normal or non-diagnostic ECG.[6;27] The ECG has an overall diagnostic sensitivity for AMI of 70 - 81%.[7;8] However, when changes of AMI are present on the admission ECG (ST elevation and new Q wave) they are highly specific and have a very high positive predictive value for the diagnosis of AMI. Cardiac markers are formidable tools for the diagnosis of AMI.[9;10] The number of cardiac markers in use is growing rapidly. This is an important area of cardiology and researches in this field are expanding rapidly.

2.2 HISTORICAL DEVELOPMENT OF CARDIAC MARKERS OF ACUTE MYOCARDIAL INFARCTION

The utilisation of cardiac markers for the diagnosis of AMI has developed over the years from the use of non-specific and non-sensitive late markers to the use of highly sensitive and specific cardiac markers. The first biochemical cardiac marker used in the identification of AMI was aspertate aminotransferase in 1954. This was joined and gradually taken over by creatine kinase in 1965. Together with Lactate dehydrogenase (LDH), these three markers became the cornerstone for biochemical diagnosis of AMI in the early days. The development of electrophoresis lead to the identification of LDH and CK isoenzymes (LDH1, CK-MB activity), as cardiac

markers with higher specificity. Myoglobin measurement became available in 1976. The first mass assay for CK-MB to be evaluated in AMI patients was in 1985. Cardiac-TnT as a marker for AMI was first evaluated in 1989, three years later (1992), cTnI came into use. Creatine kinase-MB isoforms underwent evaluation in 1994 for the triage of patients with AMI from A&E department (Figure 2).

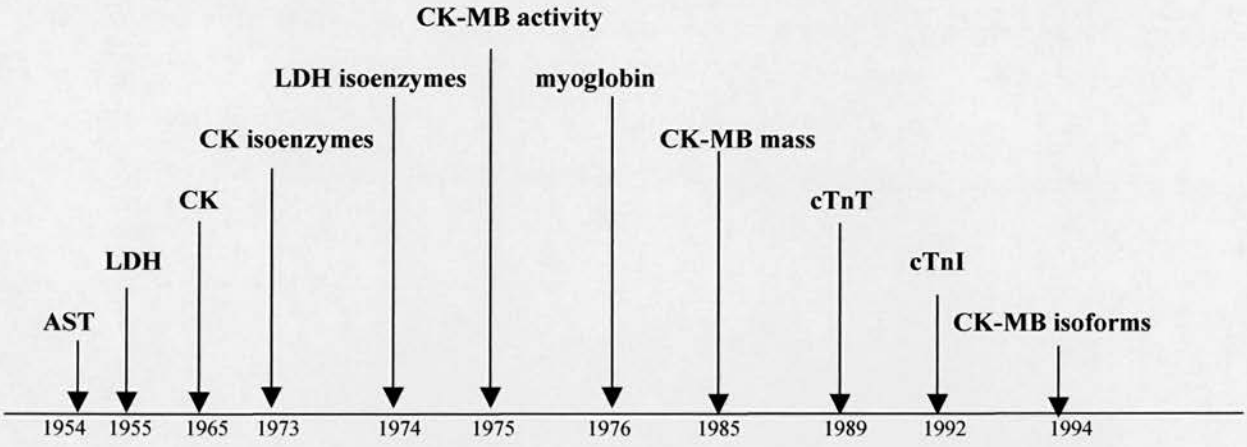

Figure 2. Illustrates the historical development of biochemical cardiac markers over the past five decades. Earlier non-specific and non-sensitive cardiac markers are gradually replaced by more specific and sensitive markers.

2.3 CHARACTERISTIC FEATURES OF BIOCHEMICAL MARKERS OF MYOCARDIAL INJURY

The ideal characteristics of a marker of myocardial injury are; (1) It should be abundant in myocardium and not present in other tissues. This gives it a high specificity for the myocardium and reduces the rate of false positive results; (2) It should have a high concentration in myocardium and low or undetectable concentration in the blood in the absence of disease. This gives it a high sensitivity so that the release of only a small amount can be readily detected thereby reducing the rate of false negatives; (3) It should be released completely and quickly when

myocardial damage occurs. This will allow its utilisation for the early detection and quantification of injury; (4) It should persist in the circulation to give a convenient diagnostic window, but not too long to prevent the detection of complications such as early re-infarction; and (5) The assay must have a high analytical sensitivity and specificity and a short turn around time, so that results could be obtained fast to influence decision-making process regarding patients' triage and management.

2.4 BIOCHEMICAL MARKERS AND EARLY DETECTION OF ACUTE MYOCARDIAL INFARCTION

Cardiac markers play an important role in the detection of AMI when the patient's history and ECG are non-diagnostic or equivocal.[11] Diagnosing AMI early i.e. within 6 hours after symptom onset is difficult, because some time must elapse after symptom onset for markers to exceed values above the normal level. The diagnosis of AMI based only on one single value at presentation or soon after admission is unreliable, and serial sampling is the most effective method. The sensitivity and specificity of the cardiac markers for the early diagnosis of AMI is influenced by several factors such as:

1. **Time of presentation**. Early presentation after symptom onset is likely to show a relatively increased sensitivity for markers like myoglobin, which are released very early in the course of AMI, and less sensitivity for markers like CK-MB or cTnI, which are released slightly later. A delayed presentation is likely to have the opposite effect. Therefore, the onset of symptoms should be used as the reference point when commenting on the sensitivity and

specificity of the markers for the early diagnosis of AMI rather than, for example, issues like time of presentation, and time of admission.[12]

2. **Size of infarct**. Cardiac markers are released in proportion to the volume of myocardium in jeopardy, the bigger the infarct the greater the quantity of cardiac markers released. This will lead to improved sensitivity compared to small infarcts where limited release of markers may be close to the threshold for detection.[13]

3. **Selection criteria and prior probability of acute myocardial infarction**. Selecting certain group (s) of patients for the study with a high possibility of AMI, such as those admitted to the CCU, can also influence sensitivity of a marker.

4. **Treatment**. Treatment can have an influence on the sensitivity of the marker. For example, patients who have been successfully thrombolysed show a greater and earlier peak of some of the markers compared to those who were not successfully reperfused.

5. **Diagnostic threshold**. The selection of an appropriate diagnostic threshold requires careful consideration of concentrations seen in the normal and disease free population and those seen in diseased populations. There is always a continuous balance between sensitivity and specificity of any marker. If the cut-off concentration used is low, the sensitivity is improved at

the expense of specificity unless the marker is 100% cardiac specific, and only present in diseased populations.

6. **Kinetic factors.** Kinetic factors like the molecular size of the marker, the biological compartment of the marker (i.e. whether it exchanges freely in the cytoplasm or is attached to structural elements within the cell), the volume of distribution, and whether the marker is released directly into the blood or cleared by the lymphatic system can all influence plasma concentration. Markers that have small molecular weight, lie free in the cytoplasm, and released directly into the circulation, show better early sensitivity compared with larger molecules that are attached to structural elements and/or cleared slowly by the lymphatic system.

It is not surprising therefore that different studies report differing results for sensitivity and specificity for the various cardiac markers for the early diagnosis of AMI. The variations in the reported results will predominantly be influenced by the above factors.

This chapter of the thesis will discuss the currently available markers of myocardial damage like CK, CK-MB mass, CK-MB activity, cTnT, cTnI, myoglobin, LDH, and LDH1. The discussion will focus on the suitability of these markers as tools for the diagnosis of ACS. In particular; (1) Their suitability for early diagnosis of AMI (within 6 hours); (2) Their ability to detect myocardial damage during ischaemic episode in patients presenting with UA or in patients undergoing cardiac intervention

like percutaneous coronary intervention (PTCA); (3) Their ability to detect reperfusion and re-infarction; (4) Their specificity and limitations and (5) Future directions with these markers. The discussion will also focus on some of the recent markers that have undergone evaluation as very early makers of AMI like CK-MB isoforms.

2.5 CREATINE KINASE

Creatine kinase is an enzyme composed of two subunits, M and/or B. Three different pairs of these units combine to give rise to three different isoenzymes, each with molecular weight of about 80 Kilodalton (KDa); CK-BB, CK-MB and CK-MM. Creatine kinase-BB is the brain isoenzyme and is present in large quantity in the brain. Creatine kinase-MB is the heart specific isoenzymes and has been the gold standard method for the diagnosis of AMI in many laboratories around the world. It exists in large quantity in heart muscle but is not totally cardiac specific and exists also in skeletal muscles and other tissues. About 15-40% of the total CK activity of heart muscle is due to CK-MB, the rest is largely due to CK-MM isoenzyme. Creatine kinase-MM is the skeletal muscle isoenzyme. It has the highest distribution in skeletal muscles. The three isoenzymes are present in varying concentrations in the smooth muscle of the colon, ileum, stomach, and urinary bladder.[14]

Fractionation of CK isoenzymes by electrophoresis results in two more additional isoenzymes, called mitochondrial CK (mit CK) and macro CK (type 1 and 2).[12] Mit CK is the mitochondrial form of the enzyme. Macro CK type 1 is believed to be the fab' fragment of the immunoglobulin IgA or IgG bound to two molecules of CK-BB.

Macro CK type 2 is a polymer of mit CK. The presence of mit CK and macro CK in serum does not have much diagnostic significance but when they are present in large concentrations they could interfere with the analysis of total CK or its isoenzymes.[15-16] The normal range for CK is approximately 80 - 200 IU/L for men and 60 - 140 IU/L for women. This is the result of the normal turnover of this enzyme in skeletal muscles. The normal range is influenced by factors like muscle mass and physical work.

2.5.1 CREATINE KINASE AND ACUTE MYOCARDIAL INFARCTION

Creatine kinase was introduced in 1965 as a biochemical marker for myocardial damage and it is one of the oldest markers in this field.[17] It has a clinical sensitivity for the diagnosis of AMI of 90%. Unfortunately, this is not matched by high specificity. It is released within 12 hours after symptom onset of AMI, peaks in serum at 24 - 36 hours, and returns to normal in 48 - 72 hours. As a result of these release kinetics, measurement of total CK is not suitable for the early diagnosis (within 6 hours) of AMI. Creatine kinase as a marker is also unsuitable for the detection of myocardial damage that may occur in patients presenting with UA, or in patients undergoing PCI.

A marker that is suitable for the early diagnosis of AMI, and the detection of small injuries to the heart should have; high cytoplasmic (cell) to vascular (plasma) ratio; very low or undetectable normal plasma concentration; and total cardiac specificity. Creatine kinase does not fulfil these criteria since it has a moderate cytoplasmic to vascular ratio of 60,000:1, high resting normal range values up to 200 IU/L, and is

widely distributed in the body. To improve on the cardiac specificity of CK for the diagnosis of AMI, it was combined with CK-MB (the cardiac specific isoenzyme of CK) in a ratio. A CK-MB to CK ratio > 6% is reported to be specific for myocardial injury, whereas a ratio < 6% is consistent with skeletal muscle damage or non-cardiac cause.

In some clinical settings a CK-MB test is not requested unless the total CK activity is elevated. The use of total CK as a screening test before ordering CK-MB could miss some patients with AMI and should be used with caution. As there are cases of AMI without total elevation of CK concentration but the total CK-MB fraction and the CK-MB to CK ratio in these patients is diagnostic for AMI. This is likely to happen in situations when there is a small MI in a small sized person with a low muscle bulk, and a low base line value of total CK. The normal reference range of CK is very broad and these patients could release small amounts of the enzyme, insufficient to raise the concentration above the normal reference range. If this possibility is not borne in mind the diagnosis could be missed when CK is used. Unless the base line total CK is known in a patient presenting with highly suspicious diagnosis of ischaemia, a low total CK should not exclude the diagnosis nor it should preclude requests for CK-MB.[12]

2.5.2 LIMITATIONS OF CREATINE KINASE MEASUREMENT

Creatine kinase enzyme is widely distributed and there are various causes of elevated total CK in the absence of myocardial injury.[18] Haemolysis leads to the release of adenylate kinase enzyme, which interferes with the assay and causes false elevation

of total CK activity. Various forms of skeletal muscle injury can lead to increased CK activity including strenuous exercise, intramuscular injection, rhabdomyolysis, burns, and trauma. Chronic muscle disease like Duchenne muscular dystrophy, polymyositis, dermatomyositis, and myopathy can all lead to increased concentrations of CK. Total CK activity is a very sensitive indicator of injury to skeletal muscles. Drugs like cocaine and alcohol can also raise CK concentrations to abnormal values, presumably due to the associated myopathy that can occur with the use of these drugs. Neurological conditions like myasthenia gravis also elevates total CK activity. Other miscellaneous conditions including pregnancy, hypothermia, and sepsis can increase total CK concentrations. In many of these situations the activity of CK-MB is also increased, giving a ratio of CK-MB to CK that remains below the 6% cut-off point required to differentiate between skeletal muscle injury and cardiac muscle injury.[49]

2.5.3 FUTURE DIRECTION WITH CREATINE KINASE ASSAYS

Creatine kinase measurement is a relatively cheap assay that is widely available. For this reason total CK measurement will probably continue to be used as a marker for myocardial injury especially in situations such as; (1) In the absence of cTnI, cTnT, and CK-MB assays; (2) In patients with unequivocal ECG diagnosis of myocardial infarction where non-specific marker such as CK can be used to monitor the progress of the patient in hospital and to gauge infarct size.

2.6 CREATINE KINASE MUSCLE BRAIN ISOENZYMES

Creatine kinase-MB isoenzyme has been considered the gold standard for the diagnosis of AMI. It is the isoenzyme of CK specific for heart muscle. It is measured in serum or plasma by one of two methods; **(1) CK-MB activity measurements:** This measures the total activity of the enzyme in serum/plasma by methods like electrophoresis, column chromatography, immunoinhibition or immunoprecipitation. The results are reported as international units per litre (IU/L). These methods are non-specific, measure only active enzymes, and have a low analytical sensitivity (5 IU/L); **(2) CK-MB mass measurement:** This measures the protein mass in serum/ plasma using specific antibodies against the M, B or MB subunits. The results are reported in nanogram per millilitre (ng/ml) or microgram per litre (μg/l). These assays are highly specific and have a high analytical sensitivity (0.3 ng/ml) and can measure both active and inactive enzymes. The normal range for CK-MB activity using various methods has been reported to be 8 - 16 IU/L. The normal relative index of CK-MB activity to total CK activity is < 6%. The normal range for CK-MB mass measurement is 5 - 10 ng/ml. The normal relative index of CK-MB mass to total CK activity is < 4%.

2.6.1 CREATINE KINASE-MB ACTIVITY VERSUS CREATINE KINASE-MB MASS

Different groups have reported that measurement of CK-MB using mass is better than that measuring CK-MB activity.[19] The recent guidelines for the redefinition of AMI mentioned at the beginning of this chapter recommend the use of CK-MB mass as opposed to CK-MB activity.[20] The problems inherent in measuring activity are:

(1) The enzyme may become deactivated by experimental manipulations leading to a low value or underestimation. This is likely to happen in the low range of

CK-MB activity such as in patients with small AMI or with minor myocardial damage. In the extreme situation a false negative result could result in the missed diagnosis of AMI. Unlike CK-MB activity assays, CK-MB mass assays measure both active and inactive enzyme.

(2) In haemolysed blood samples interference from adenylate kinase, an enzyme released from red blood cells and catalyses the same reaction as CK-MB, could result in false positive results. This problem is irrelevant with mass assays because the antibodies are specific for the CK-MB and the presence of adenylate kinase will have no effect on the results of the assay.[18]

(3) Creatine kinase-MB measurement using mass have better early sensitivity for the diagnosis of ST elevation AMI. They have also been reported to be more sensitive in detecting small injuries to the myocardium that occur in patients with non-ST elevation ACS.[21]

(4) The presence of CK-BB, macro CK type 1, or macro CK type 2 in high concentrations could interfere with the result and lead to false elevation of CK-MB when activity measurement is used. These interferences have no such effects when mass assays are used because the antibodies are specific for CK-MB and do not cross react with these compounds.[22;23]

(5) Creatine kinase-MB activity measurement is non-specific, whereas CK-MB mass measurement is specific. This higher specificity allows a lower cut-off

concentration to be used giving it higher sensitivity.

(6) Creatine kinase-MB activity measurement requires technical expertise and the turn around time is prolonged. With CK-MB mass measurement the results could be available in as little as 13 minutes, using new point of care instruments, and can be used to influence decisions regarding patients' management.[24]

2.6.2 CREATINE KINASE-MB AND ST ELEVATION ACUTE MYOCARDIAL INFARCTION

Creatine kinase-MB follows the same release kinetics as CK and has a sensitivity and specificity for the diagnosis of AMI of more than 90%. This sensitivity and specificity changes with the time of presentation after symptom onset. Creatine kinase-MB activity measurement is most reliable in the 12 - 24 hours period after symptom onset.[25] A negative result for CK-MB activity before 12 hours from the start of symptoms is too early to rule out AMI, and a negative result after 24 hours is too late. Studies comparing the utilisation of these assays for the early diagnosis of AMI have shown that CK-MB using mass measurement reaches the cut-off point in serum several hours before CK-MB activity measurement and have claimed its superiority within 4 - 8 hours after symptom onset.[21,26] The CK-MB mass assay have been shown to be sensitive for the diagnosis of AMI even in situations were the ECG is equivocal and can increase the number of diagnosis made within the time scale required for the administration of thrombolytic treatment.[27;28]

2.6.3 CREATINE KINASE-MB AND UNSTABLE ANGINA

Creatine kinase-MB activity tends to be normal in patients with UA, whereas CK-MB mass is increased in a proportion of these patients. This observation has been substantiated by different research groups.[38;39;40] A study by Seo et al (1993) compared CK-MB mass vs CK-MB activity and concluded that CK-MB mass is more sensitive when CK-MB concentration is in the low range.[41] This is important especially in cases of myocardial injury in UA and following complicated PCI, and in radiofrequency ablation of arrhythmias, when the amount of CK-MB released can be small.

Sensitive CK-MB mass assays can detect prolonged ischaemia in UA and have been used for risk stratification in these patients.[38] One small study has shown very high death rate (64%) during a four years follow-up in patients who were admitted to the CCU with chest pain, positive CK-MB mass, and non-diagnostic ECG changes for AMI.[42] Several investigators have also studied the release of CK-MB mass following PCI.[43;44-46] They demonstrated that CK-MB mass is a sensitive indicator of myocardial injury following PCI. One study reported that 40% of patients showed evidence of myocardial damage following PCI using both CK-MB mass and cTnT.[47]

2.6.4 LIMITATIONS AND FUTURE DIRECTION OF CREATINE KINASE-MB ASSAYS

Creatine kinase-MB measurement is not totally specific for myocardial infarction and there are various causes for elevated CK-MB concentration other than AMI.[18,48] Cardiac pathologies like congestive cardiac failure and arrhythmia lead to elevated CK-MB concentration. Severe skeletal muscle damage e.g. acute muscle trauma,

skeletal muscle disorders like myositis, polymyositis, chronic inflammatory and degenerative muscle disorders can lead to CK-MB elevation. In these situations the CK-MB to CK ratio or cardiac troponins can be used to differentiate cardiac and non-cardiac pathologies.[49] Creatine kinase-MB measurement is a widely accepted assay that is both sensitive and relatively specific for the detection of AMI. However, CK-MB (activity or mass) is not sensitive in the first 6 hours after symptom onset for the early diagnosis of AMI. Measurement using CK-MB mass is the preferred method and is likely to be the dominant method in the future.[20]

2.7 CREATINE KINASE MUSCLE BRAIN ISOFORMS (SUBFORMS)

Creatine kinase-MB isoforms are variants of CK-MB isoenzyme, which result from post-synthetic modification of the M subunit.[50] They were discovered by Weavers in 1972.[51] They have recently been evaluated as markers of myocardial damage in 1994.[52] After ischaemic damage to the heart, CK-MB isoenzyme is released from the damaged heart muscle into the blood.[50] This isoenzyme is converted into other forms by the action of the plasma enzyme carboxypeptidase N (CPN) according to the following reaction:[53;54;55;56]

$$\textbf{CK-MB2 (tissue form)} \xrightleftharpoons{\textbf{CPN}} \textbf{CK-MB1 (plasma form)} + \textbf{-Lysine}$$

In this reaction CPN enzyme removes one lysine amino acid from the M subunit of the released CK-MB2 to produce CK-MB1. It is suggested that this post-synthetic change prepare the molecule for clearance from the body.[57] Creatine kinase-MB2

and CK-MB1 exist in equilibrium [1:1 ratio] in the serum of normal healthy people. Therefore, measurement of total CK-MB at any point in time is equivalent to 50% CK-MB2 and 50% CK-MB1.[58;61]

Most assays that measure CK-MB use a relatively high upper limit of normal (10 ng/ml, 14 IU/L). Humans differ in their background level of CK-MB and some people may express normal concentrations as low as 1 - 2 IU/L (or ng/ml). Therefore, in the event of myocardial injury, it will require several-fold increases in the marker before it exceeds the upper limit of normal of the assays. Creatine kinase-MB being a relatively large molecule means it will take even longer time to reach the circulation and becomes important diagnostically. When myocardial injury occurs there is a sudden release and rise of the tissue isoforms i.e. CK-MB2 compared to the plasma form CK-MB1 leading to a rise in the ratio of CK-MB2 to CK-MB1. By using CK-MB isoforms effectively each patient acts as their own control, and only a release of a small amounts of the marker is required to raise the ratio to significant level much earlier. The requirement for the diagnosis of AMI is two-fold; (1) Increase of CK-MB2 > 2.6 IU/L; (2) Increase of CK-MB2 to CK-MB1 ratio > 1.7.

2.7.1 CREATINE KINASE-MB ISOFORMS AND ACUTE MYOCARDIAL INFARCTION

Creatine kinase-MB isoforms are reported to be released within 1 hour after symptom onset and peak at 4 hours. Evidence of AMI can be detected as early as 1 - 2 hours post-infarction, several hours before total CK-MB reaches diagnostic level.[52;59] These release kinetics make CK-MB isoforms potential markers for the early diagnosis of AMI. The sensitivity and specificity of CK-MB2 to CK-MB1 ratio

for the diagnosis of AMI is reported to be 92% and 95% respectively within 6 hours of infarction.[60] During this time interval total CK-MB mass or activity would just be approaching the upper limit of normal. One study reported a sensitivity and specificity of 95.7% and 93.9% respectively with a high positive predictive value and a high negative predictive value within 6 hours of infarction.

One hundred and fourteen out of 118 patients with AMI were identified using CK-MB isoforms within 6 hours. The sensitivity and specificity of conventional CK-MB during this time interval was 48% and 94% respectively.[61] Seventeen patients who were discharged from the emergency department fulfilled the criteria for the diagnosis of AMI using CK-MB isoforms (missed diagnosis). The assays also identified a substantial number of patients with UA (26 out of 133) with abnormal CK-MB isoforms results but no evidence of AMI on the basis of conventional CK-MB activity results. The test was also positive in patients with hypothyroidism and rhabdomyolysis.[61] These findings were also substantiated by other studies.[62]

Some investigators however, have questioned the value of CK-MB isoforms for the early diagnosis of AMI.[26] A study by Laurino et al (1996) showed no difference between CK-MB isoforms and conventional CK-MB isoenzyme at 6 hours after symptom onset.[63] Another study by Bhayana et al (1993) using comparison between CK-MB mass, CK-MM3 to CK-MM1 ratio, and CK-MB2 to CK-MB1 ratio found no significant advantage of isoforms over CK-MB mass for the diagnosis of AMI within 6 hours.[64]

2.7.2 LIMITATIONS OF CRATINE KINASE-MB ISOFORMS ASSAYS

There are still some unresolved issues surrounding the use of CK-MB isoforms for the early diagnosis of myocardial infarction. There is still some concern regarding the stability of these isoforms in serum/plasma after their release from damaged tissues.[65] There are also some reports of possible variations in the activity of the CPN enzyme among humans.[66;67] This may have profound effect for the use of the isoforms as markers for the early diagnosis of AMI and could lead to false positive or false negative results.[52;65;68] The other drawback is that the use of isoforms does not obviate the need to analyse total CK and the relative index of CK-MB to total CK to exclude false positive results.[69] Future work should be directed towards the resolution of the above concerns and to the development of more sensitive and reliable assays that can offer rapid results to aid in the early diagnosis and triage of patients.

2.8 MYOGLOBIN

Myoglobin is a small heme protein (17 K Da) that functions in oxygen binding and transport. It stores oxygen in red muscles (skeletal and cardiac) and under conditions of severe oxygen deprivation, it releases the oxygen to be used by muscle mitochondria for synthesis of Adenosine triphosphate (ATP). The myoglobin content of heart muscle is reported to be 2.5 mg/g wet weight of tissue and the skeletal muscle content is twice this value 4.0 mg/g wet weight of tissue.[70] Myoglobin constitutes 2% of the total cytosolic protein content of cardiac muscle. The normal range of serum myoglobin is about 20 - 80 ng/ml. Males have higher levels than females because they have bigger body size and muscle bulk.

2.8.1 MYOGLOBIN AND ACUTE MYOCARDIAL INFARCTION

Myoglobin is one of the best available early markers of AMI within 3 hours after symptom onset. The relationship between AMI and high myoglobin level was first reported in 1975.[71] Myoglobin becomes abnormal within 2 hours after symptom onset of AMI, peaks at 6 - 9 hours, and return to normal within 24 hours. This early release feature of myoglobin is attributed to its small size and localisation within the cytosol of the cell. Several investigators have confirmed a significant role for myoglobin in the early diagnosis of AMI, the most promising role being in the early rule out of AMI in patients presenting within 6 hours after symptoms onset.[72;73-80] Within this period the overall diagnostic sensitivity and specificity ranged from 77 - 97% and 90 - 97.9% respectively.

The variation in sensitivity is most dependent on the time of presentation after symptom onset and drops considerably with very early (< 2 hours) or late presentation (> 15 hours) to the hospital.[13] A consistently negative result within this time interval has such a high negative predictive value for ruling out AMI, that confident decisions regarding patients' management can be based on it. However, a positive result should be used with caution, as there are many situations that could give rise to myoglobin elevation in the absence of AMI.

2.8.2 LIMITATIONS OF MYOGLOBIN MEASUREMENT

Myoglobin is a non-specific marker protein for myocardial injury. The serum concentration of myoglobin is raised in skeletal muscle damage including intramuscular injection, exhaustive exercise, muscle trauma, direct current shock

cardioversion, and also in patients and carriers of genetic muscle disease. Severe renal disease leads to failure of clearance of myoglobin from the circulation. The concentration tends to rise and the circulation time is prolonged in these patients. Although these factors interfere with the specificity of the test, in clinical practice most of them could be ruled out by careful attention to history taking and simple blood tests like urea and creatinine to exclude renal failure. In situations where clear ambiguity exists, myoglobin could be combined with more specific investigations. The specificity is increased when myoglobin measurement is combined with other diagnostic methods like ECG or more specific cardiac markers like CK-MB, cTnT, or cTnI.

2.8.3 MYOGLOBIN AND THE DETECTION OF REPERFUSION

Ideally all AMI patients should be treated with intravenous thrombolytic treatment or PCI.[81] The success of thrombolytic treatment in establishing reperfusion is reported to be 50 - 80% (depending on the thrombolytic agent).[82] Establishment of reperfusion after the initiation of thrombolytic treatment is of important interest to the clinicians in terms of treatment and prognostic implications. Coronary angiography is the most definitive method to assess the success of thrombolytic treatment, but this procedure is invasive, carries morbidity and mortality risks, requires catheterisation laboratory team, and is not widely available.

Biochemical markers like myoglobin (among others) offer an alternative non-invasive, safe and potentially sensitive method for the detection of reperfusion. This can be done by monitoring the changes in serum concentration immediately before

and 60 or 90 minutes after initiation of thrombolysis. Patients who successfully reperfuse their occluded artery show a higher and early concentration peak of the biochemical marker compared to those who fail to reperfuse.[83] In patients with uncomplicated myocardial infarction who reperfuse, achieve peak value of myoglobin at 1 hour, whereas those who fail to reperfuse reach peak value about 5 hours later.[84] A two-fold increase in myoglobin concentrations within 60 minutes compared to base line pre-thrombolysis value is associated with 95% predictive accuracy for the detection of reperfusion.[85;86] The existence of "no-reflow" is an important indicator of impaired reperfusion and functional recovery. Thus a patent artery does not invariably indicate tissue reperfusion. Accurate markers of tissue reperfusion are required. The combination of serial 12-lead ECG, clinical features, and serial cardiac markers testing offer a practical alternative to coronary angiography for assessment of reperfusion status.

2.9 LACTATE DEHYDROGENASE AND ISOENZYMES

Lactate dehydrogenase is a vital enzyme of approximately 135 kilodalton and is present in the cytoplasm of almost all cells of the body. There are five isoenzymes of LDH [LDH1 to LDH5]. Lactate dehydrogenase 1 and LDH2 are the predominant isoenzymes in the heart muscle, red blood cell, and kidney. The level of LDH in normal healthy people is about 95 - 200 IU/L. Under normal circumstances LDH2 is the predominant isoenzyme in the circulation with LDH1 concentration less than LDH2. When myocardial damage occurs there is more release of LDH1 than LDH2 leading to the reversal of the ratio. This is called LDH1 to LDH2 flip, and is used as an index of AMI. Lactate dehydrogenase 1 starts to appear in serum 8 - 12 hours

after symptom onset and peaks at 24 - 72 hours.[87] The half-life of LDH activity in blood is prolonged, and it does not return to pre-infarction levels until 5 - 10 days post-infarction. Confirmation of AMI using LDH can be based on one or combinations of the following methods of measurements; (1) Increased total LDH concentration; (2) Increased LDH1 concentration; (3) Flipped LDH1 to LDH2 ratio; (4) Increased LDH1 to total LDH ratio.

The LDH1 to LDH2 flip has been reported to have a sensitivity and specificity that varies between 70 - 100% and 80 - 99% respectively. That depends on the time of presentation, methods used and the selected ratio. However, the presence of a flipped ratio in the absence of total increase of LDH is of equivocal significance. The clinical sensitivity of LDH1 is 89%, with specificity of 95%.[88] Reliance on a single value of LDH1 is useless and serial samples that show a rise and fall are the most sensitive. A combination of absolute LDH1 value and as a ratio with LDH (LDH1 to total LDH ratio), or with LDH2 (LDH1 to LDH2 ratio), or LDH4 (LDH1 to LDH4 ratio) has been reported to be the most sensitive method for this marker.[87;89]

Various types of anaemia with associated haemolysis show elevated concentrations of LDH1, and LDH1 to LDH2 ratio that could mimic AMI.[90] Other conditions such as liver disease, cancer, and congestive cardiac failure also lead to LDH elevation in the absence of AMI.[91] Some patients with skeletal muscle damage like muscular dystrophy, myositis or following severe physical exercise, and cardioversion also show elevated concentration of LDH activity.[92-94] Other conditions that lead to elevated concentrations of LDH activity include pregnancy and alcohol overdose.[95;96]

The LDH and isoenzymes are less specific than CK-MB, cTnI, and cTnT. The use of one single marker like cTnT or cTnI is more sensitive, specific, convenient, cost effective, and obviates the need to perform several other less specific tests like serial LDH, LDH1, or LDH1 to LDH ratio.

2.10 CARDIAC TROPONINS

The troponin complex is found on the thin filament (actin) of all types of striated muscle (fast, slow, and cardiac). Its function is to regulate calcium dependent contraction of muscles. There are three types of troponins; troponin T (TnT); troponin I (TnI); and troponin C (TnC). They are designated with a letter that refers to the function of the troponin protein; Tn**C,** binds **C**alcium; Tn**I, I**nhibits the action of the enzyme actomyosin Adenosine triphosphatase; TnT, binds to **T**ropomyosin.[98] They are called isoforms and have a pre-fix to indicate the muscle type they are in e.g. cTnT, sTnT, fTnT stands for **c**ardiac muscle, **s**low twitch skeletal muscle, and **f**ast twitch skeletal muscle TnT respectively. Each troponin protein within these muscles has different molecular weight, amino acids, and amino acid sequence unique to that muscle type. The different isoforms of TnT and TnI share between 40 - 55% amino acid sequence homology.[99]

2.10.1 CARDIAC TROPONIN T AND ACUTE MYOCARDIAL INFARCTION

Cardiac-TnT (34 KDa) was first introduced in 1989 as a marker for AMI.[100] The upper limit for cTnT has been reported as < 0.1 μg/l, but concentration between 0.03 - 0.1 μg/l may also have significance as markers of adverse outcome.[101] Cardiac-TnT appears in the serum within 12 hours after symptom onset in patients with AMI. It

shows similar release kinetics to CK-MB and cTnI, and thus does not provide an earlier detection for AMI than CK-MB or cTnI within the first 6 hours after symptom onset.[102;103;104] Once in the circulation it persists for a long time (2 - 3 weeks) after symptom onset. The half-life of cTnT in circulation is 120 minutes and this long diagnostic window is thought to be due to the continuous release of the marker from myocardial cells after necrosis, and not due to slow clearance from the circulation.[103;105] The clinical sensitivity of cTnT for the diagnosis of AMI approaches 100% at about 12 hours after symptom onset and remains elevated at 100% for at least 4 days.[105;106]

2.10.2 CARDIAC TROPONIN T AND UNSTABLE ANGINA

Unstable angina carries significant morbidity and mortality risks and early detection and treatment are essential to minimise complications.[29-33] Cardiac-TnT has been shown to be elevated in some patients with UA (non-ST elevation ACS) and the magnitude of elevation can be used for diagnostic purposes, risk stratification, and for selection of appropriate patient groups for treatment. Elevated cTnT in patients with UA is associated with poor prognosis.[107;108] The increase correlates well with the severity of coronary artery lesions determined by angiography.[109] In one important study, the prognostic value of cTnT was assessed in 967 patients with UA. It was found that the group that had elevated concentration of cTnT had an increased risk for cardiac events and the higher the cTnT the more frequent the complications. Patients were followed-up for 6 months for cardiac complications. The risk of AMI and death was 4.3% in patients with cTnT less than 0.06 μg/l and 16.1% for those with cTnT equal or greater than 0.18 μg/l.[110] In another study by Stubbs et al (1996),

62 UA patients were followed-up for about three years after their admission with cTnT concentration greater or equal to 0.2 ng/ml. The incidence of complications in this group was very high, cardiac death (12 patients), coronary revascularisation (22 patients), death and non-fatal AMI (18 patients).[111]

Cardiac-TnT measurement can help select the appropriate patients for treatment with antithrombotic treatment. Treatment of UA patients with thrombolytic therapy had not been found to be useful.[34;35] However, treatment of UA with antiplatelet and antithrombotic drugs like aspirin, low molecular weight heparin (LMWH), glycoprotein IIb/IIIa (GP IIb/IIIa) receptor antagonist, and clopidogrel is associated with 30% reduction in the incidence of mortality and AMI.[36;37] Those patients with cTnT < 0.1 ng/ml fared equally well whether they were treated by the LMWH deltaparin or placebo [4.7 vs 5.7% - 40 day mortality respectively]. However, patients with cTnT greater than or equal to 0.1 ng/ml had reduced AMI and mortality if they received deltaparin rather than placebo [7.4 vs 14.2% respectively].[112]

2.10.3 SPECIFICITY OF CARDIAC TROPONIN T ASSAYS

Elevated cTnT concentration has been reported in a significant numbers of patients with chronic renal failure. These levels do not seem to be affected by haemodialysis, with elevations persisting after treatment.[113-116] Vigorous exercise [like marathon runners], rhabdomyolysis, inflammatory muscle disease [like polymyositis and dermatomyositis], and degenerative muscle disease [like Duchenne/Becker muscular dystrophy] have been reported to show elevated concentrations of cTnT.[117] High cTnT and cTnI concentrations have been reported in critically ill patients who had

not been diagnosed with comorbid AMI.[118] Blunt trauma to the chest, closed heart massage, external defibrillation is also reported to result in elevation of cTnT.[119;120] Elevations have also been reported in cases of myocarditis and drug induced cardiac toxicity.[121;122]

The increase in cTnT concentrations in the above conditions can be due to; (1) Genuine elevation due to the release of cTnT from the heart; (2) False elevation due to cross reactivity with sTnT using the first generation cTnT assays which have 3.6% cross reactivity with sTnT;[123] (3) Elevation due to the re-expression and release of foetal isoforms of cTnT during muscle regeneration.[113] Indeed, some of these elevation in cTnT have been shown to be due to cross reactivity with sTnT using the second generation assays which is more specific and have < 0.005% cross reactivity with sTnT.[101] The advantage of the cTnT immunoassay is that it is marketed by one source only hence there are well-established standards in terms of normal range, detection limits, and clinical cut-off concentrations. There is however, a slightly higher rate of positive results with cTnT assays in some patients with chronic renal failure and acute or chronic muscle disease.[113-117;124]

2.10.4 CARDIAC TROPONIN I AND ACUTE MYOCARDIAL INFARCTION

Cardiac-TnI (24 KDa) is reported to have a unique segment containing 31 amino acids that makes it different to either sTnI or fTnI (19 KDa). During foetal development both sTnI and cTnI are expressed in the myocardium. At birth however, only the cTnI remains as the only isoform present in the human myocardium.[125;126] Cardiac-TnI has not been shown to be expressed in any type of skeletal muscle

during either development or disease stimuli.[127] This makes cTnI 100% specific for the myocardial tissue, and an excellent marker for the detection of myocardial injury in serum.

Cardiac-TnI was first reported as a biochemical marker of myocardial injury in 1992, and has since been shown to be a very sensitive and specific marker for the diagnosis of AMI. It has similar release kinetics to CK-MB and to cTnT and does not provide an earlier detection for AMI within the first 6 hours after symptom onset.[128] Cardiac-TnI peaks between 12 - 36 hours after onset of AMI and remains elevated for 3 - 7 days after AMI. The half-life of cTnI is < 2 hours and the prolonged diagnostic window is due to the continuous release of this marker from the myofibril. Cardiac-TnI is more sensitive and specific than LDH1 to LDH2 ratio for the detection of myocardial injury up to 5 days after admission.[129]

2.10.5 CARDIAC TROPONIN I AND UNSTABLE ANGINA

Cardiac-TnI is cardiac specific and its concentration in normal and disease free populations is undetectable or very low. This makes it a suitable marker for the detection of myocardial injury in UA patients as well as other situations where myocardial injury is expected but the amount released could be small e.g. following PCI.[130] Many studies have also shown cTnI to have prognostic value in patients with UA similar to that of cTnT. Those patients showing higher levels at admission have more complications [increased mortality and AMI] on subsequent follow-up.[131;132] The prognostic risk in these patients is significantly altered if early intervention

[pharmacological and invasive] was undertaken in these patients compared to conservative treatment.

2.10.6 SPECIFICITY OF CARDIAC TROPONIN I ASSAYS

Cardiac-TnI was not found in patients who underwent uncomplicated angioplasty.[133] Cardiac-TnI was not elevated in patients who undergo non-cardiac surgery, but was raised in patients undergoing CABG due to surgical injury of the myocardium. Cardiac-TnI or cTnT may be the preferred markers of choice to detect myocardial injury in patients who undergo surgery. In the situation of surgery, cTnI (or cTnT) being specific to the myocardium will also help to distinguish elevation of CK-MB due to skeletal muscle damage alone from elevation due to myocardial injury.[134] In patients with chest trauma, cocaine associated chest pain, and hypothyroidism where CK, CK-MB are elevated; cTnI is able to show true myocardial injury from those with false elevation. In marathon runners, more than 80% of samples were positive for CK-MB and in all of these samples cTnI was negative. The main problem with cTnI is that there are several commercial assays available, and each assay differs with respect to normal range, detection limit, and medical decision cut-off limits. Thus there is a lack of standardisation of methodology for cTnI assays. However, cTnI has been reported to show slightly better specificity in situations where there is severe skeletal muscle injury and renal failure.

2.11 CONCLUSIONS AND REMARKS

Based on the aforementioned discussion the markers that are well suited for the early diagnosis of AMI within the time interval 0 - 6 hours after symptom onset are

myoglobin, and CK-MB isoforms. Creatine kinase-MB mass measurement is suitable in the 6 - 24 hours interval, CK-MB based on activity measurement is more sensitive in the 12 - 24 hours interval,[25] and the other cardiac markers like total CK, LDH and isoenzymes, cTnT, and cTnI are most reliable after 12 hours from symptom onset. Decision-making regarding triage and treatment of patients should not be based on a single measurement of cardiac markers alone because of the time delay required for the marker to exceed the upper limit of normal (false negatives).[135] Based on the recent recommendations by the ESC/ACC, cTnI and cTnT are the best markers for the confirmation of AMI. Creatine kinase-MB (preferably mass) is the second best marker in the absence of troponins assays.[20]

The management of patients who present with chest pain suspicious of ACS is best dealt with in specifically designated units that have rapid access to specific equipment (ECG, echocardiogram) and facilities (to measure cardiac markers), and with appropriate staffing. Acute chest pain units attached to A&E departments can accommodate patients for 12 hours, so that appropriate investigations can be carried out quickly to identify patients with evolving infarction and unstable angina who are at increased risk early after presentation.

A serial combination testing of a sensitive early marker (e.g. myoglobin) and one of the cardiac specific troponins offers the best approach.[135;136] Two serial testing at least within 12 hours after symptom onset provide reliable sensitivity and specificity for detecting ischaemia and evolving infarction within the time interval required for the implementation of reperfusion therapy. This 12 hours strategy also identifies

patients at low-risk for acute ischaemic events.[137;138] These serial testing should not be viewed as an expensive strategy for the following reasons; (1) It is cost effective because within 12 hours after admission, decisions can be made regarding triage, implementation of treatment and discharge with great confidence and without compromising the safety of the patient. This strategy will optimise treatment and result in a reduction of inappropriate admissions and discharges; (2) This protocol will not be applicable to patients with unequivocal diagnosis of AMI i.e. significant ST segment elevation, but will be used in selected groups of patients with chest pain and either atypical clinical history or non-diagnostic ECG on admission. Early rule in of AMI (or UA) patients based on serial cardiac marker results or evolving ECG changes will optimise the early implementation of appropriate care.

Figure 3 shows flow chart with a suggested protocol for triage and management of patients who present to the A&E department with acute chest pain. High clinical probability of AMI means typical prolonged ischaemic symptoms in patients of the appropriate age and sex with evidence of risk factors or documented evidence of CAD or previous history of ACS. Low clinical probability of AMI means atypical chest pain in a young male or female without risk factors or documented evidence of CAD or previous history of ACS. Positive ECG changes of AMI means the presence of significant ST segment elevation ± new Q-waves or new left bundle branch block (LBBB). Atypical or equivocal ECG changes of AMI mean ST segment depression, T-wave inversion, ST elevation in one lead only, and Q-waves in patients with known cardiomyopathy or left ventricular hypertrophy. Confounding ECG means the presence of old LBBB, or paced rhythm, or any other conditions that may preclude the correct interpretation of the ischaemic changes present on the ECG.

Patients with high clinical probability of AMI but no definite ECG changes of AMI are admitted to chest pain units for rapid monitoring of evolving ischaemic changes. Serial blood sampling at least twice during the first 12 hours after symptom onset with serial ECG recordings will allow appropriate management of most patients. Positive ECG changes of AMI at any time interval during this first 12 hours ± cardiac marker rise consistent with AMI result in the rapid implementation of reperfusion therapy and transfer to CCU. Patients with elevated cardiac markers + ECG changes of ischaemia e.g. ST segment depression, T wave inversion (no definite ischaemic ECG changes of AMI) are treated as non-ST elevation MI with newer antiplatelet and antithrombotic medications and admitted to CCU. These patients may be considered for early investigations by means of coronary angiography ± angioplasty.

Patients with no increases in cardiac markers and without conclusive evidence of ischaemia on ECG can either be discharged or admitted and considered for further investigations of CAD, or investigated for alternative diagnoses other than ischaemic chest pain e.g. pulmonary embolism or peptic ulcer disease. Borderline cases with no clear cut ECG or cardiac markers changes are admitted to regular unit for further investigation by means of exercise tolerance test (ETT), stress thallium, or angiography. Patients with chest pain and low clinical probability of AMI, no significant ECG changes of ischaemia, are screened for cardiac ischaemia using specific cardiac marker between 0 hour and 12 hours. Negative screening results in the discharge of patients, whereas positive screening results in their admission and further investigation and treatment.

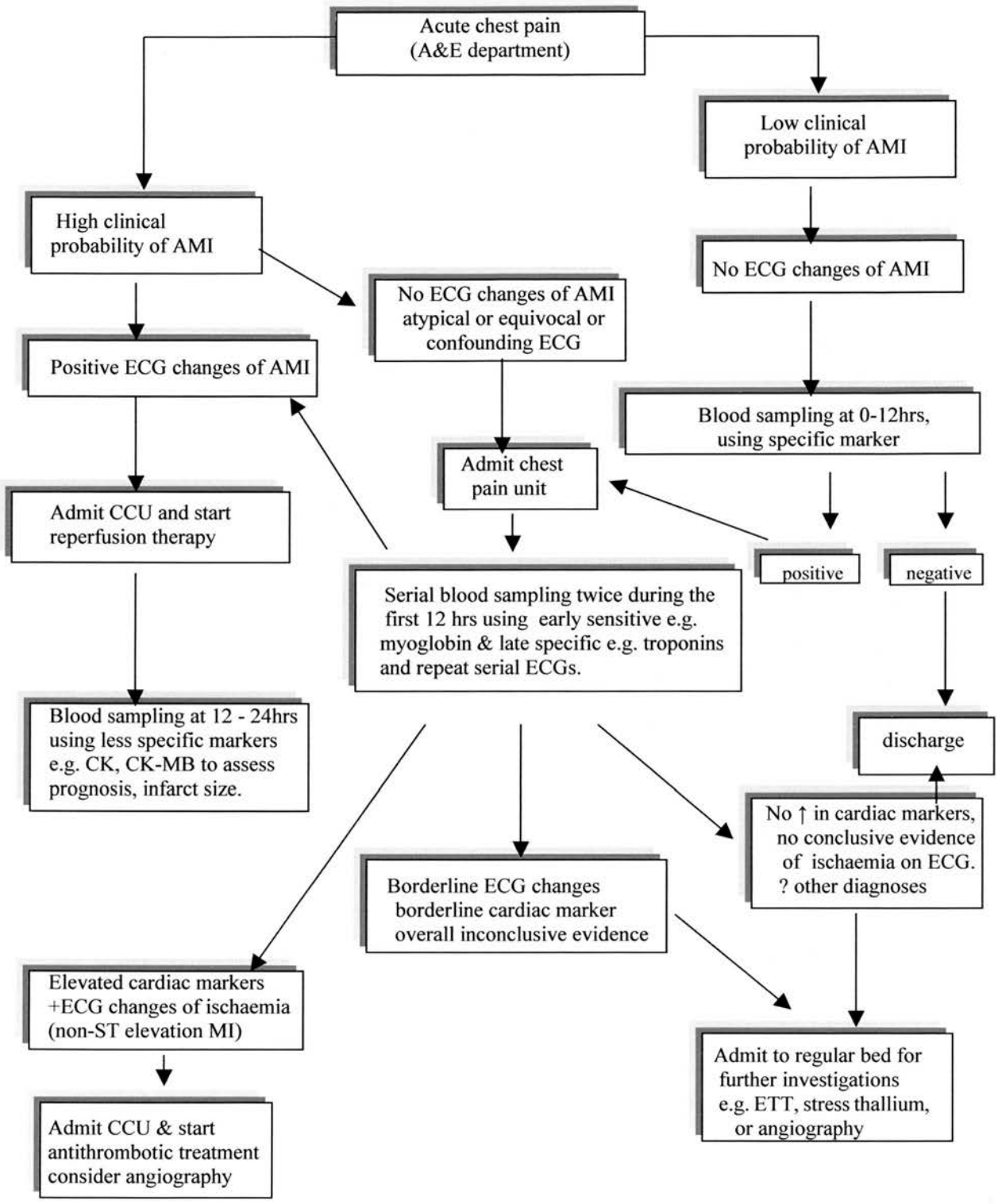

Figure 3. Shows flow chart with a suggested protocol for triage and management of patients who present to the A&E department with acute chest pain. Abbreviations; A&E, accident and emergency department; AMI, acute myocardial infarction; ECG, electrocardiogram; +, positive.

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CHAPTER 3

DO WE NEED NEW EARLY MARKERS OF MYOCARDIAL DAMAGE: THE POTENTIAL VALUE OF HEART FATTY ACID BINDING PROTEIN

3.1 INTRODUCTION

The fatty acid binding proteins (FABP) describe a family of cytosolic proteins that show a large extent of structural homology and many important differences. They were discovered by Ockner in 1972 in studies on the intestinal absorption of fatty acids.[1] They are called FABP because they exhibit a high affinity for the non-covalent binding of fatty acids. These proteins are widely distributed and are present in the fatty acids metabolising tissues of many mammals. Their presence has also been reported in some species like birds, insects and fish.[2] There are several types and they all have low molecular weight (12 - 15 KDa), but they differ markedly in tissue distribution, concentration within tissue, isoelectric point (PI), binding capacity, and binding specificity. These proteins have been extensively reviewed elsewhere.[3-10] The FABP are relatively tissue specific and are designated by a letter, which refers to their tissue of origin e.g. L-FABP, H-FABP, I-FABP refers to liver, heart and intestine fatty acid binding protein respectively.[11] Tissue specific FABP have also been reported in muscle, adipose tissue, kidney, brain and nerve cells. Tissue specific FABP such as liver (L-FABP) and intestinal (I-FABP) have been used to detect pathologies in these tissues using specific antibodies raised against these proteins.[12;13] Different FABP share between 30 - 80% amino acid sequence homology. Heart and liver contain the highest concentrations of these proteins.[9]

3.1.1 FUNCTION

Fatty acids are the major energy source of the heart.[14] They are also important molecules for the synthesis of membrane lipids and lipid mediators such as prostaglandins, leukotriens, and thromboxanes.[15] In general, 50 - 80% of the heart

energy is provided by lipid oxidation. In extreme cases (sustained exercise), this may rise to 100%. The heart is a poor fatty acid synthesiser and contributes only 0.1% of the total body fatty acid synthesis,[16] yet 10% of the total body turnover of fatty acids is by the heart.[17] Fatty acids are insoluble in the intravascular and extravascular space, and also in the intracellular space. In plasma they are transported bound to albumin, or as part of the lipoproteins complex.[14;18] Heart-FABP is believed to be the intracellular equivalent of albumin for the intracellular transport of the insoluble fatty acids within the cells. Sixty per cent of fatty acids are bound to H-FABP and 50 - 70% of bound fatty acids are of the unsaturated type. These proteins are truly cytoplasmic in the sense that they do not exist anywhere else e.g. plasma or extracellular space under normal conditions.[19;20] Recent work has suggested more complex regulatory functions for these proteins other than just lipid transport.[21;22-27] The precise physiological functions of these abundant proteins are not fully understood at the present time.

3.2 ISCHAEMIA AND ITS EFFECT ON THE HEART

Under normal circumstances the oxygen supply to and the demand of the heart are in balance. During myocardial ischaemia there is a reduction of blood flow through the coronary arteries resulting in the decline of the oxygen supply to the heart tissue, thus causing imbalance between oxygen supply and oxygen demand. Initially this is compensated for by an increase in heart rate and vasodilatation of the coronary arteries. When the oxygen shortage becomes severe and prolonged multiple enzymes in the mitochondria oxidation pathways become inhibited leading to disturbance of fatty acids oxidation and ATP production.[28] Fatty acids and their derivatives fatty

acyle coenzyme A, and fatty acyle carnitine accumulates in the ischaemic cells.[29;31] In the absence of oxygen the cell produces ATP through anaerobic metabolism with accumulation of lactic acid, causing cellular acidosis.

Ischaemia is also associated with increased catecholamine hormone production due to increased sympathetic activation.[28;30;31] The metabolic effects of this hormonal change is further increase in plasma and intracellular fatty acids due to catecholamine induced lipolysis.[31;56] Phospholipases are activated in the ischaemic myocardium and lead to break down of membranous phospholipids, with the formation of lysophosphatides, which are thought to play a role in the pathogenesis of some of the cardiac abnormalities seen in ischaemic heart disease.[32]

The combinations of the following pathological processes; (1) Accumulation of fatty acids; (2) Reduction of ATP; (3) Activation of enzymes that hydrolyse phospholipids ; (4) Increased cellular acidosis; (5) Accumulation of lipid degradation products and their integration with the cell membrane; and (6) The prevention of the wash out of these degradation products during the ischaemic phase, interferes with many cardiac functions. Many ion channels become inhibited leading to failure of ion homeostasis.[33] This leads to cell swelling and the integrity of membranes is disturbed.[34] If reperfusion is not restored quickly during this reversible phase, the ischaemic cells reach the point beyond which the damage becomes irreversible and restoration of reperfusion at that stage has little effect because cell rupture and death occurs. Early reperfusion supplies oxygen and nutrients, washes out degradation and waste products, and helps the cell reverse the damage.

3.2.1 FACTORS THAT AFFECT THE RELEASE OF CARDIAC MARKERS FROM THE MYOCARDIAL CELL AFTER DAMAGE

When the ischaemia is prolonged, cell death occurs. The cell enzymes and proteins are released into the intercellular space. The release of these proteins is thought to be influenced by several factors like their size, location within the cell, their behaviour after release, and route of elimination from the intercellular space. Small proteins like H-FABP (15 KDa), and myoglobin (17 KDa) are released faster than larger molecules like CK-MB (80 KDa) or LDH (130 KDa). Cardiac markers that lie free in the cytosol of the cell like myoglobin, H-FABP, and CK-MB are released faster than compounds that are attached to the structural elements of the cell like cTnT, cTnI, or myosin light chain, which require prior degradation before release.

After their release from the cell some proteins like CK-MB may partially bind to structural elements.[35] Further delay may also result from the route of elimination from the interstitial space. Compounds that reach the circulation by traversing through the endothelium (trans-endothelium) are faster than those compounds that are drained by the lymphatic system. Once in the circulation the marker protein requires to build up concentration to sufficient quantities to exceed the upper limit of normal range for that particular assay. This is influenced by degradation and the rate of elimination from circulation.

3.3 EARLY DIAGNOSIS OF ACUTE CORONARY SYNDROME AND ITS IMPACT ON PATIENTS' CARE

Early diagnosis of ACS based on multiple samples would contribute to patients' care in the following ways.

Triage of patients from accident and emergency department

Biochemical markers of early damage can help with the triage of patients from the A&E department. Those patients with positive results for ischaemia need to be admitted to the CCU or to one of the monitored beds. Those with "true negative" results i.e. after sufficient time for liberation of marker into the circulation can be considered for early discharge if there is a low probability of ischaemia and the patient remain free of recurrence. These strategies will optimise the effective use of expensive resources in the CCU and other acute units for the appropriate groups of high and moderate risk patients.[36-38]

Acute myocardial infarction and non-diagnostic electrocardiogram

Early cardiac markers can be helpful in the diagnosis of AMI in the following situations when there is a high clinical suspicion of infarct, but the diagnostic value of the admission ECG is limited; (1) When the ECG cannot be interpreted or has reduced diagnostic accuracy e.g. the presence of conduction disorders including left bundle branch block (LBBB) or paced rhythm; (2) If Q waves and ST-T changes are already present e.g. old infarcts and digoxin toxicity respectively; (3) With ST-T changes of marked left ventricular hypertrophy (LVH); (4) In posterior infarct or right ventricular infarct which may produce no clear-cut diagnostic ECG changes on the standard 12-lead ECG; (5) When diagnostic changes of AMI are present in one lead only; and (6) In the 30% of patients who have no diagnostic changes on their admission ECG.[36-38;39;40] In clinical practice today reperfusion therapy, thrombolysis or PCI, is only given to patients with clinical evidence of ischaemia and ST segment elevation.

Unstable angina and non-Q wave myocardial infarction

Clinical trials have shown most benefit of treatment in the UA and non-Q wave MI groups with positive biochemical marker evidence of ischaemia. Those patients with UA but no biochemical marker evidence of ischaemia show least benefit (or no benefit) from treatment compared to placebo.[43] Cardiac markers can help with risk stratification of patients early in the course of ischaemia.[43;44] In those patients with UA and non-Q wave MI, early diagnosis results in the admission of these patients to the CCU or to a monitored bed in a higher dependency area. Administration of antithrombotic agents (aspirin, clopidogrel, LMWH, and GPIIb/IIIa receptor antagonists) is associated with a significant reduction of subsequent complications (AMI and death).[45;46] In addition to early identification and implementation of treatment, these patients can have further risk stratification assessments e.g. exercise tolerance test, perfusion scans or angiography and where appropriate PCI or CABG.

Most of the trials evaluating early benefits of antithrombotic treatment in UA and non-Q wave MI patients were based on patients recruited to these trials within 24 - 48 hours of their admission. There have been few studies evaluating these treatments very early in the course of UA and non-Q wave MI i.e. within the first 6 - 12 hours in patients with biochemical marker evidence of ischaemia. This is partly because this group is very difficult to identify with certainty in the early stages. It is not known whether antithrombotic treatment in UA and non-Q wave MI is time-dependent just like thrombolytic treatment in AMI patients. Both conditions share a common pathophysiology although differing in severity. Theoretically, it is possible to assume that early treatment in UA and non-Q wave MI might have better outcome compared

to patients receiving no or delayed antithrombotic treatment. The precise answer to this question is not possible unless a biochemical marker that can identify UA and non-Q wave MI patients in the first 6 - 12 hours is available. Only then may the full benefits of very early antithrombotic treatment in UA and non-Q wave MI patients be realised. Thus biochemical markers could have a potential role in identifying such patients early adding to risk stratification and prognostic accuracy and optimising treatment.

Prevention of inappropriate discharge of patients

In the very early stages of AMI some patients may present with atypical chest pain and non-diagnostic ECG changes. Without an appropriately timed biochemical marker to rule out AMI, these patients could be misdiagnosed and inappropriately discharged. It is estimated that between 2 - 10% of patients with AMI are discharged from A&E departments.[47;48;49] This is more likely to happen in high volume medical institutions where the turn over of patients is high and there is limited availability of beds. Common features of cases of missed AMI include factors like age [young patients], sex [females], ethnic factors, atypical history of chest pain, absence of previous cardiac history, and being reviewed by inexperienced physicians.[47;48]

Financial implications

It is estimated that less than 30% of patients admitted to the CCU with suspected AMI are eventually diagnosed with AMI.[49] Conservative policies that opt for the safe admission of patients without clear-cut diagnosis of ischaemia rather than risk inappropriate discharge, result in the admission of a large number of patients without

ACS. The cost of caring for such patients is very substantial.[49] It has been shown that decisions based on cardiac markers for the triage of patients result in a considerable reduction of this financial burden without compromising the safety of patients.[50]

3.4 HEART FATTY ACID BINDING PROTEIN

Heart-FABP is a small (15 Kda) soluble non-enzyme protein. It is composed of 132 amino acids.[52] It is one of the most abundant proteins in the heart and comprises 5 - 15% of the total cytosolic protein pool in the aqueous cytoplasm. This is equal to 0.5 mg/g wet weight of tissue.[51;53;60;65] Heart-FABP specific to the mitochondria has also been reported.[54] The gene is located on chromosome 1.[55] Heart-FABP binds two molecules of fatty acids and is involved with the delivery of fatty acyle coenzyme A for oxidation with the generation of heart energy in the mitochondria.[53]

Myocardial ischaemia results in a significantly higher level of fatty acids in the plasma and the myocardial tissue, which can be harmful to the heart.[56;57-59] The presence of H-FABP is believed to serve a protective function for the myocardial cells against the oxidation of these fatty acids while still having these substances readily available for the cells metabolic needs. During ischaemia (e.g. AMI), H-FABP leaks out of myocardial tissue and their concentration increase in plasma.[60] The leakage of H-FABP from the myocardium may make the myocardium more vulnerable to the harmful effects of fatty acids during reperfusion and may account for some of the complications seen during reperfusion e.g. arrhythmias. Some reports have suggested another protective role for H-FABP as scavengers of free radicals that are present in the heart during ischaemia.[61;62]

Heart-FABP exists in high concentrations in the heart only. However, this protein is not totally cardiac specific and occurs in other tissue although in a much lesser concentrations.[63;64] It occurs in skeletal muscles in concentrations varying between 0.05 - 0.2 mg/g wet weight of tissue, depending on muscle fibre type studied.[65] It has also been reported in very low concentrations in tissues like the kidney, aorta, testes, mammary glands, placenta, brain, adrenal glands, adipose tissue, and stomach.[63;64]

It must be noticed however that, the presence of H-FABP in these tissues does not necessarily means its presence in all cells of that tissue. Also the evidence was obtained in some of these studies by immunohistochemical methods using antibodies to H-FABP. The different FABP from heart, liver and intestine share between 20 - 35% amino acid sequences homology. Heart, nerve, and adipose tissue FABP share 60 - 80% amino acid sequence homology.[9] Antibodies raised against heart, liver, or intestine in the earlier studies may have up to 5% cross reactivity with each other and have a detection limit of around 1 ng/ml. In addition, antibodies raised against H-FABP have 80% cross reactivity with skeletal muscle FABP.[71]

It is therefore possible that cross reactivity with other FABP or other as yet unidentified protein in these tissues is an alternative explanation for the reported presence of H-FABP in these tissues.[66-70] The newer assays have a much improved sensitivity and can detect H-FABP in concentrations as low as 0.25 ng/ml and the cross reactivity with other tissues FABP is < 0.005%.[72;73] The use of these newer assays might show an accurate picture of the true distribution of H-FABP in the various tissues outside the heart.

3.4.1 THE RATIONALE FOR THE USE OF HEART FATTY ACID BINDING PROTEIN AS A MARKER FOR THE EARLY DIAGNOSIS OF MYOCARDIAL INJURY

The rationale for the use of H-FABP as a marker for the early diagnosis of myocardial injury is based on the following features; (1) The presence of this soluble protein in the myocardium in high concentration; (2) Virtual confinement to the cytoplasmic space; (3) Small molecular size; (4) Relative tissue specificity. The relative distribution of H-FABP outside the heart has been equated with that of CK-MB;[65] and (5) Early release into plasma and urine (within 2 hours) after onset of myocardial injury. Heart-FABP bears a considerable resemblance to myoglobin (a well accepted early marker of myocardial injury within 6 hours) in terms of size, location within the cell, release and clearance kinetics. However, H-FABP is more cardio-specific and this advantage makes it more suitable cardiac marker than myoglobin.[74;110;125]

3.4.2 MEASUREMENT OF HEART FATTY ACID BINDING PROTEIN AND NORMAL RANGE

The method of measurement is based on sandwich enzyme linked immunosorbent assay (ELISA) using two monoclonal antibodies specific for the H-FABP.[23;63;72;75;76] The reasons for using immunological methods for measurement are; (1) Heart-FABP is non-enzyme protein and measurement using enzyme activity is not possible; (2) Using functional capacity of binding fatty acids is also not possible due to the presence of albumin (another fatty acids binding protein) in a large concentration in the serum; and (3) Other methods that require prior extraction of H-FABP could introduce inaccuracies of measurements.

The normal ranges reported for H-FABP in plasma and serum are assay and method dependent. Tanaka et al (1991) has reported the normal range for H-FABP to be 0.0 - 2.8 μg/l;[77] Wodzig et al (1997) reported 0.3 - 5 μg/l as the normal limit;[72] and Tsuji et al (1993) used 3 μg/l [normal range 0.0 - 0.6 μg/l].[79] One study used a cut-off concentration of 19 μg/l [mean ± 2 SD of controls].[78] Fatty acid binding proteins from heart and striated muscle are reported to be similar.[71;80] Heart-FABP is not likely to be found in the blood stream under normal conditions. The normal plasma H-FABP is likely to be due to the continuous release of this protein from damaged skeletal muscle cells.

3.4.3 PLASMA HEART FATTY ACID BINDING PROTEIN AND ACUTE MYOCARDIAL INFARCTION

Heart-FABP was introduced by Glatz in 1988 as a potential novel biochemical marker for the early diagnosis of AMI.[81] This assumption was soon confirmed in many studies.[60;65;78;82;83;77;110] Under normal conditions H-FABP is not present in plasma or interstitial fluid, but is released into the blood upon cellular injury. The cytoplasmic to vascular concentration of H-FABP is of the order of 200,000:1.[84] The plasma concentration of H-FABP under normal conditions is < 5 μg/l. This makes the plasma estimation of H-FABP suitable for the early detection and quantification of myocardial tissue injury. The H-FABP is released into plasma within 2 hours after symptom onset and is reported to peak at about 4 - 6 hours and return to normal base line value in 20 hours.[83] Within the period of 30 - 210 minutes after symptom onset, H-FABP has > 80% sensitivity for the diagnosis of AMI.[78] Within the interval of 0 - 6 hours after symptom onset, the other cardiac markers such as CK, CK-MB mass or

activity, cTnI and cTnT will only be starting to accumulate in the plasma, and their sensitivity has been reported to be around 64%.[85]

3.4.4 URINARY HEART FATTY ACID BINDING PROTEIN AND ACUTE MYOCARDIAL INFARCTION

Urinary indicators of myocardial injury are almost unknown and only myoglobin has been tried as a urinary indicator of myocardial injury.[86-88] Heart-FABP is eliminated from the circulation by the kidney but the precise mode of renal handling of H-FABP is unknown. A rise in serum and urine H-FABP concentration above normal values is seen in patients who present with AMI as early as 1.5 hours after symptom onset.[77] Studies in animals have also shown decreased myocardial tissue content and rising plasma and urine concentrations of H-FABP very early after coronary artery ligation.[60;129] Measurement of plasma or urine concentration of H-FABP was diagnostic of AMI as early as 30 minutes after ligation.

Assays that measure H-FABP in urine samples were able to accurately diagnose patients with AMI and provide reliable estimation of infarct size.[89] The measurement of infarct size from urinary H-FABP may be influenced by several factors like renal blood flow, perfusion pressure, glomerular filtration rate, tubular absorption, and diseases of the kidney. Measurement of urinary and plasma H-FABP in the presence of kidney diseases may lead to underestimation and overestimation respectively of the size of infarct due to impairment of excretion of H-FABP.[128] Heart-FABP circulates for longer time (> 25 hours) after AMI in the presence of renal failure.[78]

Several sensitive assays that can measure H-FABP in urine samples are available.[76;77;79;89]

3.4.5 LIMITATIONS OF HEART FATTY ACID BINDING PROTEIN ASSAYS

The human skeletal muscle FABP has been reported to be identical or very similar to that of H-FABP.[71] The 'H-FABP' content of skeletal muscle is variable and is reported to range between 0.05 - 0.2 mg/g wet weight of tissue, depending on muscle type.[80;90] Skeletal muscle damage during the course of AMI e.g. intramuscular injections, electric cardioversion, and traumatic cardiopulmonary resuscitation may result in the leakage of H-FABP and this could interfere with the results of the assays.[90] Diagnosis of AMI in these groups of patients using H-FABP alone can be difficult. Heart-FABP is increased in the plasma of healthy volunteers after strenuous exercise as a result of release from skeletal muscle, but in these patients the ratio of myoglobin to H-FABP is above the 6% cut-off value considered specific for skeletal muscle injury.[91] One study however did not report any increase of H-FABP in urine or serum in a patient with crush injury, whereas myoglobin was markedly elevated.[77]

Surgery (both cardiac and non-cardiac) causes elevation of H-FABP concentration. Heart-FABP is excreted by the kidney and renal insufficiency results in decreased clearance of H-FABP thereby elevating the concentration and prolonging the circulation time.[92] In situations of AMI and renal failure, measurement of plasma H-FABP could lead to overestimation of myocardial infarct size, and could interfere with its use for the detection of re-infarction.[128] Fortunately most of these (if not all) could be ruled out by careful attention to history taking and routine blood tests like

urea and creatinine to exclude renal failure and should not interfere with the specificity of H-FABP assay for the vast majority for clinical purposes.

3.4.6 ISOFORMS OF HEART FATTY ACID BINDING PROTEIN

Heart-FABP could be an ideal early marker of myocyte injury in ACS if there is an isoform of this protein that is 100% specific to the heart. Several investigators have addressed the possibility for the existence of possible isoforms of H-FABP.[93] Glatz et al (1985) isolated FABP from the human heart. This protein had molecular weight of 15000 Dalton; isoelectric point of 7.5; and the amino acid sequence was not reported.[94] Unterberg et al (1986) reported the isolation of H-FABP with a molecular weight of 15500 Dalton; PI of 5.3; and amino acid sequence (2 residues of cysteine).[95] Offner et al (1988) also reported the isolation of H-FABP with a molecular weight of 14768; PI of 5.25; and amino acid sequence (contains no cysteine residues).[52] These results suggest that isoforms of H-FABP may exist in the human heart. This view is also supported by some animal studies in rats by the finding that the nucleotides sequence of two rat hearts FABP cDNA differ in the 5' and 3' untranslated regions. Existence of H-FABP isoforms have also been reported in bovine species.[96-98]

There is still some lack of clarity as to whether FABP from heart and skeletal muscle are identical or very closely related. In an abstract Said and Schatz (1986) reported the purification of FABP from rat muscle and heart.[99] Although immunologically related the proteins were different as judged by their ultraviolet spectra, electrophoretic mobility, and their capacity to bind fatty acids. Fatty acid binding

protein from heart and skeletal muscle show a strong cross reactivity (80%) with each other in immunochemical studies and therefore must be similar or closely related.[71] Other investigators have claimed both proteins to be similar.[100;101] Further studies using more sensitive techniques are needed to resolve this matter.

3.4.7 HEART FATTY ACID BINDING PROTEIN AND MYOGLOBIN

Myoglobin has been introduced as a marker for the early diagnosis of AMI within 3 hours after symptom onset.[102-106;123] In a study by Bhayana (1994) myoglobin was found to be superior to CK-MB mass and cTnT for ruling out AMI within the period of 3 - 6 hours after symptom onset.[107] Myoglobinuria has long been known to be useful in the diagnosis of AMI.[86;108] Myoglobin and H-FABP share many key features;[109] (1) Low molecular weight proteins (17 and 15 Kda respectively); (2) Found in abundant concentrations in the cytosol of myocardial cell; (3) Provide substrate for mitochondrial oxidation (oxygen and fatty acids respectively); and (4) Both are released within 2 hours after symptom onset, peak early (6 hours) and return to normal base line concentration within 24 hours.

Both proteins are present in the heart and skeletal muscle in different concentrations. The concentration of myoglobin in heart and skeletal muscle is 2.5 and 4.0 mg/g wet weight of tissue respectively. The H-FABP concentration in heart and skeletal muscle is 0.5 and 0.05 - 0.2 mg/g wet weight of tissue respectively.[90;109] The myoglobin content of skeletal muscle is twice that of the heart. The H-FABP content of skeletal muscle is only 10 - 50% of that of the heart. The normal plasma concentration of H-FABP (< 5 μg/l) is 10 to 15 fold lower than that of myoglobin (20

- 80 μg/l). Heart-FABP is therefore more cardio-specific than myoglobin and because of this superior specificity, the use of H-FABP as a marker for the early diagnosis of AMI seems preferable.[74;109;110]

The normal ratio of myoglobin to H-FABP in myocardium is about 5, whereas the ratio of myoglobin to H-FABP in skeletal muscle is in the order of 21 - 70 (depending on muscle type).[90] The main disadvantage of myoglobin and H-FABP as early markers of myocardial injury is lack of total specificity due to their presence in skeletal muscle. Severe skeletal muscle injury may result in the release of both proteins in sufficient quantity to interfere with the specificity of the assay. Both proteins are released into plasma after injury at about the same time and in a ratio similar to the concentration of the proteins in the tissue of origin, therefore the measurement of the myoglobin to H-FABP ratio could be a useful index for the discrimination between cardiac and skeletal muscle damage.

A myoglobin to H-FABP ratio that is around 5 is considered to be specific for the heart and a ratio between 21 - 70 is more specific for skeletal muscle damage.[90] The combination of the two markers in a ratio has been reported by some investigators to increase the diagnostic specificity for the diagnosis of AMI more than relying on either marker alone. However, the use of this ratio should not be a rigid criterion as overlaps do occur. Some investigators did not support additional value of myoglobin to H-FABP ratio over the measurement of H-FABP alone.[90;112;110]

3.4.8 HEART FATTY ACID BINDING PROTEIN AND UNSTABLE ANGINA

Heart-FABP may be useful for the identification of patients with UA. However, there have been no detailed studies evaluating its usefulness for the diagnosis, or risk stratification in patients with UA. In a study by Tsuji (1993) using H-FABP with a normal range of 0.0 - 0.6 μg/l and an upper limit of normal of 3 μg/l, in patients suspected with a diagnosis of UA, the concentration of H-FABP was 3.5 ± 1.7 μg/l. In patients with AMI the range was 12.3 ± 9.6 μg/l.[79] Other investigators have also observed an increase in H-FABP serum concentration in UA patients.[110] One study reported that H-FABP was normal in a patient diagnosed with UA.[78] In this study a relatively high upper limit of normal concentration was used (19 μg/l) and this high cut-off concentration may have affected the sensitivity or could be due to UA without myocardial necrosis. At present we have limited information on the ischaemic threshold at which H-FABP leaks out. Preliminary results from our pilot study have suggested a possible role for H-FABP in the diagnosis of UA. There is a need for studies that are properly designed to look specifically at the role of H-FABP for the diagnosis of patients with UA.

3.4.9 HEART FATTY ACID BINDING PROTEIN AND ACUTE MYOCARDIAL INFARCTION AFTER SURGERY

Surgery is associated with various complications. Peri-operative or post-operative development of AMI is one of more serious. The risk of peri-operative AMI with major non-cardiac surgery is only 1 - 2% in patients over 40 years. This risk increases to 3 - 10% in elderly patients and those with cardiovascular diseases.[113] In cardiac surgery peri-operative myocardial injury is the most common cause of

morbidity and mortality. The diagnosis of peri-operative or post-operative AMI poses a challenge in cardiology. The ECG may show non-specific ST segment and T wave changes only. The diagnosis of AMI using biochemical markers in the setting of surgery is difficult because there are many factors that could interfere with the tests. Inflammation and per-operative trauma could lead to the release of markers from the myocardium as well as from skeletal muscle. The ability of the cardiac markers to diagnose myocardial injury will depend primarily on their cardiac specificity and on the surgery itself i.e. cardiac or non-cardiac. In non-cardiac surgery the marker that is cardiac specific will detect myocardial injury, because its concentration in the blood is low or undetectable before surgery and its source of release after surgery is the heart only.

The situation is different after cardiac surgery because there is damage to the heart as well as to other tissue structures including skeletal muscle. The release of markers is from both infarcted cardiac tissue (as a result of vessel occlusion) and the injured non-infarcted cardiac tissue (as a result of surgical trauma). In this setting the post-operative concentration of the specific marker is set to increase even in the absence of AMI due to the traumatic release as a result of the surgery itself. This will require a careful validation of a cut-off value to diagnose AMI and to take into account this 'expected' increase in the cardiac marker concentration.

Heart-FABP has been shown to peak early and may be useful for the early detection of myocardial injury after surgery. The plasma concentration of H-FABP is increased relatively early compared to CK-MB and cTnT after aortic declamping in CABG

surgery. The time to reach the peak concentration was significantly shorter for plasma H-FABP [1.4 ± 0.5 hours] than for CK-MB [2.5 ± 0.5 hours] or cTnT [6.6 ± 1.3 hours].[115] Similar finding were also reported in other studies.[116] Heart-FABP was not increased in low-risk patients after CABG surgery without cardiopulmonary bypass.[117] The discriminative value of myoglobin to H-FABP ratio for the detection of AMI after cardiac surgery is less clear and ranged between [11.3 ± 4.7] to [32.1 ±13.6].[90]

3.4.10 HEART FATTY ACID BINDING PROTEIN AND DETECTION OF REPERFUSION

Establishment of reperfusion in the infarct-related artery is associated with significant reduction in morbidity and mortality. However, thrombolytic treatment is associated with successful reperfusion in only 50 - 80%.[118;137] New alternative treatment options are being examined to try to see the best way to deal with patients who do not reperfuse after the first course of thrombolytic treatment.[119-121] Clinical trials are now underway randomising patients who do not reperfuse to either another course of thrombolytic treatment, PCI, or conservative treatment.

In clinical practice reperfusion is ascertained indirectly by the reliance on clinical features such as disappearance of chest pain, resolution of ST segment elevation, and occurrence of reperfusion arrhythmias (e.g. accelerated idioventricular rhythm, bradycardia, and atrioventricular block).[122] The reliance on clinical features alone is not sensitive for the detection of reperfusion. Heart-FABP has been reported to be a sensitive marker for the detection of reperfusion after thrombolytic treatment. Abe et al (1996) has demonstrated that a rise of H-FABP ratio greater than 1.5 or more

(compared to pre-treatment concentration), 30 minutes after thrombolytic treatment is associated with 100% accuracy for the detection of reperfusion. This accuracy drops to 94% at 60 minutes after thrombolytic treatment.[82] The advantage of using H-FABP is that reperfusion is ascertained very quickly and in some studies as early as 15 minutes. In a study by Ishii et al (1995) the predictive accuracy of H-FABP ratio > 1.8 for the detection of reperfusion within 60 minutes of the initiation of treatment was 93% at 15 minutes, 98% at 30 minutes, and 100% at 60 minutes after reperfusion.[124] The few other studies that examined the role of H-FABP for the detection of reperfusion also support this view.[125]

3.4.11 HEART FATTY ACID BINDING PROTEIN AND DETECTION OF RE-INFARCTION

Re-infarction is a well-recognised complication of initial AMI. This may be attributable to re-occlusion of the infarct-related artery after an initial successful reperfusion or to a vessel occlusion at another site. Re-infarction can manifest as a recurrence of chest pain or haemodynamic deterioration such as hypotension, acute pulmonary oedema, and arrhythmia with or without new ST segment changes. In the presence of AMI, recurrence of chest pain with or without ST segment changes could be misinterpreted and without a confirmatory test, the diagnosis of re-infarction could be missed. Re-infarction carries a worse prognosis, and necessitates further intervention with thrombolytic treatment, PCI, or urgent CABG. It is vital that this complication is recognised and appropriate interventions implemented. The most conclusive method for the confirmation of re-infarction is coronary angiography. Alternatively, the diagnosis of re-infarction can be confirmed by cardiac markers

measurement. The high sensitivity, simplicity, cost and safety profile make cardiac markers a practical option for the detection of re-infarction.

The ideal marker that can detect early re-infarction must be released early and cleared rapidly from the circulation, thus permitting a quick return to pre-infarction level. Heart-FABP fulfil these features appearing within 3 hours after infarction, peaks early at about 5 hours and returns to base line concentration in about 20 hours after symptom onset.[78] Re-infarction is shown by a rapid rise in H-FABP concentration in serum compared to the previous value. Heart-FABP can detect re-infarction if it occurs 10 hours after symptom onset.[90] The other cardiac markers like CK-MB, cTnI, cTnT, and LDH take several days to return to the pre-infarction level, and thus are not reliable for the detection of re-infarction.

3.4.12 HEART FATTY ACID BINDING PROTEIN AND ESTIMATION OF INFARCT SIZE

The measurement of infarct size after AMI can have important prognostic implications.[111;126;127] It may also have therapeutic applications in the selection of patients for the implementation of ACE inhibitor or anticoagulation treatment. Those patients with large infarcts who are deemed at higher risk for complications such as congestive cardiac failure, adverse remodelling of the ventricles or intramural thrombosis may be selected for these treatment options. The measurement of infarct size however is not done routinely. This may be partly due to the complicated blood sampling protocol that is both prolonged (over several days) and time consuming, but is necessary to establish a complete time-concentration curve profile used in this type of measurement.

In clinical practice infarct size is estimated indirectly (or qualitatively) by methods like nuclear perfusion imaging, echocardiography (wall motion abnormalities, measurement of ejection fraction), ECG changes (like the number of leads involved or the presence of conduction abnormalities in anterior infarction), the presence of heart failure, and by reference to the maximum rise of cardiac markers concentrations after infarction. Accurate measurements of infarct size is possible using nuclear studies but are again not a practical option for routine use, because it is expensive, requires high technology and exposes patients to radiation.

Cardiac markers offer an alternative possibility for the estimation of infarct size. The rapid and quantitatively robust release of H-FABP into plasma after symptom onset and its rapid clearance from the circulation within 24 hours, makes it potentially suitable for the early estimation of infarct size, provided that blood is sampled sufficiently frequently.[128] Sohmiya et al (1993) showed good correlation between myocardial infarct size measured from plasma H-FABP and infarcted myocardium estimated from triphenyl tetrazolium chloride (TTC) staining.[89]

A study by Glatz et al (1994) using H-FABP for the early estimation of infarct size, showed good correlation between H-FABP, CK-MB and α-hydroxybutyrate dehydrogenase (α-HBDH) for the estimation of infarct size. The advantage of H-FABP is that, this measurement is completed much earlier than the other two markers 24 hours, 48 hours, and 72 hours for H-FABP, CK-MB, and α-HBDH respectively.[84]

3.4.13 EXCRETION OF HEART FATTY ACID BINDING PROTEIN

It is not clear at present whether H-FABP reaches the circulation trans-endothelially, via the lymphatic system or both, after its release from the cell into the intercellular space. The rapid appearance in blood may suggest the former route. The route of elimination from the circulation is assumed to be the kidney. This assumption was based on direct and indirect evidence. The indirect evidence comes from observations in clinical studies. (1) Patients presenting with AMI demonstrated rising levels of plasma and urine H-FABP within 1.5 hours after symptom onset.[79] (2) Patients with renal insufficiency have raised levels of H-FABP, and the circulation time is prolonged compared to those who have normal renal function.[78;128]

The direct evidence comes from radioactive iodine-H-FABP excretion studies in animals. The compound is concentrated within the kidney and appears in the bladder within very short period after intravenous injection.[89] However, the reported amount of radioactive H-FABP excreted in the urine is variable. One study reported that only 14 - 29% of the total intravenous dose injected was excreted in the urine. The total clearance was 0.33 ml/min and the half-life value of total elimination is estimated to be 270 minutes.[129] A study by Sohmiya (1993) reported only 6.5 ± 1.0 recovery of the radioactive H-FABP in the urine, and a disappearance half-time was 27.5 ± 8.4 minutes. They suggested that the administered H-FABP might be degraded elsewhere in the body and the undegraded H-FABP is excreted in the urine. The authors concluded that their results were comparable to the excretion studies of myoglobin (known to be excreted by the kidney).[130] More studies are required to establish the exact route of elimination from the intercellular space and the

circulation. Moreover, the metabolism of H-FABP has not yet been fully elucidated and is necessary to study the metabolism and excretion of H-FABP under normal and disease conditions.

3.4.14 PATHOLOGICAL CONFIRMATION OF ACUTE MYOCARDIAL INFARCTION USING ANTI-HEART FATTY ACID BINDING PROTEIN ANTIBODIES ON AUTOPSY MATERIALS

Pathological confirmation of AMI is possible using autopsy materials of the heart. The diagnosis can be established microscopically or macroscopically using immunohistochemical methods. Haematoxylin & Eosin (H&E) staining of the tissue sections establish the microscopic diagnosis, whereas the macroscopic diagnosis is based on nitrue blue tetrazolium (NBT) staining methods. This macroscopic technique reflects the intracellular activity of the enzyme dehydrogenase. In viable tissue this enzyme is able to convert the NBT into dark blue insoluble pigment (formazan), while infarcted tissue remains unstained.[131] These two methods are only positive after at least 4 - 6 hours after AMI.

Anti-H-FABP has been used for the confirmation of AMI on autopsy materials. Using anti-H-FABP it was possible to diagnose infarcts less than 4 hours old. In all biopsies that were positive by either H&E or NBT staining, the anti-H-FABP staining showed an absent or noticeably decreased staining of H-FABP in these tissues. Some biopsy material from patients with AMI who died within 4 hours were positive using anti-H-FABP but the H&E and NBT staining were negative. The authors of the study concluded that anti-H-FABP antibody is more sensitive than

either H&E or NBT staining methods for the detection of subtle changes of AMI or very small or very recent (< 4 hours) AMI on autopsy materials.[132;133]

3.5 DISCUSSION AND SUMMARY

Heart-FABP is a novel protein with potential for the early diagnosis of AMI, early detection of re-infarction, detection of reperfusion, and early estimation of infarct size. However, there is still some skepticism in the clinical practice about its additional value compared to the currently available markers such as myoglobin, CK-MB and troponins.[85] Many studies have convincingly shown that the latter markers (with the exception of myoglobin) are relatively insensitive for the early detection of AMI in the first 6 hours after symptom onset.[85]

Heart-FABP is a small protein that is abundant in the heart and has low concentrations in the blood and in tissues outside the heart. It appears in the blood as early as 1.5 hours after symptom onset, peaks around 6 hours and returns to base line values in 24 hours. These features of H-FABP make it an excellent marker for the detection of AMI. A consistently negative serial samples within 6 hours after symptom onset can be used to rule out AMI with great confidence. Measurement of H-FABP before and at 30 or 60 minutes after the administration of thrombolytic treatment can detect reperfusion of the infarct-related artery with high sensitivity and permit further reperfusion therapy to be planned for those patients who do not reperfuse successfully. Early re-infarction is a well-recognized complication after initial infarct. Given the release kinetics of H-FABP it is more suited for the detection of re-infarction than other markers. The accurate estimation of myocardial

infarct size has important prognostic and therapeutic applications. Heart-FABP can provide a reliable estimate of infarct size. The advantage of H-FABP over other markers is that this measurement can be provided within 24 hours of admission.

Anti-H-FABP antibodies may also have a role in the pathological confirmation of AMI. This is especially so when there is a high clinical suspicion of AMI but the H&E and NBT staining are negative. It is premature to attach specific clinical value for the detection of AMI from measurement of H-FABP in the urine. Further studies are needed to examine and establish the exact renal handling and metabolism of H-FABP under normal and disease states. Until such essential details are available it can only be assumed that, if H-FABP is excreted mainly by the kidney, urinary H-FABP might offer an alternative and important method for the detection of AMI. Urinary H-FABP testing might be useful as a qualitative test for general practitioners to rule in or rule out AMI in the community.

Heart-FABP distribution outside the heart has been equated with that of CK-MB, which is currently regarded as the gold standard marker for the diagnosis of AMI.[65] Creatine kinase-MB lacks the required sensitivity to be of value for the very early diagnosis of AMI in the first 3 hours after symptom onset. During this interval the sensitivity of CK-MB and H-FABP for the diagnosis of AMI was 20% and 91.4% respectively.[79] Heart-FABP shares many key features with myoglobin but is more cardio-specific because its concentration in the skeletal muscle is only a fraction of that of myoglobin. The normal concentration of H-FABP in the blood is 10 - 15 fold lower than myoglobin. As compared to the troponins, H-FABP is less cardiac

specific. The value of cTnT and cTnI for the late diagnosis of AMI and for the diagnosis and prognosis and risk stratification of patients with UA is well-established. However, these markers have little role in the early diagnosis of AMI i.e. within the first 6 hours after symptom onset. They achieve their greatest sensitivity and specificity in the interval 12 - 16 hours after symptom onset. Creatine kinase-MB isoforms (CK-MB1 to CK-MB2 ratio) have also been reported to be sensitive early markers for the diagnosis of AMI within 6 hours. So far there have been no studies comparing H-FABP and CK-MB isoforms for the early diagnosis of AMI. The initiation of such study is very important and will help provide useful comparison between these two early markers.

SUMMARY

Heart-FABP is a sensitive marker for the detection of AMI but is not 100% cardiac specific, because of its presence in tissues outside the heart. However the features of H-FABP, which combine very early release after onset of symptoms and relative cardiac specificity, makes it one of the markers with great potential. Serial measurement of H-FABP within 24 hours after symptom onset can; (1) Define patients with AMI who need CCU admission; (2) Distinguish patients who reperfuse their infarct-related artery from those who do not and in need of further intervention as early as 30 minutes after starting thrombolytic treatment; (3) Detect re-infarction if it occurs 10 hours after symptom onset; (4) Permit accurate estimation of myocardial infarct size and thus provide information concerning prognosis.

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CHAPTER 4

MATERIALS AND METHODS

4.1 MEASUREMENT OF HEART FATTY ACID BINDING PROTEIN

4.1.1 HEART FATTY ACID BINDING PROTEIN ANTIBODY

The H-FABP antibody used for the assay of H-FABP in serum was purchased from HyCult biotechnology b.v (Hbt HK401, Cambridge-UK). The Hbt human H-FABP ELISA kit is intended for the quantitative and qualitative measurement of natural human H-FABP in plasma or serum and in autopsy materials respectively. The principal of the assay is based on a sensitive, non-competitive ELISA of the antigen capture type (Sandwich ELISA) (Figure 1). The analytical sensitivity of the assay determined by 24 replicates of the sample dilution buffer within one ELISA plate was 0.206 ± 0.047 μg/l (mean ± 2SD (i.e. 97.5 percentile) = 0.301 μg/l). The assay has a measurable concentration range of 0.25 - 25 μg/l and shows no cross reactivity with human intestinal or liver FABP.[1]

4.1.2 PRINCIPLES OF THE TEST

Diluted samples and standards are incubated together with peroxidase conjugated second antibody (tracer) in microtiter wells coated with antibodies recognising human H-FABP. During this incubation, human H-FABP is captured by the solid bound antibody. If human H-FABP is present in the sample, the tracer antibodies will bind to the captured H-FABP. Unbound material present in the sample and excess tracer is removed by washing, and substrate tetramethylbenzidine (TMB) is added to the wells. Colour develops proportionally to the amount of H-FABP present in the sample. The enzyme reaction is stopped by the addition of citric acid and the absorbence at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbence versus the corresponding concentrations of

defined standards. The human H-FABP concentration of diluted samples with unknown concentrations, which are run concurrently with the standards, can be determined from the standard curve. The final concentration of H-FABP in the sample is obtained by multiplying the value obtained by the dilution factor.

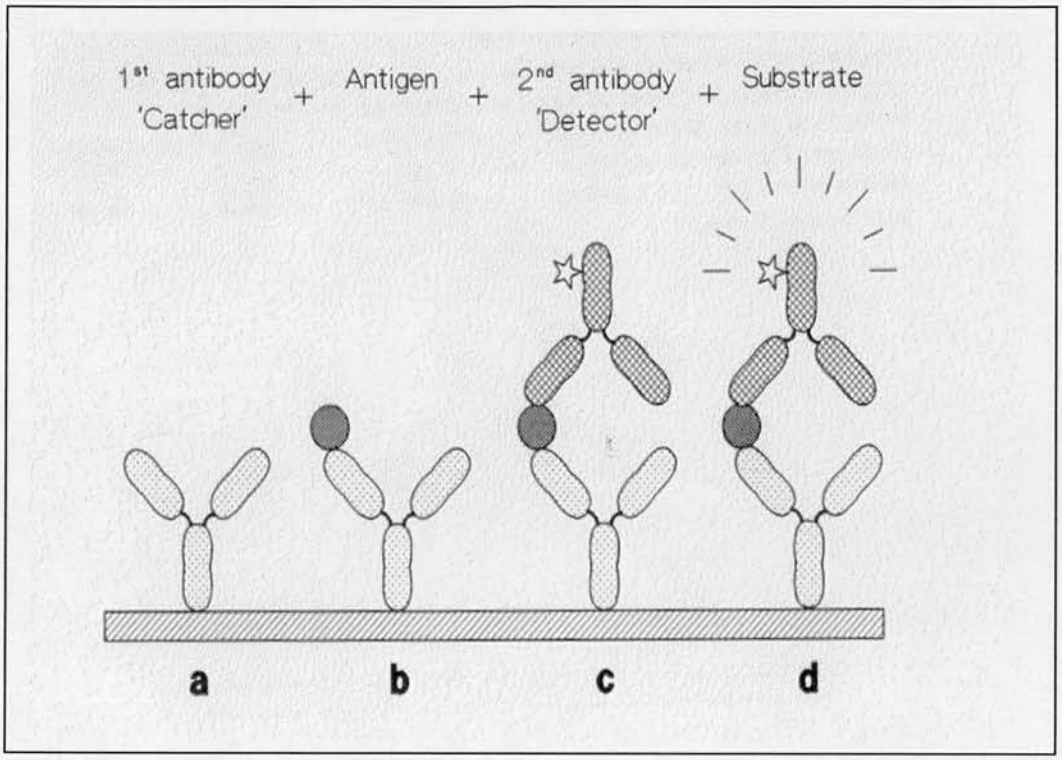

Figure 1. Direct ELISA method (capture or sandwich type). A - the monoclonal H-FABP antibody is immobilised on the microtiter plate. The sample containing H-FABP is incubated with this antibody, b - the H-FABP protein present in the sample adhere and is bound by the immobilised H-FABP antibody, c - incubation of the wells with a second H-FABP antibody labeled with an enzyme, thus the H-FABP become sandwiched between the two H-FABP antibodies. The second H-FABP antibody is directed against different epitope of the H-FABP protein, and d - the last step involves incubation of the complex with a substrate solution suitable for the enzyme, followed by stopping the enzyme reaction and spectrophotometric reading of the wells.

4.1.3 MATERIALS FOR HEART FATTY ACID BINDING PROTEIN MEASUREMENT

Concentrated wash buffer (20 ml) containing Tween 20; concentrated dilution buffer (5 ml); protein stabilised phosphate buffered saline; preservative, 2-chloroacetamide;

freeze-dried human H-FABP standard concentration, approximately 60 ng/ml in protein stabilised buffer; freeze-dried tracer, peroxidase-conjugated antibody to human H-FABP in protein stabilised buffer; tetramethylbenzidine Substrate (6 ml); substrate buffer (6 ml); blocking solution (22 ml) containing citric acid 2.0 M; ELISA Plate (96 wells) coated with anti-human H-FABP antibody (12 strips per plate); Frame for microwell strips and adhesive covers for microtiter plates.

4.1.4 PREPARATIONS

Samples and reagents are brought to room temperature (18 - 25°C) before use. Heart-FABP microtiter plates are removed from the storage bags and the strips to be used are numbered with a laboratory marker. Unused test wells are returned to the storage bag with desiccant, sealed and stored at 2 - 8°C. The standard and tracer are reconstituted by an injection of 1 ml distilled water into each vial. The concentrated dilution buffer is diluted by adding 5 ml of concentrated dilution buffer to 50 ml with distilled water, which is enough for 96 tests. The tracer is reconstituted by adding 5 ml of the dilution buffer to the vial of reconstituted tracer. Twenty milliliters of 20X concentrated wash buffer is diluted with 400 ml of distilled water, which is enough wash buffer for 96 tests.

4.1.5 ASSAY PROTOCOL

1- Preparation of the heart fatty acid binding protein standard series. The standard series is made by diluting the reconstituted standard in dilution buffer in polypropylene tubes in order to achieve a standard range from approximately 250 to 25,000 pg/ml (0.25 - 25 µg/l). Eight polypropylene tubes are used numbered 1 - 8.

Tube 8 is set aside with 250 µl dilution buffer and used as control value. Tubes 2 - 7 are filled with 150 µl dilution buffer. One hundred microlitres reconstituted standard is transferred to tube number 1 and diluted 2:5 further by mixing well and pipetting 100 µl over to tube 2 and again 100 µl to the next tube and so on until tube number 7. Tubes 1 - 8 are used as the standards in the assay.

2- Dilution. Serum samples to be tested are diluted 1:10 in dilution buffer in polypropylene tubes. The desired number of marked tubes is filled with 180 µl dilution buffer. Twenty microlitres serum is added to the corresponding tube.

3- Fifty microlitres of the diluted tracer is added to each well.

4- Subsequently 50 µl in duplicate is transferred from each standard, each unknown diluted sample and the controls to the assigned wells, following the scheme on previously prepared data collection sheet. A clean pipette tip is used for each transfer.

5- An adhesive cover is applied to the tray. The tray is gently taped to eliminate any air bubbles trapped in the wells.

6- The tray is incubated for 60 minutes at room temperature.

7- The TMB colouring solution is prepared just prior to the end of the incubation period by mixing 6 ml TMB with 6 ml substrate buffer in a clean container, which is

enough for 96 tests. Tetramethylbenzidine substrate solution is kept in the dark (i.e. wrapped in aluminum foil) and used within 15 minutes after preparation.

8- Washing. The adhesive cover is removed carefully. The plate is emptied by inverting the plate and shaking contents out over the sink. The plate is kept inverted and taped dry on a thick layer of tissues. Two hundred microlitres of diluted wash buffer is added to each well, left for 20 seconds, and the plate is emptied as above. The washing process is repeated three times, and then the plate is emptied to remove the remaining wash buffer.

9- One hundred microlitres of the prepared TMB substrate solution is added to each well.

10- The tray is covered with the adhesive cover and incubated for 15 minutes at room temperature (18 - 25°C) avoiding exposure to strong light (i.e. wrapped in aluminum foil or placed in dark space).

11- The reaction is stopped by adding 100 µl of blocking solution with the same sequence and timing as used in step 9. The tray is gently tapped to mix the solutions in the wells and to eliminate any air bubbles trapped in the wells.

12- The tray is placed in a spectrophotometer and the absorbence is measured at 450 nm following the instructions provided by the instrument manufacturer.

4.2 MEASUREMENT OF CREATINE KINASE MUSCLE BRAIN MASS, CARDIAC TROPONIN I, MYOGLOBIN, AND CARDIAC TROPONIN T

Measurement of cardiac markers CK-MB mass, cTnI, and myoglobin were performed on the Stratus CS stat flourometric analyser machine using commercially available assays (Dade Behring - Germany), according to the manufacturer instructions. The method of measurement for all markers is based on the sandwich ELISA described previously. The essential materials required to perform the analysis (e.g. solid-phase antibody, glass fibre paper, substrate wash solution) are assembled into small test packs (3 x 6 x 1 centimetre), each containing all the reagents for one test, which are ready and easy to use. These test packs are bar-coded and are inserted in the Stratus CS together with the sample for measurement of the particular cardiac marker.

The Stratus CS machine is fully automated and is designed for low volume sample analysis. It can perform one test in 13 minutes or 4 tests in 22 minutes. The accuracy and reproducibility of the Stratus CS assays for the measurement of CK-MB mass, myoglobin and cTnI have been validated previously.[2-6] The cTnI used in this assay is of the second generation type, which is highly sensitive. The analytical sensitivity of cTnI is 0.03 ng/ml with a measurement range of 0.0 - 50 ng/ml.[7] The previous generation of cTnI assays (for use on Opus Plus- Dade Behring) had a sensitivity of 0.5 ng/ml.[8] The minimum detection limit of CK-MB mass assay is 0.3 ng/ml.[9] The assay range is 0.4 - 150 ng/ml. The assay range of myoglobin is 1 - 900 ng/ml and the analytical sensitivity is 1 ng/ml.[10]

Frozen samples were allowed to thaw slowly at 2 - 8°C and at room temperature, and were analysed within one hour. The samples were mixed thoroughly by pipetting the sample in and out gently a couple of times, or inverting the tube several times. The mixed samples were then centrifuged at 6000 rotations per minutes (r.p.m) for 1 - 5 minutes to remove any debris or clot materials that may still be present in the serum. After centrifugation, 400 µl of sample was transferred into a sample cup for analysis. Samples with values above the assay range were diluted with pooled human serum (PHS), a gift from Mr. Simon Palker, SCIPAK-Kent. Nine hundred and fifty microlitres of PHS was added to 50 µl of sample (1:10 dilution). This dilution factor was taken into account in the final reporting of the results. This dilution serum has no cTnI proteins, but has a small trace concentration of myoglobin (33 µg/l) and CK-MB mass (1.4 µg/l). A correction factor was made for all the diluted samples according to the following equation (1:10 dilution).

The Final concentration (µg/l) = measured value (µg/l) – 9 [Y] (µg/l)

Y- represents the concentration of proteins in the dilution serum.

There was a good correlation between concentrations of samples diluted with PHS and those diluted with dilution packs provided specifically by Dade Behring for dilution with these assays [$r = 0.988$, $p < 0.0005$]. The coefficient of variations (CV) was always less than 10%. The use of other dilution methods that do not contain any marker proteins were not successful. Several dilution methods like phosphate buffered saline (PBS) [PH - 7.4] alone; PBS [PH - 7.4] containing 0.1% bovine serum albumin; and normal saline were used. With all these dilution methods the CV

was unacceptably high [> 10%]. These dilution methods are not suitable for this type of measurement and are not recommended with these assays.

The dilution materials come in bar-coded dilution packs similar to the test packs, and each marker has its own specific dilution packs (e.g. cTnI DilPak, for dilution with cTnI test packs). The instrument will automatically perform 1:5 dilution of the sample. The results obtained will already be corrected for the dilution factor. The dilution packs provided by Dade Behring were not used with samples above the assay range for the following reasons; (1) There was good correlation between results obtained by using dilution packs and those obtained by using PHS, thus obviating the need to use dilution packs; (2) The use of dilution packs doubled the cost of the analysis. The cost of the dilution pack was the same as the cost of the test packs itself; (3) Use of dilution packs will double the time required to complete the analysis. Since the machine can accommodate a maximum of four test packs at one time (e.g. 2 test packs and 2 dilution packs or 4 test packs). This time delay is avoided by using previously diluted samples; and (4) Dilution was only needed in a small fraction of samples.

The Stratus CS has to pass through stringent checks before being used for the analysis of samples.[11] First, the machine is kept in check daily by running system checks (equivalent to electronic quality control). This ensures good alignment of components, and the right pressure and temperature. The system check is completed in 5 minutes. Second, having passed this check test, the machine is calibrated for

each assay. The instrument will automatically perform the calibration update procedure using calibration packs (CalPak), and three test packs for each cardiac marker to generate an updated calibration curve. After calibration, the recovered value is calculated from these stored calibration curves. The calibration is stable for three months. The machine must pass calibration before it can allow analysis to proceed. Third, having passed calibration for that particular assay, quality controls are run first to ensure a successful calibration. Three levels of quality control materials (Table 1) were used for each marker protein [Dade TRU-liquid cardiac control level 1, 2, and 3].[12] Quality control were run twice daily (two different levels) with each batch of tests, at the beginning and end of each run, to ensure no drift. All quality control values fell within the acceptable ranges.

Cardiac-TnT was analysed on Elecsys 2010 immunoanalyser using commercially available assays (Roche - Germany). The detection limit of cTnT assays was 0.01 ng/ml. Quality control [Elecsys PreciControl CARD 1 and 2] were run with each batch of tests. All quality control concentrations fell within the acceptable ranges [0.117 - 2.03 ng/ml] and [3.92 - 6.26 ng/ml]. The analysis was performed by specially trained medical staff. The clinicians caring for the patients were unaware of the results of the markers in the study, and the person as performing the analysis was unaware of the clinical outcome or the routine tests e.g. CK-MB, and troponins.

4.3 VALIDATION OF NORMAL RANGES AND PRECISION OF ASSAYS

The normal reference ranges quoted by the manufacturer for CK-MB mass [0.6 - 3.5 μg/l], cTnI [0.0 - 0.06 μg/l], myoglobin [20 - 82 μg/l], cTnT [< 0.01 μg/l], and H-

FABP [< 5 μg/l] were validated by assaying the normal ranges of 20 healthy blood donors samples [10 males and 10 females] obtained from the blood donor unit. The mean concentrations ± SD of these markers were CK-MB mass = 1.52 ± 0.8 μg/l, cTnI = 0.015 ± 0.006 μg/l, myoglobin = 41.5 ± 13.3 μg/l, cTnT = 0.011 ± 0.002 μg/l, and H-FABP = 6.86 ± 2.21 μg/l [n = 46, 22 females and 24 males]. The 99th percentile of control values for these assays were cTnI = 0.1 μg/l, CK-MB mass = 4.5 μg/l, and myoglobin = 95 μg/l. Quality control materials (Dade TRU-liquid cardiac control level 1, 2, and 3, Elecsys PreciControl CARD 1 and 2) were used to test the precisions of these assays (Table 1).

Within-assay (intra-assay) CV for Stratus CS were tested by measuring quadruplicate of three levels (level 1 - 3) four times (n = 16), and between-assay (inter-assay) CV were tested by measuring the three levels on 16 different days. Similar method was used to test the CV for cTnT assays. As shown in Table 1, the CV of these assays was within the acceptable 10% limit. The stability of calibration on Stratus CS was tested by running two levels of quality controls (level 1 and 3) for 12 weeks. The CV were cTnI [level 1 = 7.1%, level 3 = 5.3%], CK-MB mass [level 1 = 5.5%, level 3 = 6.0%], and myoglobin [level 1 = 7%, level 3 = 6.9%]. The calibration curve was stable for up to three months. Patients' samples in level 2 and 3 (Table 1) were used to check for H-FABP drift across the ELISA plate. These samples were assayed in two rows in the ELISA plate, in the middle and edge. There was no significant drift across the plate or at the edges. The inter-assay precision for H-FABP at the concentrations ranges 47.46 ± 9.39 μg/l was 19.8%. The average recovery of purified human H-FABP, added to human plasma was 90.6 - 105.5% (Table 2).

Level	CK-MB	CTnI	CTnT	Myoglobin	H-FABP
Level 1 (μg/l)	3 – 4.5	0.24 - 0.36	0.117-2.03	38 – 57	11.1 ± 0.9
Within-assay CV (%)	2.9	7.4	4	4.7	7.8
Between-assay CV (%)	4.5	6.8	9.1	5.8	-
n	16	16	10-31	16	10
Level 2 (μg/l)	11.3 - 16.9	4.6 - 6.9	3.92-6.26	157 - 235	19.4 ± 2
Within-assay CV (%)	2.8	7.2	5.5	3.2	10
Between-assay CV (%)	7	6.7	7.8	3.4	-
n	16	16	10-31	16	10
Level 3 (μg/l)	47 - 71	13 - 19	-	405 - 608	235 ± 13.7
Within-assay CV (%)	6.2	4.9	-	5.7	4.8
Between-assay CV (%)	4.8	4.0	-	7.26	-
n	16	16	-	16	10

Table 1. This table illustrates the concentrations of the various quality controls at each level (1,2, and 3), and the precision (coefficients of variations) of the assays at that particular concentration.

Sample	H-FABP added (μg/l)	H-FABP recovered (μg/l)	H-FABP % recovery
A	55	58	105.5
B	96	87	90.6
C	179	183	102.2
D	220	200	90.9

Table 2. Shows the percentage recovery of purified H-FABP added to human plasma.

4.4 RECRUITMENT OF PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

Patients with an initial diagnosis of AMI admitted to the CCU, the Royal Infirmary of Edinburgh between September 1999 and March 2000, were included in the study. The purpose of the study was explained to the patients or members of the family and informed consent was obtained before beginning the study. The recruitment process started within 20 minutes of patients' arrival in the A&E department, before being admitted to the CCU and before the administration of thrombolytic therapy. Thereafter, five serial blood samples were taken at 0 hour (at presentation), 2 hours, 4 hours, 8 - 10 hours, and 16 - 24 hours after presentation. Blood samples were processed and serum was stored until analysis. All patients underwent serial clinical evaluations by the CCU team. Standard 12-lead ECGs were obtained on arrival and in the CCU at least once each day. Serum CK, and CK-MB activity ± cTnI were routinely measured at admission, at least twice daily within the first 24 hours, and once daily for the first three days.

4.4.1 INCLUSION AND EXCLUSION CRITERIA

Patients presenting with chest pain suggestive of AMI were evaluated by A&E department physician. The evaluation includes rapid history taking and very brief physical examination and recording of 12-lead ECG. The time of onset of symptoms was carefully recorded for each patient at the time of presentation. Patients were included in this study if they presented within 6 hours after the onset of symptoms, and had significant elevation of ST segment or new Q waves or other ischaemic changes highly suggestive of AMI on the admission ECG (e.g. suspected posterior

AMI), and prolonged ischaemic chest pain (often combined with radiation of pain to the left arm) in combination with transpiration, nausea, vomiting and/or shortness of breath. A total of fifty-six patients were included in this study. Eleven patients were later excluded from the analysis because of incomplete blood samples (four patients), inconclusive diagnosis of AMI (two patients), delayed presentation more than six hours after the onset of symptoms (three patients), or had cardiac arrest during the first 24 hours requiring repeated DC cardioversion ± prolonged cardiopulmonary resuscitation (two patient). The final study population consisted of forty-five patients admitted within 6 hours after the onset of chest pain with confirmed AMI.

In addition to the above mentioned four specific exclusion criteria [i.e. incomplete blood sampling protocol, delayed presentation, cardiac arrest at presentation, and inconclusive diagnosis], patients were also not eligible for the study if: (1) They had recent severe muscle trauma or repeated intramuscular injection; (2) They had acute or chronic skeletal muscle disorders; (3) They had recent major surgery, CABG or AMI (< 1 month), because of the associated prolonged healing inflammatory process involved. Patients with recent AMI may have sustained release of troponins for several weeks; (4) They had any evidence of renal impairment defined as a creatine and urea concentration rise above 138 mmol/l and 8 mmol/l respectively. This may interfere with the excretion of H-FABP and myoglobin leading to prolonged and exaggerated concentration rise; or (5) They had biochemical hypothyroidism as reflected by thyroxine (T_4) concentration < 8 pmol/L and thyroid stimulating hormone (TSH) concentration > 3.5 mU/L.

Patients who were admitted to CCU with suspected AMI and were later diagnosed with non-Q wave myocardial infarction or unstable angina were included in these study groups (see later).

4.4.2 DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION

The diagnosis of AMI was established independently by a consultant cardiologist without knowing serum concentrations of H-FABP, myoglobin, CK-MB mass, cTnT or cTnI in the study. The diagnosis was based on the criteria set by the WHO and must include at least two of the following three findings.[13] (1) Clinical history of ischaemic chest pain $\geq$ 30 minutes in duration. (2) Evolution of typical changes in at least two adjacent leads of the ECG, appearance of new Q waves > 1 mm wide and 2 mm deep or an R wave increment leading to an R/S ratio > 1 in leads V1 and V2 defined as Q wave AMI or ST segment elevation > 2 mm 0.08 seconds after J point persisting for at least 24 hours. (3) Time-dependent rise in routine concentration of CK and CK-MB activity (CK $\geq$ 400 IU/L, and CK-MB activity > 6%) and subsequent fall. In addition, if patients had evidence of recent coronary artery occlusion at angiography, this was taken with the above criteria to support the diagnosis of AMI.

4.5 RECRUITMENT OF PATIENTS WITH ACUTE CHEST PAIN (NON-Q WAVE MYOCARDIAL INFARCTION, UNSTABLE ANGINA, AND ATYPICAL/ANGINAL CHEST PAIN)

Consecutive patients presenting with acute chest pain to the Royal Infirmary of Edinburgh between September 1999 and March 2000 were included in the study.

Patients were principally recruited from the acute receiving medical unit. a few patients were recruited from the acute chest pain clinic, CCU, and cardiology wards. The study was explained to each patient and informed consent was obtained from each participant. Thereafter, five serial blood samples were collected by venepuncture or via in situ venflon (if this access existed) at 0 hour (at presentation), 2 hours, 4 hours, 8 - 10 hours, and 16 - 24 hours after presentation [see patients' classification page 188].

4.5.1 INCLUSION AND EXCLUSION CRITERIA

The time of onset of symptoms was carefully recorded for each patient at the time of presentation. Patients were included in the study if they presented with chest pain suggestive of myocardial ischaemia [$\geq$ 20 minutes in duration] within 7 hours after symptoms onset with or without ECG changes suggestive of ischaemia. There was no age limit and no specific entry criteria of ischaemic changes on the admission ECG. The intention was to study a broad and unselected group of patients representing all forms of chest pain [and ECG changes] that is commonly encountered in the A&E department that require early admission to hospital.

Patients were not included in the study if they had delayed presentation more than 7 hours after symptom onset. A total of sixty patients were initially included in this study. Five patients were later excluded from the analysis due to incomplete blood sampling protocol. The final study population consisted of fifty-five patients. None of the patients in this group had any of the additional exclusion criteria listed under section 4.4.1.

4.5.2 DIAGNOSIS OF NON-Q WAVE MYOCARDIAL INFARCTION, UNSTABLE ANGINA, AND ATYPICAL/ANGINAL CHEST PAIN

The final diagnosis of patients in these groups was established independently by a consultant cardiologist. Unstable angina symptoms were defined as; (1) Symptoms of angina at rest; (2) New onset (< 2 months) of angina on minimal exertion and increasing severity; (3) Recent (< 2 months) acceleration of angina from previously stable pattern, as reflected by an increase in severity to at least Canadian Cardiovascular Society Classification III.

Unstable angina was diagnosed when patients had two or more of the following criteria without a clear-cut ECG changes of infarction [section 4.4.2] or cardiac markers (CK or CK-MB) elevations diagnostic of AMI; (1) Ischaemic chest pain ≥ 20 minutes in duration; (2) Transient ST segment elevation ≥ 1 mm 0.08 seconds after J point less than 30 minutes in duration; (3) Transient or persistent ST segment depression ≥ 1 mm 0.08 seconds after J point in two adjacent leads; (4) Symmetrical or asymmetrical T wave inversion ≥ 1 mm excluding T wave inversion in leads III, AVR, and V1 only; and (5) Evidence of routine cardiac markers rise to level above the upper limit of normal [cTnI ≥ 0.1 μg/l or cTnT ≥ 0.1 μg/l] in the absence of CK and/or CK-MB rise diagnostic of AMI [see below]. Patients were diagnosed as having non-Q wave MI if they had one or more of the criteria listed above and in addition, they had evidence of myocardial necrosis as reflected by CK elevation greater than 400 IU/L and CK-MB activity ≥ 6% and subsequent fall.

Patients were diagnosed as having atypical or anginal chest pain if they had all the following criteria; (1) Typical or atypical presentation of chest pain (such as chest

pain which differed in character, location, duration, severity, unusual associations, relieving or precipitating factors from that of typical ischaemic pain); (2) No significant ECG changes of ischaemia; and (3) No rise in routine CK, CK-MB or cTnI concentrations.

4.6 RECRUITMENT OF PATIENTS IN THE ANGIOPLASTY GROUP

The study population consisted of a consecutive series of patients recruited from the cardiology programmed investigation unit, the Royal Infirmary of Edinburgh between March and October 1999. These were scheduled admissions for elective angioplasty with or without stenting. Ethical approval for the study was obtained from the local ethical committee. The study was explained to each patient and a written informed consent was obtained. Thereafter, five serial blood samples were collected from each patient at 0 hour (base line concentration, pre-angioplasty) and at 1 hour, 2 hours, 4 hours and 16 - 24 hours after angioplasty. Patients in this group were followed-up for 20 - 26 months after angioplasty. End points include the development of complications like angina, UA, AMI, target and non-target vessel PCI, CABG, and death.

4.6.1 INCLUSION AND EXCLUSION CRITERIA

The study group consisted of patients who were referred to our centre for elective angioplasty for both stable angina pectoris [defined as chest pain on exertion relieved with rest] not well controlled on current medications and UA [see section 4.5.2 page 116 for definition of UA]. There was no specific age limit in the study. Patients who had angioplasty and a continuous balloon inflation time for a minimum of 60 seconds

or more with or without stenting [single or multiple] constituted 91.25% of the study group. The remaining patients had an inflation time between 30 – 60 seconds with or without stenting. Patients who had an angioplasty other than elective [e.g. rescue angioplasty, primary angioplasty, salvage angioplasty, or emergency angioplasty] were not eligible for this study.

A total of eighty-four patients were initially included in the study. Two patients with increased cardiac markers concentration before angioplasty [i.e. base line or pre-angioplasty sample] were later excluded from the study. This was to ensure normal base line concentration for cardiac markers before the start of the study. Two more patients were later excluded [one with mild renal impairment and one with hypothyroidism]. The final study population consisted of eighty patients. The diagnosis of complications during angioplasty was made independently by two cardiologists. None of the patients in this group had any of the additional exclusion criteria listed under section 4.4.1.

4.7 BLOOD SAMPLING PROTOCOL

Healthy blood donors

Blood samples were collected from 20 healthy blood donors at the blood donor centre. An information sheet was given to each donor and a written consent was obtained. Only one sample was obtained from each participant and these samples were utilised for; (1) The exploration of the normal limit for H-FABP; (2) To validate the normal range quoted by the manufactures for CK-MB mass, myoglobin, cTnT, and cTnI.

Mixed population of ischaemic heart disease patients

Blood was also collected from 20 inpatients attending the Royal Infirmary of Edinburgh. This group consisted of a heterogeneous population with various medical disorders and various risk factors for ischaemic heart disease but without active presentation and without chest pain. One sample was obtained from each patient. The purpose of this group was to explore the true concentration of CK-MB mass, cTnI, cTnT, myoglobin, and H-FABP in this population with a mixed medical background but without active symptoms of ischaemia.

Pre and post cardiac catheterisation controls

Pre and post cardiac catheterisation blood samples were also collected from 12 patients undergoing cardiac catheterisation studies (ventriculography and coronary angiography) without angioplasty. The purpose of this control group is to study the effects (if any) cardiac catheterisation studies (without angioplasty) had on the release of the different markers after the study. It also validates any changes in cardiac marker concentrations after angioplasty procedures and excludes diagnostic procedures (diagnostic catheters manipulations) and mechanical trauma as the cause of cardiac markers release. Three samples were obtained from each participant at 0 hours (before cardiac catheterisation), and at 2 hours and 16 - 24 hours after the procedure.

In all the above groups and the study populations, blood samples were handled in exactly the same way. Five millilitres of blood was collected by venepuncture into a standard Starstedt Monovette serum tube. The blood sample was allowed to clot at

room temperature (18 - 25 °C) for 1 hour. It was then centrifuged at 4 °C at 3000 r.p.m for 10 minutes. The resulting serum was divided into small aliquots and stored in microcentrifuge tubes (ELKay) at – 70 °C until analysis.

4.8 VALIDATION OF THE SAMPLING PROTOCOL

The sampling protocol for the AMI and UA groups of 0 hour, 2 hours, 4 hours, 8 - 10 hours, 16 - 24 hours after presentation to hospital has been validated in other studies.[14] The angioplasty group sampling protocol is slightly different i.e. 0 hour (before angioplasty), and at 1 hour, 2 hours, 4 hours, and 16 - 24 hours. This was based on the fact that most controlled animal and human studies that have looked at the early release of H-FABP have shown that this marker peaks at about 4 hours after myocardial injury.[15] Thus this sampling protocol is ideal for detecting the changes in concentrations in the very early stages after myocardial injury.

4.9 RECEIVER OPERATOR CHARACTERISTIC CURVE ANALYSIS

The cut-off concentrations selected for the various cardiac markers in these studies were based on the following considerations. First, the normal concentrations of these markers were established in healthy blood donor and normal control groups without ACS. Second, the best cut-off concentrations for each marker were established from a series of receiver operator characteristic (ROC) curve analyses in the four groups of patients with ST elevation MI (STEMI), non-ST elevations MI (NSTEMI), UA, and atypical/anginal chest pain.[16]

Two different cut-off concentrations were chosen for each marker. The first cut-off concentration discriminates between patients with myocardial injury [UA, NSTEMI and STEMI] and those without myocardial injury [healthy blood donors, control group, and non-ischaemic chest pain group]. The second cut-off concentrations were used to discriminate between patients with myocardial injury [UA] from those with myocardial infarction [NSTEMI and STEMI]. These two cut-off concentrations were used to measure the sensitivity in Chapter 6 and Chapter 7.

The following cut-off concentrations were chosen to discriminate between patients with myocardial damage (ACS) and those without evidence of myocardial damage, CK-MB mass $\geq$ 5 μg/l [area under ROC curve (AUC) = 0.869, $p < 0.0005$, 95% confidence interval (CI) = 0.839 - 0.9, sensitivity = 71.7%, specificity = 100%], cTnI $\geq$ 0.18 μg/l [AUC = 0.974, $p < 0.0005$, 95% CI = 0.962 - 0.987, sensitivity = 80%, specificity = 100%], cTnT $\geq$ 0.1 μg/l [AUC = 0.906, $p < 0.0005$, 95% CI = 0.881 - 0.932, sensitivity =65.2%, specificity = 100%], myoglobin $\geq$ 95 μg/l [AUC = 0.867, $p < 0.0005$, 95% CI = 0.836 - 0.898, sensitivity = 68.5%, specificity = 100%], and H-FABP $\geq$ 16 μg/l [AUC = 0.894, $p < 0.0005$, 95% CI = 0.861 - 0.926, sensitivity = 81.8, specificity = 86%] (Figure 2).

The following cut-off concentrations were selected to discriminate patients with UA from patients with MI. Values above these concentrations indicate the presence of myocardial infarction, CK-MB mass $\geq$ 8 μg/l [AUC = 0.915, $p < 0.0005$, 95% CI = 0.887 - 0.943], cTnI $\geq$ 0.6 μg/l [AUC = 0.905, $p < 0.0005$, 95% CI = 0.876 - 0.934],

cTnT ≥ 0.4 μg/l [AUC = 0.821, p < 0.0005, 95% CI = 0.781 - 0.861], myoglobin ≥ 107.5 μg/l [AUC = 0.881, p < 0.0005, 95% CI = 0.845 - 0.918], and H-FABP ≥ 21.5 [AUC = 0.833, p < 0.0005, 95% CI = 0.775 - 0.891]. Figure 2 shows ROC curves that were used to derive some of these cut-off concentrations. Sensitive and specific cut-off concentrations are associated with a large area under the ROC curve, and the cut-off concentrations tend to cluster in the left upper corner indicating high rate of true positive and few false positive test results.[16]

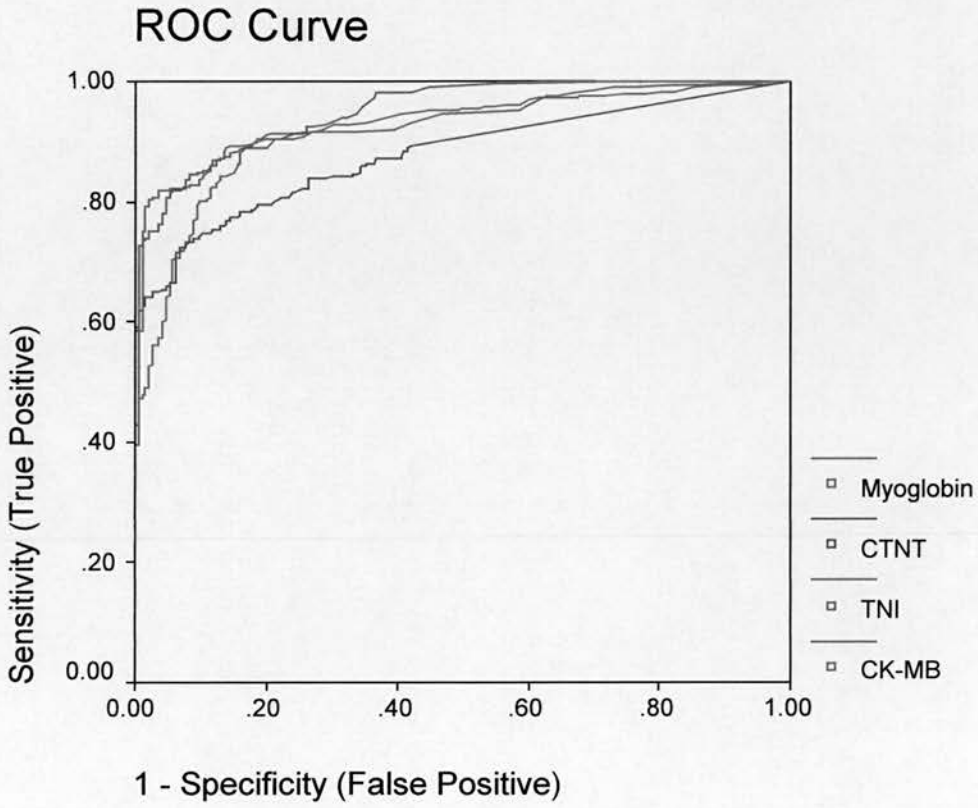

Figure 2. Shows ROC curves for the various cardiac markers used in the study. The total area under the curve is significant and the cut-off concentrations are mainly clustered in the left upper corner, both features reflect high sensitivity and specificity.[16] H-FABP is not shown here but it displayed similar characteristics.

4.10 STATISTICAL ANALYSIS

Analyses were performed using SPSS™ (Statistical Package for Social Sciences, Pittsburgh statistical software, version 10). Values were expressed as mean ± SD. The correlation coefficient was calculated by Spearman's Rank order correlation (rho). Chi-square test was used to explore the group differences with respect to categorical variables. For two by two tables Yate's correction for continuity was applied to compensate for overestimations. For categorical variables that had an expected cell frequency less than five, fisher's exact probability test was used instead. Mann-Whitney U test was conducted to compare the mean score for the continuous variables in the angioplasty cTnI positive and negative groups. A one-way repeated measure analysis of variance (ANOVA) or Friedman's test was conducted to compare the mean concentrations of cardiac markers (CK-MB mass, cTnI, cTnT, myoglobin, and H-FABP) at time 0 (at presentation or pre-angioplasty) and at 1, 2, 4, and 16 - 24 hours after angioplasty and at 2, 4, 8 - 10 and 16 - 24 hours after presentation. Post-hoc comparisons between times were performed. For multiple comparisons conducted in post-hoc a Bonferroni adjustment was used. A p value ≤ 0.05 was considered statistically significant.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each cardiac marker [H-FABP, CK-MB mass, myoglobin, cTnT, and cTnI] for the diagnosis of AMI were measured at each time point after presentation. The sensitivity for the detection of non-Q wave MI, UA and complications during angioplasty was also calculated. Sensitivity is defined as; the number of samples above cut-off concentration in AMI divided by the number of

sample in AMI. Specificity is defined as; the number of samples below the cut-off value in non-AMI divided by the number of samples in non-AMI. Positive predictive values is defined as; the probability of an AMI being present in the presence of a single marker result above the upper reference limit of the marker for each time point. Negative predictive values is defined as; the probability of an AMI not being present in the presence of a single marker result below the upper reference limit for each time point. The calculations of sensitivity, specificity, PPV, and NPV were based on the following equations:

$$\text{Sensitivity} = \frac{\text{True positive sample}}{\text{True positive sample + false negative sample}} \times 100\%$$

$$\text{Specificity} = \frac{\text{True negative sample}}{\text{True negative sample + false positive sample}} \times 100\%$$

$$\text{PPV} = \frac{\text{True positive samples}}{\text{True positive samples + false positive samples}} \times 100\%$$

$$\text{NPV} = \frac{\text{True negative samples}}{\text{True negative samples + false negative samples}} \times 100\%$$

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CHAPTER 5

THE DETECTION OF COMPLICATIONS DURING PERCUTANEOUS CORONARY INTERVENTION USING CARDIAC MARKERS HEART FATTY ACID BINDING PROTEIN, CREATINE KINASE MUSCLE BRAIN MASS, CARDIAC TROPONIN I, CARDIAC TROPONIN T, AND MYOGLOBIN

5.1 INTRODUCTION

Percutaneous coronary intervention represents a widely accepted revascularisation procedure and a clinical model of induced ischaemia. After its introduction in 1964, PCI has since been used in the treatment of many patients with stable angina, unstable angina, and acute myocardial infarction.[1] Between 1987 and 1996 the number of PCI procedures in the United Kingdom has increased to about 20,000 procedures per year and in the United States over 300,000 procedures are performed per year. In patients with recurrent angina after coronary artery bypass grafting, PCI of narrowed saphenous vein grafts is often considered since the peri-operative mortality associated with a second bypass operation is higher than that associated with the initial operation [5 - 10% vs 1 - 2%].[2] Percutaneous coronary intervention provides effective relief (90%) of stable angina in most patients with single vessel coronary artery disease.[3;4]

Among patients with UA who are treated medically, 1 - 5% die and 2 - 10% sustain AMI before they are discharged from hospital.[5-7] Percutaneous coronary intervention in UA is associated with 84% angiographic success rate (defined as < 40% residual luminal narrowing) and is usually considered if aggressive medical therapy fails.[8] In subjects with evolving AMI, percutaneous coronary intervention is associated with a one year survival of 90 - 97%.[9] When compared with thrombolytic therapy, PCI may be advantageous in patients considered to be at high risk i.e. those with massive anterior AMI, and in patients with tachycardia, in AMI complicated by cardiogenic shock, and in patients with contraindications to thrombolytic treatment.[9;10] The success rate of PCI is lower in patients with UA, advanced age, and low ejection

fraction (EF).[11] The success rate of PCI is also lower with stenoses that are long, eccentric, angulated, calcified, located at the ostium or the site of a branch point, or associated with intraluminal thrombus.[12]

5.2 COMPLICATIONS OF PERCUTANEOUS CORONARY INTERVENTION

Percutaneous coronary intervention is in general considered a safe procedure, occasionally complications occur, including AMI [3 - 5%],[4;8] the need for emergency CABG [3 - 7%],[13;14] and death [0.9%].[15] These events are usually caused by extensive arterial dissection, intracoronary thrombosis or both with resultant vessel occlusion. Acute or abrupt closure occurs in 2 - 8% of patients undergoing PCI and accounts for most of the short-term morbidity and mortality associated with PCI.[16;17] In 75% of patients with abrupt closure, it occurs within minutes after PCI when they are still in the catheterisation laboratory, in the other 25% it usually occurs within 24 hours after PCI.[18] Ultrasound imaging has shown that dissection of the arterial wall is detected in 50 - 80% of patients who have undergone successful PCI.[19;20]

The consequences of abrupt closure vary widely depending on; (1) The size of the occluded vessel; (2) The extent of myocardium at risk; (3) The presence or absence of good collaterals; and (4) The duration of the occlusion. Patients with adequate collaterals perfusion of the occluded vessel may have abrupt closure without chest pain, ECG abnormalities or haemodynamic compromise.[17] More commonly, abrupt closure is accompanied by chest discomfort and ECG evidence of ischaemia and

requires immediate revascularisation of the occluded vessel (by repeated PCI ± bail out stents or emergency CABG) to prevent or limit myocardial damage. Ten per cent of patients with extensive coronary arterial dissection after PCI require emergency bypass surgery, sustain AMI, or die. Small dissection (not compromising flow or lumen) are only associated with 2% complications.[21] Other complications of PCI include coronary vessel perforation, rupture or injury, re-stenosis of the dilated vessel, UA, arrhythmias including ventricular fibrillation, coronary artery spasm/elastic recoil, hypotension/hypertension and embolism.[22] Side branch occlusion occurs in 5% of side branches that are adjacent to a dilated coronary stenosis.[23]

5.3 BIOCHEMICAL MARKERS OF MYOCARDIAL INJURY AND PERCUTANEOUS CORONARY INTERVENTION

Uncomplicated angioplasty is not associated with any significant cardiac markers release in the majority of patients, because the duration of coronary occlusion and subsequent myocardial ischaemia is brief and usually well tolerated by the myocardium.[24;25] However, the development of new and more sensitive cardiac markers has increased the numbers of patients diagnosed with myocardial injury after PCI. Myocardial infarction diagnosed by elevation of cardiac markers is relatively common and is reported in approximately 7.7 - 15% of patients undergoing PCI.[26] These infarclets, which were initially thought to be benign, were associated with increased risk of future complications.[27-29;30-33] Minor increases of CK and CK-MB after an apparently successful coronary intervention have been reported in 5 - 15% and 11.5 - 26% of cases respectively.[27;30;34-36;40;50] Creatine kinase-MB elevation after

PCI was associated with increased risk for cardiac death and AMI during follow-up.[27]

Cardiac-TnI and cTnT are more sensitive and specific markers of myocardial injury and have been shown to be independent predictors of early and late adverse clinical outcomes in patients with ACS.[37;53;55-57] Elevations of cardiac troponins were detected in 13 - 44% of patients undergoing PCI.[33;38-40] Some of the increases in cardiac markers have been correlated with clinical variables e.g. low EF,[31] or procedural variables e.g. total inflation time,[33] side branch occlusion,[32] the use of stents and the occurrence of in-hospital complications (e.g. AMI).[41] Increased cTnT concentration post-PCI was correlated with a higher incidence of complex lesion morphology [73% of patients], intracoronary thrombus [29%], abrupt closure [10%], and side branch occlusion [15%] during angioplasty.[42]

There are still some unresolved issues concerning the role of these cardiac markers in interventional cardiology clinical practice. There is little information on the relationship between cardiac markers elevations after PCI and; (1) Correlations with demographic, angiographic and procedural variables; (2) Correlations with long-term complications. Also the definition of myocardial injury following PCI using biochemical markers is not clear. For CK and CK-MB, some studies have recommended the use of concentrations 1.5 – 3 times the upper limit of normal.[26;27;31] There is also little information about the best marker(s) that will define myocardial injury post-PCI especially with regards to cTnI and cTnT and the optimum cut-off concentrations that carry prognostic value. Some investigators used concentrations

above the normal cut-off value of the assays (e.g. > 0.1 μg/l for cTnI). Others suggested that values twice or three times the upper limit of normal are associated with more significant complications.[34;48] Heart-FABP is a novel cardiac marker introduced for the early identification of myocardial damage after AMI.[43] The value of H-FABP as an early marker for the identification of myocardial damage during PCI has not yet been studied.

5.4 AIMS OF THE STUDY

1- Examine the relation between elevations of cardiac markers (H-FABP, cTnT, cTnI, CK-MB mass, and myoglobin) post-PCI and complication rates during and after PCI.

2- Determine whether elevated cardiac markers (H-FABP, cTnT, cTnI, CK-MB mass, and myoglobin) post-PCI are related to demographic, angiographic and procedural variables.

3- Compare and contrast the release kinetics of the new cardiac marker H-FABP in this model of induced ischaemia with standard markers of myocardial damage (cTnT, cTnI, CK-MB mass, and myoglobin) and determine whether H-FABP has advantage in terms of early release characteristics.

5.5 PATIENTS AND METHODS

5.5.1 STUDY POPULATION

The patients group consisted of a consecutive series of 80 patients. The recruitment process of patients in this group, the inclusion and exclusion criteria are described in details in Chapter 4 materials and methods pages 117 – 118. Five serial blood samples were obtained from each patient at 0 hour i.e. basal or pre-angioplasty and at 1 hour, 2 hours, 4 hours, and 16 - 24 hours post-angioplasty. The following cardiac markers cTnI, cTnT, CK-MB mass, myoglobin, and H-FABP were measured at each time point, and the changes between basal and maximum post-angioplasty cardiac markers concentrations were compared. A control group of 12 patients who underwent coronary angiography without angioplasty at the same period were also included and analysed consecutively. These were patients who had coronary artery disease (CAD) but were not found suitable for angioplasty. The purpose of this control group is to validate the results and exclude diagnostic procedure as the cause of cardiac markers elevation.

5.5.2 METHODS

Patients were categorised into two main groups according to the presence (cTnI positive) or absence (cTnI negative) of elevated cTnI concentrations ≥ 0.18 μg/l. The frequency of abnormal results was determined for each marker. The concordance and discordance and the predictive accuracy of the different cardiac markers (cTnT, myoglobin, CK-MB mass, and H-FABP) for complications were compared between the two groups. Base line and maximum post-procedural cardiac markers concentrations changes in the two groups were compared and related to demographic, angiographic and procedural variables. The two groups were also compared with respect to the frequency of complications during PCI and the in-

hospital period post-procedure. Patients were followed-up for approximately two years after discharge from hospital and the numbers of cardiac events in each group were compared. Patients were also classified into cTnT, CK-MB mass, myoglobin, and H-FABP positive (increased above cut-off concentrations) and negative (below cut-off concentrations) groups and compared in a similar manner to cTnI positive and negative groups. The diagnosis of complications during angioplasty was made by two cardiologists. The diagnosis of AMI in this group was based on the presence of two or more of the following findings; (1) Clinical history of typical chest pain ≥ 30 minutes in duration; (2) Evidence of ischaemic ECG changes such as ST segment elevation or new Q wave or left bundle branch block; (3) Time-dependent rise in concentrations of CK and CK-MB activity (CK ≥ 400 IU/L, CK-MB activity ≥ 6%) and subsequent fall; (4) Evidence of new vessel occlusion at angiography within 24 hours of PCI.[44]

Patients were classified as UA if they had one of the following presentations, symptoms of angina at rest; new onset (< 2 months) exertional angina; recent (< 2 months) acceleration of stable angina as reflected by an increase in severity.[45] The diagnosis of in-hospital recurrent ischaemia was based on chest pain associated with transient ST segment and/or T wave changes without cardiac markers elevation diagnostic of AMI. Angiographic success was defined according to the American College of Cardiology/American Heart Association (ACC/AHA) task force guideline on angioplasty as < 50% residual diameter stenosis and without the occurrence of death, AMI, or the need for emergency bypass operation.[12] Clinical success was

defined as Angiographic success without in-hospital complications (death, AMI, emergency CABG, or ischaemia driven repeat PCI).

5.5.3 PERCUTANEOUS CORONARY INTERVENTION

Diazepam (10 mg) was given orally as a pre-medication. The angioplasty procedure was performed under local anaesthesia using the Judkins transfemoral approach and conventional techniques of intracardiac recording and pacing. Ten thousands units of heparin (or a slightly modified dose if the patient already received some heparin) were given at the start of the procedure. A guide wire was then introduced into the artery. A 6 - 7 French guide catheter (2 - 2.3 mm in diameter) is advanced through the sheath to the ostium of the coronary artery to be dilated. With the guide catheter positioned in the coronary ostium, angiography of the diseased artery is assessed using multiple angiographic views to visualise the stenosis and the arterial segment proximal and distal to it. A flexible guide wire (0.25 - 0.46 mm in diameter) is then advanced through the guide catheter, navigated across the stenosis and positioned in the distal arterial segment. The guide wire is navigated by rotating and advancing its angulated tip. With the guide wire across the stenosis, the deflated balloon catheter is advanced over the wire and positioned at the stenosis. The position of the guide wire and balloon catheter is confirmed periodically by visualisation of the artery as contrast material is injected through the guide catheter. Once positioned, the balloon is usually inflated for 20 - 60 seconds at 6 - 8 atmospheres of pressure with a mixture of saline and contrast materials, so that the inflation can be visualised fluoroscopically. Occasionally other inflation variables are used. If the stenosis is adequately dilated the guide wire and balloon catheter are removed. If the dilation is

not adequate the guide wire remains across the stenosis and the balloon catheter may be replaced by a larger one, or a coronary stent is deployed using higher pressures (10 - 12 atmospheres of pressure). When the inflation has been completed, a final angiogram is obtained to confirm the result is satisfactory and the other segments of the artery including branches have not been compromised.

Electrocardiographic monitoring using leads I, II, and III were used to monitor the patients during PCI. Heart rate, systolic and diastolic blood pressure were monitored continuously throughout the procedure. When indicated, extra readings of diastolic and systolic blood pressure were taken by an automated machine (Dinamap). Intracoronary nitroglycerin (0.2 mg) was given during the procedure as required for pain or suspected coronary spasm. Cyclomorphine (2.5 - 5mg) was given intravenously as required for chest pain. Base line 12-lead ECG was recorded before angioplasty and immediately after angioplasty. Intravascular ultrasound and pressure wire studies were undertaken in selected patients to assess the success of angioplasty and guide further intervention or to assess the degree of complications. After PCI, patients were monitored at the programmed investigations unit or CCU, depending on the occurrence of complications during the procedure. In patients with chest pain after PCI, additional 12-lead ECG were recorded to diagnose myocardial ischaemia and infarction and additional blood samples for CK, CK-MB, or cTnI measurements were collected as part of the routine monitoring of patients. After PCI and stenting most patients were started on either ticlopidine, clopidogrel, abciximab or combinations of these treatments and the study did not interfere with this protocol. Abciximab was used mostly on a rescue basis. Following the procedure, patients

were reviewed during the first 24 hours. Clinical notes, blood tests and ECGs were also reviewed.

5.5.4 FOLLOW-UP PROTOCOL

Out of hospital clinical outcomes for up to 26 months were obtained by serial telephone interviews by research nurses. The following were considered as cardiac complications; hospitalisation for cardiac events (angina, UA, and heart failure), target and non-target vessel revascularisation, CABG, AMI, and death. The degree of angina control post-PCI was also assessed by the patient reporting whether his/her angina is worse, better or unchanged since PCI. The diagnosis of these events during follow-up was based on hospitalisation records and documented discharge summaries according to the guidelines mentioned earlier.

5.5.5 LABORATORY ANALYSIS

Peripheral blood samples for serum analysis were collected in white Starstedt Monovette vacutainer tubes. The blood samples (5 mls) were taken through a peripheral line (intravascular access). The extracted samples were allowed to clot at room temperature for 1 hour and then centrifuged at 4 °C, and the resulting serum was divided into small aliquots and frozen at – 70 °C until analysis. Routine urea and electrolyte were reviewed to exclude renal failure. Cardiac-TnI, CK-MB mass, and myoglobin were analysed on Stratus CS flourometric analyser machine (Dade Behring), using commercially available test materials as described in Chapter 4 pages 106 - 109. Heart-FABP was analysed by an ELISA method using commercially available assays (Hycult - Cambridge) as described in Chapter 4 pages

101 - 105. Cardiac-TnT were analysed on Elecsys 2010 using commercial assays (Roche - Germany) as described in Chapter 4 page 109.

The optimal cut-off concentrations of cardiac markers were based on ROC curve analysis between patients with and without complications after PCI, and also considerations of cardiac markers concentrations in the normal healthy blood donor group, the control group, and the basal or pre-angioplasty concentrations. The following cut-off concentrations were used to indicate myocardial injury following angioplasty (cTnI $\geq$ 0.18 μg/l [AUC = 0.753, SE = 0.03, 95% CI = 0.693 – 0.813, sensitivity = 41.2%, specificity = 92%], cTnT $\geq$ 0.1 μg/l [AUC = 0.672, SE = 0.037, 95% CI = 0.599 – 0.746, sensitivity = 23%, specificity = 99.7%], CK-MB mass $\geq$ 5 μg/l [AUC = 0.656, SE = 0.036, 95% CI = 0.586 – 0.726, sensitivity = 14%, specificity = 98.1%], myoglobin $\geq$ 95 μg/l [AUC = 0.654, SE = 0.034, 95% CI = 0.588 – 0.721, sensitivity = 30%, specificity = 90%], and H-FABP $\geq$ 16 μg/l [AUC = 0.683, SE = 0.038, 95% CI 0.609 – 0.758, sensitivity = 42%, specificity = 92.7%]). All these cut-off concentrations were associated with statistically significant areas under the curve ($p < 0.0005$). The AUC for cTnI was greater than other markers. Cardiac-TnI was also the most frequent abnormal marker and was therefore chosen for comparison in this study.

5.5.6 STATISTICAL ANALYSIS

Continuous variables were presented as mean ± SD. Comparisons between cTnI positive and negative groups demographic, angiographic and procedural variables were conducted by the Mann-Whitney U test for continuous variables and chi-square

or Fisher's exact test for categorical variables. Comparison of serial cardiac markers (CK-MB mass, cTnI, cTnT, myoglobin, and H-FABP) mean concentrations changes at 0 hour (before angioplasty) and at 1, 2, 4, and 16 – 24 hours after angioplasty was conducted by Friedman test. Comparison between basal and maximum concentration of cardiac markers in the control and angioplasty groups was compared with Wilcoxan Signed Rank Test. Correlations between the concentration changes of cTnI and cTnT, CK-MB mass, myoglobin, and H-FABP in the cTnI positive group was studies by Spearman's Rank order correlation. Significance was defined as p value $\leq$ 0.05. The rate of event-free survival was estimated from the Kaplan-Meier survival method and was compared with the log rank test. The sensitivity, specificity, positive predictive value and negative predictive value for the detection of complications were determined for each cardiac marker.

5.6 RESULTS

The study group included 21 females (26%) and 59 males (74%). The mean age of the group was 61.1 ± 7.5 years. The control group consisted of 12 patients, 5 females and 7 males, mean age 61.9 ± 8.7 years. The serum concentrations CK-MB mass, myoglobin, cTnI, H-FABP, and cTnT in the angioplasty and the control groups were determined and compared. There were no significant releases of any of the cardiac markers in the control group who had angiography procedure alone without angioplasty (Figure 1). This provides evidence that exclude diagnostic procedure as the cause of cardiac trauma and support angioplasty as the primary cause of cardiac markers release. There was also no significant difference in cardiac markers concentrations between healthy blood donors and the control group (Figure 2).

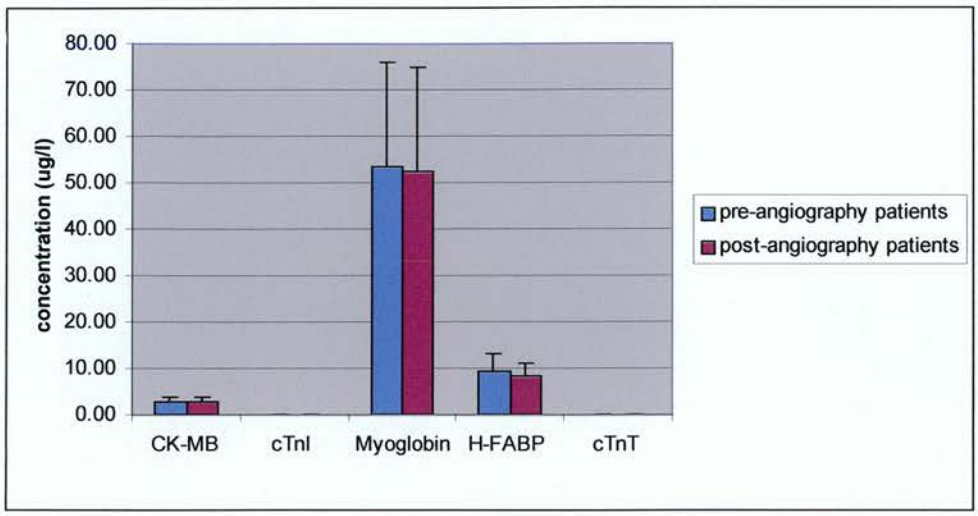


Figure 1. This graph illustrates the concentrations of cTnI, CK-MB mass, H-FABP, cTnT and myoglobin before and after angiography. There was no significant release of markers after angiography compared to the base line value.

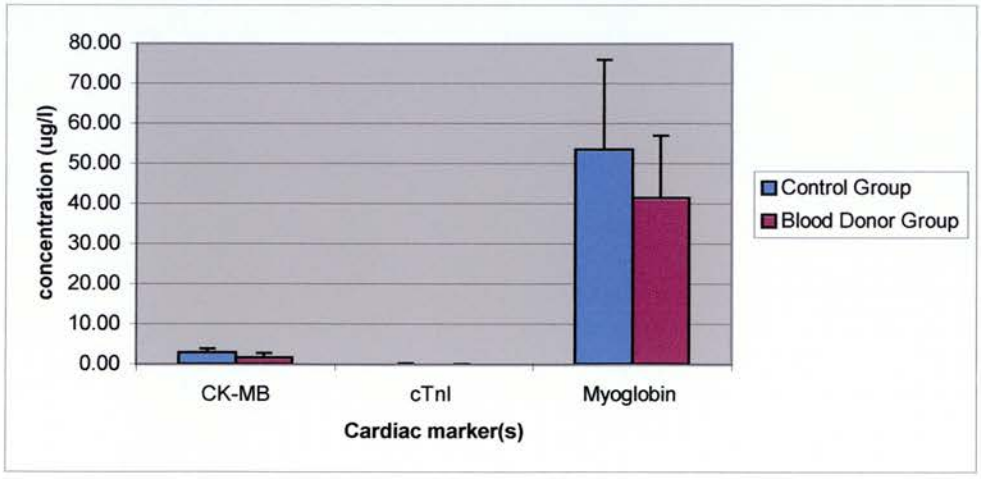


Figure 2. Illustrates the differences in serum concentrations between the healthy blood donor group and the control group.

The following demographic, angiographic and procedural variables were analysed in every patient. The ratios of these variables in cTnI positive and cTnI negative groups were compared. Demographic data included age, sex, type of angina, prevalence of risk factors, previous cardiac events and interventions, and current treatments. Angiographic variables that were analysed included type of CAD i.e. single or multiple vessels CAD, the anatomic site of angioplasty, type of PCI i.e. single or multiple vessels PCI, and the ejection fraction. Procedural variable included balloon dimensions, the total numbers of balloon inflations per procedure, total duration of inflations, maximum inflation time and pressure, and the total duration of PCI. Other variables included, the incidence and type of complications reported by the operator during PCI, complications reported within the first 24 hours period post-PCI, the numbers of stents used and the reason for stenting, the use of intravascular ultrasound during PCI, the reported clinical and angiographic successes and the type of antiplatelet treatment post-PCI.

Four patients were excluded from the analysis because of an initial increase in cTnI concentration in the pre-angioplasty sample, suggesting another cause for the increase in cTnI e.g. active ischaemia. Out of these four patients, three had further increase in cTnI concentration in subsequent samples post-PCI compared to base line. One patient had elevated cTnI concentration of 0.75 μg/l in the base line sample. The value remained relatively unchanged throughout the sampling period. There was no complication reported during PCI. The myoglobin concentration was also markedly increased in this patient (range 201 - 317 μg/l). Creatine kinase-MB mass concentration was normal. This patient was a diabetic and his renal function

showed a slightly raised urea (7.7 mmol/l) and creatinine (131 mmol/l). He had severe angina poorly controlled with medications, with angina occurring every night at rest and on minimal exertion. The elevated cTnI concentration is most probably due to UA with minor myocardial damage. One patient was biochemically hypothyroid with TSH of 52 mU/L and T_4 of 5 pmol/L. The pre-angioplasty sample showed increased concentration of myoglobin of 104 μg/l, which remained elevated throughout. Creatine kinase-MB mass concentration was normal but cTnI was only abnormally increased at 16 - 24 hours post-PCI (0.27 μg/l). The slight increase in myoglobin could be due to a subclinical myopathy that is often associated with uncontrolled hypothyroidism. Cardiac-TnI being more specific to the heart can differentiate myoglobin rise due to myocardial injury and other injuries.

Out of the 80 patients studied, 10 [12.5%] developed ST segment changes, or T wave inversion or new conduction abnormality and chest pain suggestive of ischaemia during angioplasty, 15 [18.75] developed chest pain but no reported ECG changes of ischaemia. Fifty-five [68.75%] patients had no chest pain or ECG changes reported during PCI (Table 1). Eleven out of the 55 patients were given Cyclomorphine (2.5 - 5 mg) as prophylaxis at the beginning of angioplasty and this may have affected the frequency of chest pain reporting in this group during PCI.

No. of patients (%)	Clinical findings
10 (12.5)	Had ECG changes of ischaemia and chest pain
15 (18.75)	Had chest pain but no ECG changes of ischaemia
55 (68.75)	Had no chest pain or ECG changes of ischaemia reported

Table 1. Illustrates the percentages of patients with chest pain ± ECG changes of ischaemia during angioplasty.

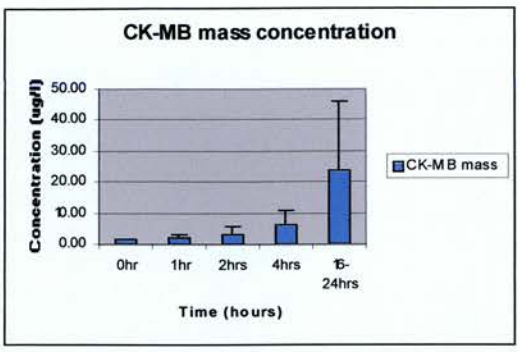
Table 2 shows the basal and maximum concentrations of cardiac markers in the control and angioplasty groups. There was no difference between the basal concentrations in the two groups. However, the maximum concentrations were significantly elevated in the angioplasty group only [$p < 0.001$] (Table 2). Cardiac-TnI showed the most frequent abnormal values. The maximum increase in cTnI concentration occurred at 16 - 24 hours. In 37 out of 80 patients (46.25%), cTnI concentration was ≥ 0.18 µg/l. An increase in cTnI concentration [> 0.06 - ≤ 0.17 µg/l] was observed in 22 patients (27.5%). Myoglobin was the second most frequent abnormal marker. Myoglobin was increased in 14 (17.5%) patients, H-FABP was increased in 6 (13.3%) patients, and CK-MB mass in 9 (11.25%) patients. The concentrations changes of cTnI at 16 - 24 hours were closely correlated to changes in CK-MB mass [$r = 0.784$, $p < 0.0005$] at 16 hours, cTnT [$r = 0.529$, $p < 0.001$] at 16 hours, myoglobin [$r = 0.484$, $p < 0.002$] at 4 hours, and H-FABP [$r = 0.612$, $p < 0.002$] at 2 hours. In all cases where CK-MB mass, cTnT, H-FABP or myoglobin were elevated, cTnI was also elevated.

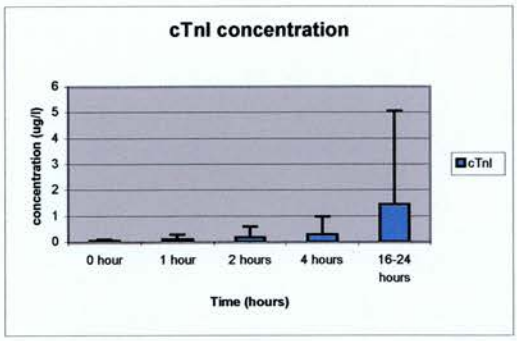
	Control group		*Angioplasty group*	
Marker(s)	*Basal* µg/l	*Maximum* µg/l	*Basal* µg/l	*Maximum* µg/l
H-FABP	6.86 ± 2.21	8.00 ± 2.50	9.70 ± 5.8	30.0 ± 15.12
CK-MB mass	2.55 ± 1.2	2.22 ± 1.1	1.60 ± 1.2	23.70 ± 29.10
Myoglobin	54.5 ± 24.7	51.3 ± 23.9	54.8 ± 33.4	113.78 ± 60.0
CTnI	0.065 ± 0.054	0.058 ± 0.054	0.04 ± 0.04	1.44 ± 3.61
CTnT	0.011 ± 0.003	0.012 ± 0.007	0.01 ± 0.003	0.49 ± 0.61

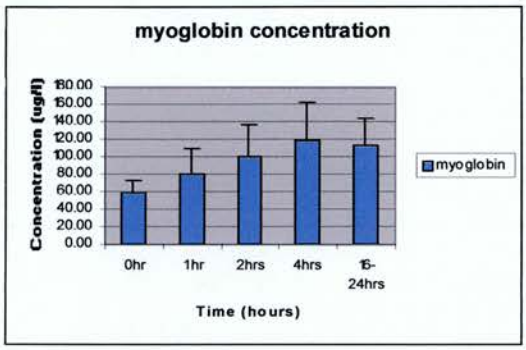
Table 2. Illustrates basal and maximum concentrations of the different cardiac markers in the control and angioplasty groups.

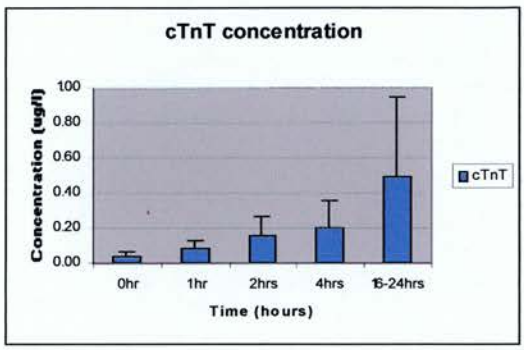
As compared with cTnI, cTnT was increased in only 6 (7.5%) patients, and five of them had significant complications. Cardiac-TnT was elevated to concentrations between 0.01 - 0.06 μg/l in 36 patients, and between ≥ 0.06 - < 0.1 μg/l in 4 patients. The complications reported in these two groups were in 10 and 3 patients respectively. All 6 patients with cTnT > 0.1 μg/l and the 13 patients in the last two groups had cTnI ≥ 0.18 μg/l. There was a contrast between cTnI and cTnT peak concentrations levels. In two patients who had PCI complicated by AMI, the cTnI concentration was 19.77 and 6.66 μg/l, and the corresponding cTnT concentration was 1.68 and 0.66 μg/l respectively. This may reflect different standardisation between the two assays. In the cTnI negative group [43 patients, 53.75%], no increase in cTnI, cTnT or CK-MB mass concentration was observed. Myoglobin was elevated in two patients, and H-FABP (concentration = 21 μg/l) in one patient. The PCI procedure in one of the two patients with elevated myoglobin (myoglobin = 117 μg/l, cTnI = 0.15 μg/l) was complicated by minor dissection but no complications were reported in the other patient, or in the patient with elevated H-FABP.

The release patterns of cardiac markers in the cTnI positive group are shown in Figure 3. These markers were significantly elevated compared with the base line concentrations. Peak concentrations of H-FABP, myoglobin, cTnI, cTnT, and CK-MB mass were achieved at 2 hrs, 4 - 16 hrs, 16 - 24 hrs, 16 - 24 hrs, and 16 - 24 hrs respectively. This release pattern shows the early release characteristics feature of H-FABP after myocardial injury compared with other markers including myoglobin.









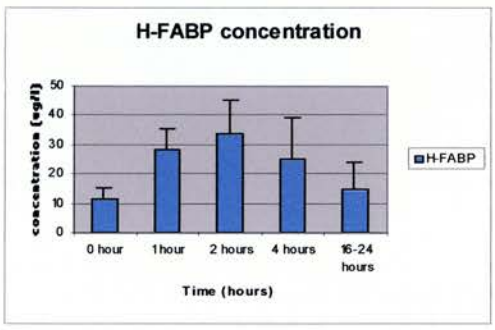


Figure 3. These graphs illustrate the release patterns of CK-MB mass, myoglobin, cTnT, H-FABP, and cTnI before (0 hour), and at 1, 2, 4, and 16 - 24 hours after angioplasty in the cTnI positive group. There was a significant change in cardiac markers concentrations before and after PCI.

Concomitant increases of all markers were associated with more peri-procedural and post-procedural adverse events, and the higher the concentration the more frequent and more serious were the complications (Table 3). Out of the 3 patients with elevations in all five cardiac markers, 2 developed AMI and 1 had major equipment failure. One patient with simultaneous increase in cTnI and CK-MB mass had an important dissection during PCI. Two of the 7 patients with elevations in cTnI and myoglobin had complications including dissections, transient vessel closure (TVC), side branch occlusion (SBO) and recurrent chest pain requiring revascularisation. Of the remaining five, 2 had minor dissections and 3 had no complications reported. Two patients had simultaneous increases of cTnT and cTnI, one patient had major dissection with SBO and bail out stents, and the other patient died 4 days after catheterisation from massive stroke.

Eighteen patients had an increase in cTnI concentration alone. Eight patients had some complications during PCI, whereas in 6 patients no specific complications were reported during PCI. However, in these patients the angioplasty procedure was described as technically difficult and prolonged or the lesion was complex, and three patients had failure of stent deployment, sudden drop of blood pressure after sheath removal, and limb ischaemia post-procedure. Two patients in this group had total occlusion of the vessels and two had osteal lesions. In 4 patients with cTnI elevations [range 0.18 - 0.65 μg/l] no specific complications were reported to explain this rise.

The use of newer and potent antiplatelets treatment after PCI was liberal in this group of patients. Fifty-five patients (68%) received some form of antiplatelets treatment after stenting. Thirty-one patients (56.4%) received ticlopidine, 15 (27.3%) received clopidogrel, 5 (9.1%) received Abciximab and ticlopidine, and 4 (7.2%) received Abciximab and clopidogrel. The demographic and angiographic data of the cTnI positive and negative groups are shown in Table 4 and 5. Patients in the cTnI positive group were older ($p < 0.02$). The type of proceeding angina, antianginal treatment and the prevalence of risk factors were similar between the two groups.

# Patients (37)	# CM elevated	Complication(s)
3	I, T, MB, Myo, H-FABP	2- AMI. 1-major equipment failure. *
4	MB, I, Myo	1-AMI.(H-FABP increased in this patient) 2-major dissection ± SBO. 1-minor dissection.
1	I, T, MB	Bradycardia + T wave inversion on ECG.
7	I, Myo	2- major dissection ± TVC ± bail out stent. 2-minor dissections. 3-no complications reported.
1	I, MB	Major dissection.
2	I, T	1-major dissection ± SBO ± bail out stent. 1-see text.
1	I, H-FABP	Major dissection ± TVC ±bail out stent.
18	I	5-major dissection ± TVC ± bail out stent. 3-minor dissection ± coronary spasm. 6- complex lesion ± prolonged procedure ± minor technical problems. 4- no reported complications.

Table 3. Shows the classifications of patients with elevated cTnI (n = 37) into subgroups. In each subgroup the numbers of elevated cardiac markers are shown a long with the complication(s) reported in each subgroup. Abbreviations: CM, cardiac markers; I, T, MB, H-FABP, and Myo means cTnI, cTnT, CK-MB mass, heart fatty acid binding protein, and myoglobin respectively; AMI, acute myocardial infarction; SBO, side branch occlusion; TVC, transient vessel closure. * In this patient the angioplasty balloon catheter-tip was lost inside the coronary artery.

There was a non-significant increase in the frequency of hypertension, previous AMI, and multiple vessels CAD in the cTnI positive group. Only a few patients (11) had multiple vessels PCI and there was no difference in cardiac markers release between the two groups. There was a small difference related to the anatomical site of angioplasty with increased cTnI with angioplasty in the left anterior descending artery territory. Clinical and angiographic successes were reported more frequently in the cTnI negative group [95% vs 73%, $p < 0.013$], and [95% vs 81%, $p < 0.04$] respectively (Table 4 and 5).

Demographic data	*TnI positive group* (N = 37)	*TnI Negative group* (N = 43)	*P value*
Age (yrs)	64.35 ± 9.22	59.1 ± 7.47	0.02
Sex:			
Male	26 (70)	33 (77)	NS
Female	11 (30)	10 (23)	NS
Type of angina:			
Stable angina	19 (51)	26 (43)	NS
Unstable angina	14 (38)	17 (40)	NS
Atypical angina	4 (11)		
Risk Factors:			
Smoking	24 (65)	27 (63)	NS
Diabetes Mellitus	3 (8)	8 (19)	NS
Hypercholestrolaemia	27 (73)	37 (86)	NS
Hypertension	18 (49)	14 (33)	NS
Family history of IHD	16 (43)	23 (53)	NS
Cardiac Events:			
Previous PCI	10 (27)	16 (37)	NS
Previous CABG	4 (11)	3 (7)	NS
Previous AMI	15 (41)	14 (33)	NS
Current treatment:			
Nitrates	14 (38)	22 (51)	NS
β-Blockers	22 (60)	29 (67)	NS
Ca antagonists	23 (62)	23 (53)	NS
Aspirin	36 (97)	43 (100)	NS
K^+ channel openers	3 (8)	4 (9)	NS

Table 4. Shows the demographic data of patients with and without elevated cTnI. Abbreviations: TnI, cardiac troponin I; P, probability value; yrs, years; IHD, ischaemic heart disease; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; AMI, acute myocardial infarction.

Angiographic data.	*TnI positive group (N = 37)*	*TnI Negative group (N = 43)*	*P value*
Type of CAD:			
Single vessel CAD	6 (16)	14 (33)	NS
Multiple vessel CAD	31 (84)	29 (67)	NS
Single vessel PCI.			
Anatomic site of PCI:			
LAD	27 (73)	29 (67)	NS
LCX	11 (30)	14 (32)	NS
RCA	6 (16)	4 (9)	NS
Vein grafts	1 (3)	3 (7)	NS
Multiple vessel PCI	5 (14)	6 (14)	NS
Left ventricular EF (%)	62±12	65±14	NS
Clinical success)	27 (73)	41 (95)	0.013
Angiographic success	30 (81)	41 (95)	0.04

Table 5. This table shows the angiographic data of patients with and without elevated cTnI. Abbreviations: TnI, cardiac troponin I; P, probability value; CAD, coronary artery disease; PCI, percutaneous coronary intervention; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; EF, ejection fraction.

Table 6 illustrates the procedural variables. There was a significant difference between the two groups with respect to occurrence of the following; chest pain ± ischaemic ECG during PCI [51.4% vs 13.9%, $p < 0.004$]; numbers of stents used [42 vs 32, $p < 0.019$]; and use of intravascular ultrasound during PCI [16% vs 2%, $p < 0.045$]). These variables were more frequent in the cTnI positive group. Total duration of PCI [56.9 ± 38.3 vs 42.31 ± 19.2 minutes], and total numbers of balloon inflations per procedure [6.4 ± 4.6 vs 4.81 ± 2.8] were increased in cTnI positive group but not statistically significantly. There was no difference between the groups related to the number of lesions treated, balloon size, inflation time and pressure, and the antiplatelet regimes after PCI.

Procedure data	*TnI positive group (N = 37)*	*TnI Negative group (N = 43)*	*P value*
Chest pain ± ECG changes of ischaemia	*19 (51.4)*	*6 (13.9)*	*0.004*
Procedure information:			
Number of lesions dilated (per patient)	46 (1.24)	41 (1.24)	NS
Number of vessels dilated (per patient)	41 (1.11)	40 (1.21)	NS
Balloon diameter (mm)	3.3 ± 0.56	3.12 ± 0.45	NS
Total number of balloon inflation (n)	6.4 ± 4.6	4.81 ± 2.8	NS
Total time of balloon inflation (minutes)	3.77 ± 4.18	3.41 ± 2.86	NS
Maximum inflation time (seconds)	50.12 ± 26	55.24 ± 24.34	NS
Maximum inflation pressure (Pa)	11.21 ± 3.34	10.45 ± 3.56	NS
Total duration of procedure (minutes)	56.9 ± 38.3	42.31 ± 19.2	NS
Major complications during PCI:			
Major dissection	10 (27)	1 (2)	0.004
Side branch occlusion	5 (13.5)	2 (5)	NS
Transient vessel occlusion	7 (19)	1 (2)	0.022
Major technical failure	1 (3)	0 (0)	NS
Bail out stent	7 (19)	0 (0)	0.003
Minor complications during PCI:			
Minor dissection	8 (21.6)	7 (16)	NS
Coronary spasm/ elastic recoil	2 (5.4)	5 (12)	NS
Minor technical failure	1 (3)	2 (5)	NS
Post-procedural complications (24h):			
AMI.	3 (8)	0 (0)	0.042
Angina with re-catheterisation	5 (13.5)	1 (2)	NS
Angina without re-catheterisation	1 (3)	2 (5)	NS
Emergency CABG.	0 (0)	0 (0)	-
Total number of stents	42	32	0.019
Reason for stenting:			
Stent for dissection	15 (40.5)	8 (17)	0.05
Stent for sub-optimal result	12 (32.4)	15 (35)	NS
Elective stent	3 (8)	6 (14)	NS
Bail out stent	7 (19)	0 (0)	0.003
Use of IVUS during PCI	6 (16)	1 (2)	0.045
Post-procedural treatment:			
Ticlopidine	11 (30)	20 (47)	NS
Clopidogrel	8 (22)	7 (16)	NS
Abciximab + Ticlopidine	3 (8)	2 (5)	NS
Abciximab + Clopidogrel	3 (8)	1 (2)	NS

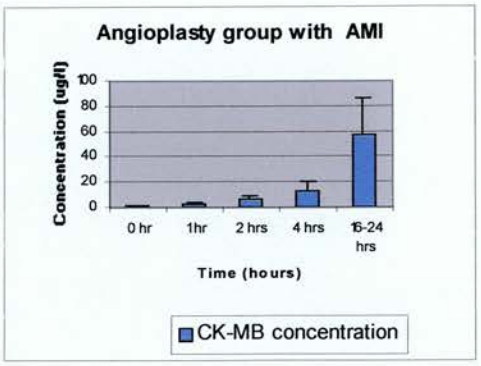
Table 6. Illustrates the procedural variables in patients with and without elevated cTnI. Abbreviations: TnI, cardiac troponin I; P, probability value; CAD, coronary artery disease; PCI, percutaneous coronary intervention; mm, millimetre; Pa, Pascal; AMI, acute myocardial infarction; IVUS, intravascular ultrasound.

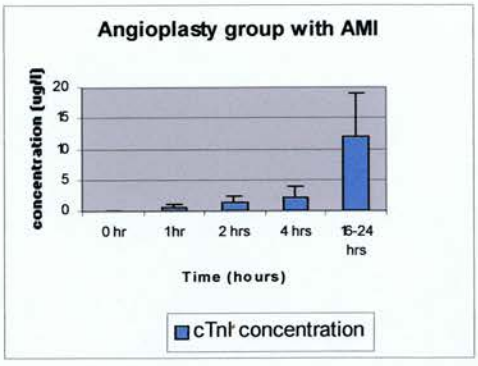
The frequency and types of complications reported during and after PCI were significantly increased in the cTnI positive group. Out of the 37 patients in this group 23 (62%) had complications compared to only 14 out of the 43 patients (32.5%) in the cTnI negative group ($p < 0.03$). These complications included one or combination(s) of the following; major dissection [27%, $p < 0.004$]; side branch occlusion [13.5%, p = NS]; transient vessel occlusion [19%, $p < 0.022$]; bail out stents [19%, $p < 0.003$]; AMI [8%, $p < 0.042$]; major technical failure [3%, p = NS]; angina requiring re-catheterisation [13.5%, p = NS]; and minor dissection [21.6%, p = NS]. One patient had major technical failure of equipment during angioplasty. The balloon tip snapped-off inside the coronary artery and was snared with difficulty. The corresponding cTnI, CK-MB mass, cTnT, H-FABP, and myoglobin concentrations were elevated in this patient 1.5, 5.9, 0.23, 31, and 129 μg/l respectively.

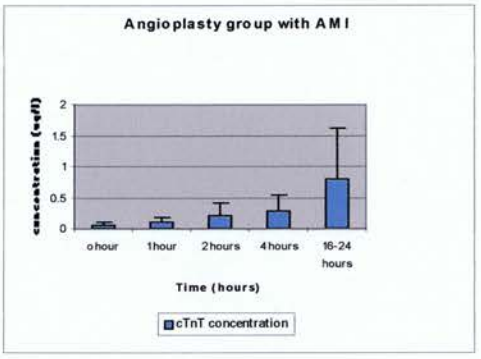
Fourty-two stents were used in this group compared to 32 stents in the cTnI negative group. Thirteen stents (31%) were used mostly on bail out bases. The increased frequency of stents in this group was mostly to treat dissections [40.5% vs 17%, p = 0.05]. The use of stents for elective and sub-optimal angioplasty results was slightly more in the cTnI negative group [49% vs 40.4%]. Seven patients had bail out stents, which were all used to treat major dissection ± acute vessel closure. Two of the 7 patients subsequently developed AMI (cTnI, cTnT, CK-MB mass, H-FABP, and myoglobin mean concentrations were 13.23, 1.17, 68.4, 38.5, and 216 μg/l respectively) and the cTnI, cTnT, CK-MB mass, and myoglobin mean concentrations were elevated in the remaining 5 patients (1.2, 0.1, 5.75, and 102.6 μg/l respectively).

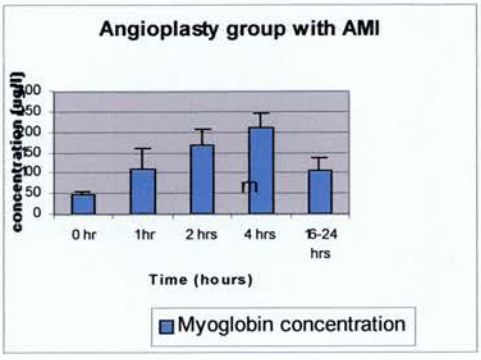
Six patients had intravascular ultrasound investigation or pressure wire studies performed during PCI, compared with one patient only in the cTnI negative group. All of them had cTnI elevation after PCI. This increase was due to another apparent complications ± intravascular ultrasound in 3 patients, but in 2 patients no explanation for the rise in cTnI could be detected other than uncomplicated angioplasty and intravascular ultrasound use. The mean concentrations of cTnI in these two patients was slightly raised at 0.185, whereas cTnT, CK-MB mass, H-FABP and myoglobin remained below the cut-off point 0.076, 2.1, 14.3, and 91.5 μg/l respectively. One patient was admitted with massive stroke two days after intravascular ultrasound investigation, and he died four days after the event. Creatine kinase-MB mass and H-FABP concentrations in this patient were normal, but cTnI, cTnT, and myoglobin were elevated (0.39, 0.14, and 123 μg/l respectively). This patient had no complications and the cause of death was not related to PCI.

Three patients developed AMI after PCI (1 Q wave and 2 non-Q wave AMI). The concentrations of cardiac markers in these patients were significantly elevated compared to the normal base line values. Heart-FABP was the first marker to appear in significant concentrations after AMI. Peak concentrations of H-FABP, myoglobin, CK-MB mass and troponins occurred at 2 hours, 4 hours, 16 - 24 hours, and 16 - 24 hours respectively after angioplasty. These release patterns were similar to the release patterns seen in Figure 3. Heart-FABP reached diagnostic concentrations for AMI 1 - 2 hours post-angioplasty. The diagnosis of AMI based on myoglobin, CK-MB mass, and troponins can be established at 2 - 4, 4 - 16, and 4 - 16 hours respectively (Figure 4).









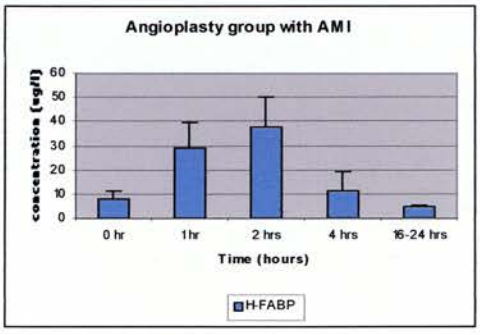


Figure 4. Shows the release patterns of cTnT, cTnI, CK-MB mass, H-FABP, and myoglobin at 0 hour (before angioplasty), and at 1, 2, 4, and 16 - 24 hours post-angioplasty, in patients with AMI after PCI. Diagnostic concentrations for AMI were noticed between 1 - 2, 2 - 4, 4 - 16, and 4 - 16 for H-FABP, myoglobin, CK-MB mass, and cTnI respectively.

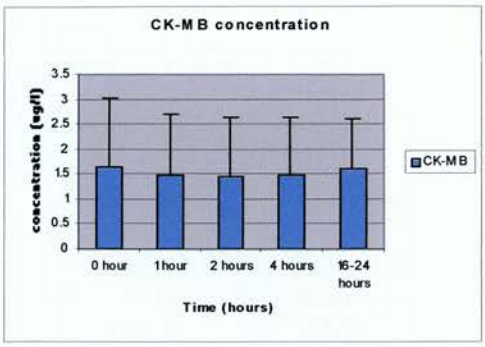
Five patients had SBO during angioplasty. In 3 patients, this complication followed an extensive dissection. Out of the 5 patients, 2 developed AMI, 2 had considerable chest discomfort and ST segment depression ± T wave inversion, and in one patient SBO was asymptomatic. Cardiac-TnI concentration was elevated in all patients. The mean cTnI increase in these three groups was 14.7 μg/l, 1.73 μg/l, and 0.18 μg/l respectively. Creatine kinase-MB mass, and myoglobin were increased in three patients, whereas H-FABP and CTnT were increased in two patients only. In the cTnI negative group two patients had asymptomatic SBO, but there was no increase in cardiac markers in any of them.

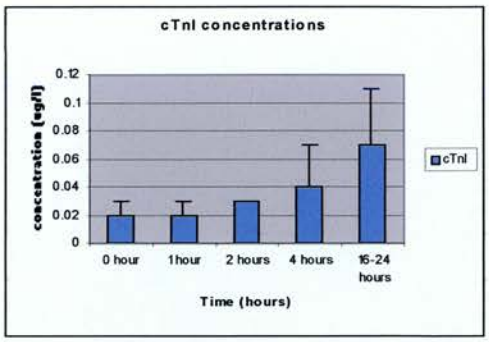
The release patterns of cTnI, cTnT, CK-MB mass, H-FABP, and myoglobin in the cTnI negative group are shown in Figure 5. There were no significant increases in cardiac markers concentrations compared to base line values. This group included 43 patients with cTnI < 0.18 μg/l. Fourteen patients (32.5%) in this group developed complications during PCI. However, the frequency and severity of complications in this group were considerably lower than cTnI positive group. These complications include one patient with major dissection, two patients with transient side branch occlusion, and one patient with transient vessel closure. The types of complications reported in the remaining 10 patients were minor dissections, coronary spasm, elastic recoil, and minor technical problems.

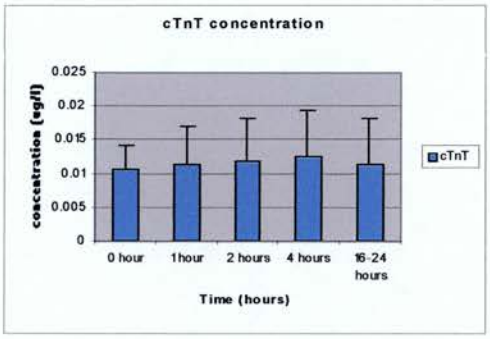
Patients were also divided into cTnT, CK-MB mass, myoglobin, and H-FABP positive and negative groups, depending on the presence or absence respectively of concentrations rise above the respective cut-off values. These groups were then

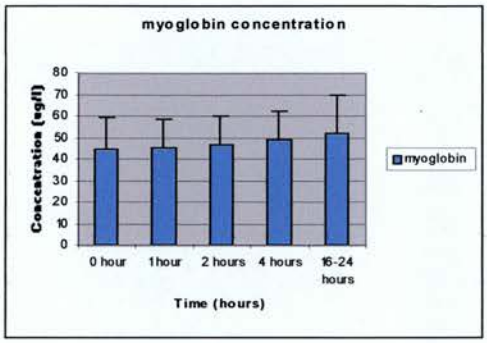
compared in exactly the same manner as patients in cTnI positive and negative groups. There were similar findings across most of these groups. The most significant and consistent predictors of elevated concentrations were increased age, development of chest pain ± ischaemic ECG changes, occurrence of in-hospital complications, occurrence of major complications during PCI (major dissection, side branch occlusion, transient vessel closure, and AMI), angina with re-catheterisation, bail out stents, number of stents used to treat dissection, and the use of intravascular ultrasound. These variables were significantly elevated across most cardiac markers positive groups. Clinical and angiographic successes were increased in cardiac markers negative groups.

In a separate analysis, patients were classified according to the degree of complications reported during or after PCI into 3 groups. Group I, includes patients who had no complications (NC); group II, includes patients with minor complications (MC), and group III, includes patients with major complications (C). The types of complications are outlined in Table 7. In group I, a total of 43 patients had no complications reported during PCI, 29 patients (67.5%) had cTnI < 0.18 μg/l and 14 patients (32.5%) had elevated cTnI ≥ 0.18 μg/l. In group II, 16 patients had minor complications during PCI, 6 patients (37.5%) had elevated cTnI concentrations, whereas 10 patients (62.5%) had no cTnI elevation. In group III, cTnI was elevated in 17 out of 21 patients (81%).









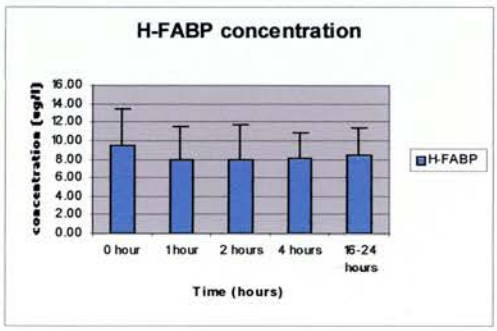


Figure 5. These graphs illustrate the release patterns of CK-MB mass, myoglobin, cTnT, H-FABP, and cTnI before (0 hour), and at 1, 2, 4, and 16 - 24 hours after angioplasty in the cTnI negative group. There was no significant change in cardiac markers concentrations before and after PCI.

Table 8 below illustrates the three different groups of patients. The percentage of patients with elevated cTnI concentration increases proportionally in relation to the degree of complications reported during or after PCI. The proportion of patients with cTnI $\geq$ 0.18 μg/l was 32.5%, 37.5%, and 81% for the group with NC, MC, and C respectively. The maximum increase in cTnI concentration occurred in the group with major complications (mean = 2.64 μg/l). The maximum concentration of cTnI in the other two groups was < 0.96 μg/l. The frequency of abnormal elevation for the other markers in these three groups is shown in Table 8 (cTnT [C = 23.8%, MC = 0%, NC = 2.3%], CK-MB mass [C = 33.3%, MC = 6.3%, NC = 2.3%], H-FABP [C = 33.3%, MC = 0, NC = 5.3%], and myoglobin [C = 38.1%, MC = 18.7, NC = 7%]).

Based on these data, the sensitivity, specificity, PPV, and NPV of the various cardiac markers for the detection of complications during angioplasty are shown in Table 9. Table 9A shows these values when the analysis was conducted specifically to detect major complications during angioplasty (patients with minor complications excluded, see Table 7 and 8). Cardiac-TnI has the highest sensitivity (81%) and negative predictive accuracy (88%) compared to cTnT, CK-MB mass, H-FABP, and myoglobin. However, the specificity and PPV of the latter markers was significantly better than cTnI. The low specificity (67.5%) and PPV (55%) of cTnI was due to the inclusion of patients with no reported complications (NC, n = 14) but with cTnI concentrations $\geq$ 0.18 μg/l. Cardiac-TnT, H-FABP, myoglobin, and CK-MB mass were highly specific (93 - 97.6%), and elevation of these markers were correlated with the presence of major complications. However, this superior specificity and positive predictive accuracy was markedly offset by poor sensitivity (24 - 38.1%)

and low NPV for major complications. The corresponding sensitivity and other values for all cardiac markers are shown Table 9A.

Table 9B, shows repeated analysis for the detection of any complications (major and minor) during angioplasty (See Table 7 and 8). There was an overall reduction in sensitivity and NPV for all markers. This reduction could be explained by the inclusion of many patients in the minor complications group with evidence of myocardial damage at angiography but without any significant release of cardiac markers. Cardiac-TnI was still the most sensitive marker (62%). Cardiac-TnT and CK-MB mass retained the highest specificity and the lowest sensitivity for the detection of minor and major complications. Heart-FABP performance closely resembled those of CK-MB mass, but with a reduced NPV.

The reduction in NPV of H-FABP (46%) is due to the inability of this marker to differentiate patients with small damage to the myocardium in the minor complication group. Overall, in terms of sensitivity, specificity, PPV and NPV for the detection of complications during PCI, the performance of H-FABP, CK-MB mass, cTnT, and myoglobin were closely related. In patients with major complications there was a strong correlations between concentrations changes of cTnI at 16 hours and changes of CK-MB mass [$r = 0.832$, $p < 0.0005$] at 16 hours, cTnT [$r = 0.672$, $p < 0.002$] at 16 hours, myoglobin [$r = 0.640$, $p < 0.002$] at 4 hours, and H-FABP [$r = 0.705$, $p < 0.003$] at 1 hour.

Minor complications (MC)	*Major complications (C)*
Coronary spasm	Major dissection
ECG changes of ischaemia only	Transient vessel occlusion
Complex lesion or prolonged procedure (> 60 minutes)	Acute myocardial infarction
Minor dissection (not compromising lumen)	Major side branch occlusion
Elastic recoil	Bail out stent
Minor technical complication e.g. failure of stent deployment, balloon burst	Major equipment failure
Asymptomatic small vessel side branch occlusion	

Table 7. Shows the types of minor and major complications in the two groups of patients.

Groups	*# Patients*	*CM status*	*CTnI*	*CTnT*	*CK-MB mass*	*Myoglobin*	*H-FABP**
I-NC	43	+ -	14 (32.5) 29 (67.5)	1 (2.3) 42 (100)	1 (2.3) 42 (97.7)	3 (7) 40 (93)	1 (5.3) 18 (94.7)
II-MC	16	+ -	6 (37.5) 10 (62.5)	0 (0) 16 (100)	1 (6.3) 15 (93.7)	3 (18.7) 13 (81.3)	0 (0) 11 (100)
III-C	21	+ -	17 (81) 4 (19)	5 (23.8) 16 (76.2)	7 (33.3) 14 (66.6)	8 (38.1) 13 (61.9)	5 (33.3) 10 (66.7)

Table 8. This table illustrates the three different groups of patients without complications (NC), with minor complications (MC) and with major complications (C). The total numbers of patients (# patients) in each group and cardiac marker (CM) status i.e. (+) above cut-off concentration or (-) below cut-off concentration is shown in the first three columns. The numbers and percentages of patients with cTnI, cTnT, CK-MB, myoglobin and H-FABP above and below the respective cut-off point are also shown. *These calculations were based on a slightly modified numbers of patients.

9A Statistical values for the detection of major complications

	CTnI	CTnT	CK-MB	Myoglobin	H-FABP
Sensitivity	81	24	33	38.1	33
Specificity	67.5	97.6	98	93	94.7
Positive predictive value	55	83	87.5	73	83
Negative predictive value	88	72	75	75.5	64

9B Statistical values for the detection of any complications (minor and major)

	CTnI	CTnT	CK-MB	Myoglobin	H-FABP
Sensitivity	62	13.5	22	29.7	19.2
Specificity	67	97.6	97.6	93	94.7
Positive predictive value	62	83	88.8	78.5	83
Negative predictive value	67	56.7	59.2	60.6	46

Table 9. Shows the sensitivity, specificity, positive predictive value and negative predictive values of the various cardiac markers for the detection of; (9A) major complications only; and (9B) any complications (major or minor) during angioplasty.

Patients were then divided into positive complications group (had any complications during PCI, minor or major, n = 37) and negative complications group (no reported complications, n = 43) and compared. The following demographic, angiographic and procedural variables were correlated with the presence of complications during PCI (previous angina pectoris [$p = 0.05$], previous AMI [$p < 0.04$], any previous cardiac event i.e. CABG, PCI, and AMI, [$p < 0.013$], use of stents for dissection [$p < 0.005$], total numbers of stents used [$p < 0.006$], use of intravascular ultrasound [$p < 0.005$], total duration of procedure [$p < 0.001$], total numbers of balloon inflations [$p < 0.035$], total time of balloon inflation [$p < 0.041$], increased cTnI concentrations [$p < 0.002$], increased CK-MB concentrations [$p < 0.01$], increased myoglobin concentrations [$p < 0.001$], and increased H-FABP concentrations [$p < 0.001$]).

After discharge, clinical follow-up was available in 79 out of 80 patients (98.75%). Mean follow-up period was 22.3 ± 1.7 months (range 20 - 26 months). Only one patient were lost to follow-up, and had not been seen as either inpatients or outpatients at the Royal Infirmary of Edinburgh since discharge following their PCI procedure. Patients in the positive and negative cTnI groups had similar periods of follow-up (22.6 ± 1.5 vs 23 ± 1.2 months). The incidence of adverse clinical events is summarised in Table 10 A. Patients who had cTnI elevation post-PCI had a non-statistically significant higher incidence of complications (angina pectoris, UA, and non-target vessel revascularisation) than patients without post-procedural cTnI elevation. Seventeen patients (46%) in the cTnI positive group had one or more clinical event(s) during the follow-up period compared with 12 patients (28%) in the cTnI negative group. The total number of clinical events per group in the cTnI positive and cTnI negative group was [29/37 (78%)] vs [21/43 (48%)] respectively, p = NS.

The prevalence of uncontrolled angina in the cTnI positive group was not statistically different. Seventeen patients (46%) described their angina after PCI as unchanged or worse, compared to 12 patients (28%) in the cTnI negative group. Three patients who were included in the cTnI negative group had several adverse clinical events during follow-up (worsening of their angina, target and non-target vessel PCI, and CABG), despite uneventful PCI. Their cTnI concentration was between 0.08 - 0.11 μg/l, which suggests that even a small rises of cTnI may carry prognostic significance and are associated with adverse clinical events post-PCI. There were three deaths in total, one death in the cTnI positive group and two deaths in the cTnI negative group.

Table 10 A. Cardiac troponin I data

Events	*CTnI (+) group (N = 37)*	*CTnI (-) group (N = 43)*	*p value*
Angina control post-procedure:			
Worse	10 (27)	10 (23)	NS
Better	20 (54)	30 (70)	NS
Unchanged	7 (19)	2 (5)	NS
Cardiac event(s) during follow-up:			
Admitted with angina	5 (14)	3 (7)	NS
Admitted with UA	5 (14)	2 (5)	NS
Admitted with AMI	1 (3)	0	-
Admitted with Heart failure	1 (3)	0	-
Target vessel revascularisation	6 (16)	7 (16)	NS
Non-target vessel revascularisation	5 (14)	4 (9)	NS
Referred for CABG	3 (8)	3 (7)	NS
Death	1 (3)	2 (5)	NS
Total No. with uncontrolled angina	17 (46)	12 (28)	NS
Total No. of patients with events(%)	17 (46)	12 (28)	NS
Total No. of events per group	29 (78)	21 (48)	NS
Average duration of follow-up (months))	22.6 ± 1.5	23 ± 1.2	NS
Patients lost to follow-up	0	1	

Table 10 B. Cardiac troponin T data

Events	*CTnT (+) group (N = 6)*	*CTnT (-) group (N = 74)*	*p value*
Referred for CABG	3 (50)	3 (4)	0.004

Table 10. Table 10 A shows the degree of angina control and the numbers of adverse cardiac events after ≥ 20 months of follow-up. Numbers between brackets indicate the percentages. Table 10 B shows a similar analysis for a subgroup of patients with cTnT positive and negative groups with respect to referral for CABG. See text for explanation. Abbreviations are similar to Table 4.

All deaths were not related to cardiac complications. There were also no statistical differences between the frequency of these cardiac events when patients were compared with respect to CK-MB mass, myoglobin, and H-FABP positive and negative groups. Surprisingly cTnT was highly predictive of the need for CABG on long-term follow-up. Although the total numbers of patients who had CABG in each group were similar (i.e. three patients in each group), the high statistical significance between the two groups can be appreciated when these numbers were translated into percentages of the overall number of patients with cTnT elevation post-PCI. Fifty per cent of patients with elevated cTnT after PCI were referred for CABG compared to only 4% of patients with normal cTnT after PCI ($p < 0.004$, Table 10 B). There were no other statistical differences between cTnT positive and negative groups when other cardiac events (see table 10 A) were compared between the two groups.

The time-dependent effect of post-procedural cTnI elevation on late clinical outcome was assessed using Kaplan-Meier survival analysis. There was a significant decrement in event-free survival with more recurrent angina, myocardial infarction, repeat PCI, and CABG in the group of patients who had cTnI elevations during angioplasty. The mean event-free survival for the group with cTnI elevation and those without cTnI elevation was 16.92 months [SE = 1.66, 95% CI = 13.67 – 20.18, median = 23 months] vs 20.67 months [SE = 1.64, 95% CI = 17.46 – 23.88, median = 27 months] respectively ($p < 0.03$). Event-free survival was also decreased in cTnT and H-FABP positive groups. There were no event-free survival differences when CK-MB mass or myoglobin positive and negative groups were compared (Figure 6).

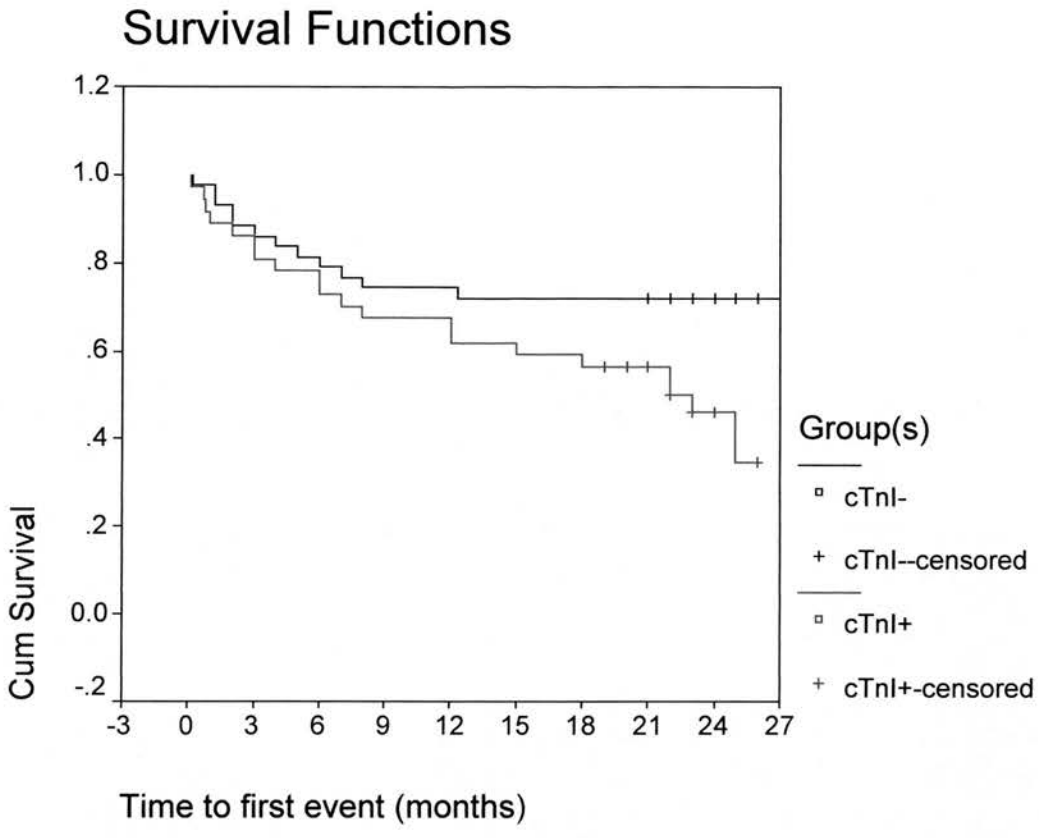


Figure 6. Shows the Kaplan-Meier event-free survival analysis for the two groups of patients with cTnI elevation (cTnI +) and without cTnI elevations (cTnI -) during angioplasty.

5.7 DISCUSSION

The frequency and prognostic value of elevation of cTnI, cTnT, CK-MB mass, myoglobin, and H-FABP were evaluated in 80 patients undergoing elective PCI. The significance of H-FABP as marker with potential for early detection of myocardial injury was also studied and compared with other standard markers. Heart-FABP has been shown in this study to be the most important early marker of myocardial injury. Its concentration was significantly elevated within 1 - 2 hours following myocardial injury and the concentration had returned to normal within 16 - 24 hours. The frequency of cTnI increases post-PCI in patients with complications was much

higher than that seen with myoglobin, CK-MB mass, H-FABP, and cTnT (62 % vs 29.7 %, 22%, 19.2%, and 13.5 % respectively). No patients with complications post-PCI had an increase in cTnT, CK-MB mass, H-FABP or myoglobin without concomitant increase of cTnI. Cardiac-TnI was the most useful marker for the detection and quantification of PCI related complications. This reflects superior sensitivity of cTnI for the detection of small releases of myocardial proteins after PCI, which were undetectable using some less specific markers.

Previous AMI or angina, increased use of stents, use of intravascular ultrasound, total numbers of balloon inflations, total time of balloon inflation, total durations of PCI, and increased cTnI, CK-MB mass, myoglobin, and H-FABP concentrations were variables that correlated with occurrence of complications during PCI. Old age, peri-procedural angina or ECG changes of ischaemia, major dissection, transient vessel closure, and AMI correlated with significant cardiac markers release during PCI. The major In-hospital adverse coronary events were AMI and angina driven revascularisation. Patients with AMI demonstrated profound increases in cardiac markers concentrations post-PCI. Heart-FABP was the earliest marker that can detect evolving AMI post-PCI within 1 - 2 hours. The diagnosis of AMI using myoglobin, CK-MB mass, and troponins can be established at 2 - 4 hours, 4 - 16 hours, and at 4 - 16 hours post-PCI respectively.

Patients who develop chest pain post-PCI are often assessed by re-catheterisation to exclude serious complications especially early re-occlusion of dilated vessels. Sometimes patients with chest pain are subjected to this invasive procedure

unnecessarily. The use of an early marker such as H-FABP may help cardiologist to select the most appropriate groups of patients with chest pain, in the early post-PCI period, for early investigations to ascertain the diagnosis of AMI and re-establish early reperfusion. No serial blood samples were taken between 4 hours and 16 - 24 hours or beyond 24 hours. Therefore, the time to peak concentration for each marker (with the exception of H-FABP) and the time taken to return to base line could not be estimated accurately. It is possible that CK-MB mass and troponins concentration may have peaked earlier than 16 - 24 hours, if serial measurements between 4 hours and 16 - 24 hours were performed in this study. Ricchiuta et al (2000) has reported that CK-MB and cTnI peak concentrations occurred at about 12 hours post-PCI.[46] The maximum concentration of myoglobin was noticed at 4 hours. It is possible that the true peak concentration of myoglobin may be somewhere between 4 - 16 hours. Serum H-FABP concentration reached peak level within 1.4 ± 0.5 hours after reperfusion following aortic declamping in CABG surgery.[51] This early peak of H-FABP after coronary reperfusion was similar to the early peak obtained for H-FABP after PCI in this study.

There were different concentrations rise of cTnI and other markers and different symptoms in different subgroups of patients with SBO. These differences may be related to the size of the side branch vessel, the extent of collaterals, and the duration of occlusion. The occurrence of asymptomatic SBO, and also absence of cardiac markers concentration rise following documented SBO has been reported before.[23;30] Cardiac-TnI was found to be the most sensitive marker for detecting and quantifying the degree of myocardial damage following SBO. Cardiac markers were also

increased in the groups that had more stents and intravascular ultrasound investigations. Cardiac markers concentration are elevated in some patients after PCI procedures involving stenting.[36;48] However, the increase in cardiac markers was probably not related to the use of stents per se, but indirectly reflects more complications e.g. dissection in this group, and the increased use of stents were to combat these complications. This is supported by the absence of significant cardiac markers elevation in the cTnI negative group that used thirty-two stents. It is not clear whether the use of intravascular ultrasound or pressure wire studies during PCI contributes to myocardial injury. The results of this study suggest that patients who were investigated by these methods during PCI should have cTnI testing afterwards to exclude myocardial damage.

Cardiac-TnI was increased in a small numbers of patients who had no complications reported during PCI. This inevitably led to low specificity and positive predictive accuracy for the detection of complications. The reason for the low specificity and PPV reported in this study was based on the assumption that patients with elevated cTnI concentrations and without any reported complications were considered "false positive" results. Cardiac-TnI is a very sensitive and totally cardiac specific marker and any increase in its concentrations should mean myocardial damage. The reliance on cardiac markers for the detection of myocardial damage during PCI is better than reliance on visual assessment alone.

The reasons for an elevated cTnI concentration in the absence of visible complications during angioplasty may be due to; (1) The formations of small thrombi

at the angioplasty site that may subsequently embolise to small distal arteries leading to small areas of focal necrosis; (2) Inability of contrast angiography to detect complications. Indeed, intravascular ultrasound has been shown to be much more sensitive for the detection of coronary dissection after PCI compared with contrast angiography (83% vs 27%).[19] Sherman et al (1986) showed that coronary angiography detected the absence of complex lesions and thrombus in normal vessels, but it detected only one of four complex lesions and one of seven thrombi in diseased arteries. They concluded that angioscopy frequently reveals complex plaques or thrombi not detected by coronary angiography;[54] (3) May be due to observers variability in complications reporting e.g. small dissection that is not clearly visible or missed on visual angiography assessment alone; and (4) May be due to mechanical trauma to the heart caused by guide wires manipulations within the coronary arteries.

Some of our results were similar to previously published findings. The majority of patients who had uncomplicated angioplasty had no significant increase in cardiac markers. This finding was similar to the study published by Hunt et al (1991) who found no increase in the concentrations of CK-MB mass and cTnI in 22 patients who had successful elective angioplasty.[25] In our study we found no apparent explanations for cTnI increases in 27% of patients (CK-MB mass = 3%, myoglobin = 8%), which was similar to findings published by Garbarz et al (1999). They have reported 34% increases in cTnI concentrations in their study, which were not accounted for by complications during PCI.[41] The increases in cTnI, cTnT, CK-MB mass in the group with SBO was in agreement with the results published by Talasz

and Genser.[30;32] These investigators reported an increase in cTnI, cTnT and CK-MB mass concentrations in patients with SBO even when this complication was asymptomatic.

The total frequency of cTnI rise (46.25%) in this study was slightly higher than that reported by Karim et al (1995) 44% and many of the other studies.[38;41] This could be explained by the use of second generation cTnI sensitive assays (< 0.03 μg/l) and the use of a relatively low cut-off concentration (≥ 0.18 μg/l) to indicate myocardial injury post-PCI. The frequency of CK-MB mass elevation in our study (11.25%) was lower than that reported previously (15 - 26%). Some of the differences in CK-MB mass results could be related to differences in assay methods used and to the inclusion and exclusion criteria. The use of CK-MB assays based on enzyme activity can give false positive results in the presence of interference (e.g. haemolysis), and may lead to overestimation of patients with positive results especially in the lower cut-off concentration range used in most of these studies.

In addition, some of the earlier studies included patients with recent UA and AMI events undergoing emergency PCI, and some studies did not perform base line measurement of CK-MB concentration.[35;42;50] Many of these patients may have increased base line cardiac markers concentrations as a result of the acute event and it is difficult or impossible to differentiate rises due to complications of angioplasty procedure from those due to the acute events. Kugelmass et al (1994) reported elevated CK-MB in 11.5% of patients following elective PCI, and there were no clinical sequels over two years follow-up. However, in a small subset with a greater

elevation of CK-MB, there was a trend towards decreased late survival compared to patients without CK-MB elevation. They also reported common CK-MB elevation after coronary stenting.[36]

Patients with increased cardiac markers concentrations had more frequent complications on long-term follow-up (p = NS). An increased frequency of cardiac events on long-term follow-up (angina, PCI, CABG) were noticed in some patients who had very small increases of cTnI concentration (0.08 - 0.1 μg/l), which suggest that even small increases of cTnI may have a significant prognostic value and may reflect more diffuse or extensive CAD. Heeschen et al (1999) found that the rate of death and AMI, in 42% of patients with UA and cTnI ≥ 0.08, occurred in 25.5% of cTnI positive patients compared to 2.9% of cTnI negative patients during 30 days of follow-up.[52]

Long-term complications in patients with elevated cardiac markers post-PCI have been suggested by Abdelmejuid and others.[27;29;31;47] However, these investigators reported more frequent and more serious complications (e.g. death and AMI) than this reported by us. These differences could be related to several factors. First, the duration of follow-up. The follow-up period in our study was relatively short compared to the study by Abdelmejuid (3 - 5 years, in some patients up to 8.5 years). This assumption is strengthened by the fact that short-term (3 - 8 months) follow-up studies of patients with elevated cardiac markers post-PCI have failed to detect significant long-term complications.[34] The follow-up period in our study was intermediate and although an increased but non-significant positive correlation with

future complications was found, it is possible that if the follow-up was extended over a prolonged period of time (e.g. > 3 years), more noticeable complication differences between the two groups may have been detected.

Second, increased use of stents. The use of stents was more frequent in the group of patients with elevated cardiac markers concentrations. The use of stents to treat complications during angioplasty e.g. dissections may alter the short-term risk of further progression to more serious complications. The long-term benefits of stents in preventing further coronary restenosis and events are also well established. Third, the use of antiplatelets therapy. The use of newer and more potent antiplatelets regimes (ticlopidine, clopidogrel, Abciximab) was also high in our group of patients (68%). The clinical benefits of these antiplatelets compounds in reducing the progression to AMI and death in patients with myocardial injury is well-established.[58-60] It may be that the combinations of improved modern techniques of angioplasty and the liberal use of stents and potent antiplatelets treatment could have affected the long-term risk of further complications as compared to conventional PCI alone without antiplatelets treatment or stenting during or post-procedure. Despite the fact that there were no statistically significant differences between the numbers of events, there was still a significant difference with respect to event-free survival between groups with and without elevations of these markers after angioplasty.

Increases in cTnT after PCI had been described previously.[40;48] Ravkilde et al (1994) found moderate increases in CK-MB mass [range 10 - 20 μg/l] in 6 of 23 patients (26%) undergoing visually successful PCI, whereas only 3 (13%) showed cTnT

elevation [range 0.25 - 1.3 μg/l].[40] In this study, the percentage of positive cTnT results [range 0.1 - 1.68 μg/l] after PCI was only 7.5%. A third generation cTnT assays that were very sensitive (< 0.01 μg/l) and specific were used. Some of the previous studies used a lower cut-off concentrations with these assays e.g. ≥ 0.04 μg/l or ≥ 0.06 μg/l, to indicate the presence of myocardial injury post-PCI.[42;53] Increased cTnT ≥ 0.06 μg/l were found to be associated with increased risk of death and AMI (10.5%) compared to cTnT ≤ 0.06 μg/l.[53] As mentioned earlier, cTnT concentration between ≥ 0.06 - ≤ 0.1 μg/l was associated with complications in 75% of patients. Based on ROC analysis a cut-off concentration of cTnT ≥ 0.06 μg/l was slightly more sensitive and equally specific to cTnT ≥ 0.1 μg/l for the detection of complication. Depending on whether the cut-off concentration selected was ≥ 0.1 μg/l or ≥ 0.06 μg/l, the frequency of abnormal cTnT elevations was 7.5% and 12.5 % respectively, which is consistent with previous reports. Even by lowering the cut-off concentration to ≥ 0.06 μg/l, there was still a discrepancy between the frequency and magnitude of abnormal cTnT and cTnI elevations.

Most studies that compared both markers post-PCI did also report some discrepancy between the results of these two markers.[48] The sensitivity of cTnT for the detection of major complications in this study was considerably lower than that of cTnI (24% vs 81%). In this model of controlled myocardial injury, this difference may be either due to delayed leakage of cTnT from myocardial tissue into the blood within this relatively short sampling period, or due to differences in assay methods. Unlike cTnT assay which is well standardised, there are several cTnI assays. The normal concentrations of these assays can vary by up to twenty-fold. Depending on which

cTnI assay was used, some discrepancy between the results of these two markers is expected. Despite the low sensitivity of cTnT, the specificity and positive predictive value for the detection of complications was very high. Event-free survival of patients with elevated cTnT concentrations was significantly lower than those without cTnT elevations after angioplasty. Cardiac-TnT was also associated with increased risk of CABG on long-term follow-up. Thus validating the prognostic significance of cTnT elevations post-PCI.

The reporting of clinical and angiographic success may be overestimated by reliance on visual assessment alone.[19;54] Even in the absence of reported complications during angioplasty, cardiac markers elevations can still be detected (sometimes as high as 2.12 μg/l, for cTnI) and these elevations may be associated with long-term complications. Measurements of cardiac markers post-PCI will be a useful adjunct to angioplasty and will help detect patients with subtle myocardial damage and may guide further management. For example, twelve patients (32%) with elevated cTnI in our study did not receive any form of antiplatelets (other than aspirin) during or after PCI. The cTnI concentrations range in these patients were 0.18 - 2.12 μg/l. Eight out of these 12 patients had worsening of their angina or further cardiac events during follow-up. Patients with acute coronary syndromes and elevated troponins are known to benefit from long-term treatment with antiplatelet therapy e.g. clopidogrel.[58-60] The availability of cardiac markers results in these patients may influence management decisions. These patients may be selected for long-term antiplatelet therapy and this treatment may alter their long-term outcome.

Finally, it is important to consider the impact of the new definition of AMI on our group of patients. The new definition of AMI published in year 2000 jointly by the ESC/ACC states that, AMI should be diagnosed in any patients with cTnT or cTnI concentration > the 99th percentile of control value on at least one occasion or maximum value of CK-MB mass exceeding the 99th percentile of a control group on two successive samples or exceeding twice the upper limit of normal on one occasion.[49] The 99th percentile value for cTnI assays on Stratus CS was 0.1 μg/l. Based on this new definition, 47 patients (58.75%) with elevated cTnI in this study will fulfil the 'biochemical' diagnosis of AMI. The incorporation of this new definition of AMI in clinical practice will have several important implications.

(1) Safety issues. The use of this definition will detect many patients who had suffered small damages to the myocardium or occasionally in patients with visually successful angioplasty (as in this study), but with small cardiac markers elevations. Classifying these patients as suffering from AMI as a result of PCI will add great concerns to the safety of angioplasty. This will inevitably have a negative impact on the wider and growing applications of this popular intervention.

(2) Social and psychological implications. Many patients will be labelled as suffering from AMI even if the damage was slight and sometimes symptomless. This will add considerable amount of stress to the patients and their families.

(3) Financial and economic implications. Labelling patients with small increases of cTnI as suffering from AMI will have many important financial consequences to the patient and the economy such as loss of jobs, sick leaves, and insurance.

(4) Legal implication. Sometimes PCI is undertaken to improve the coronary anatomy e.g. treating very severe discrete stenosis noticed at angiography even if the patient had few and stable symptoms. The diagnosis of AMI following such situations will provide grounds for litigations. This may incur huge financial losses on the part of health services and will have a negative impact on the patient-doctor relationship.

5.7.1 SUMMARY

This study has confirmed an early release characteristic feature of H-FABP (within one hour) compared with myoglobin (and other markers) following PCI-related myocardial damage. Heart-FABP measurements at 1 hour (or thereafter) post-PCI in patients with suspected complications may offer the best early chance of detecting serious myocardial damage such as evolving AMI, that may necessitate investigation by re-catheterisation in the first few hours post-PCI. Provided that serial measurements were done, myoglobin may offer reliable indication of AMI at 2 - 4 hours post-PCI, whereas CK-MB mass, cTnI or cTnT are reliable between 4 - 16 hours. In general, H-FABP was equally sensitive and specific to CK-MB mass, myoglobin and cTnT for the detection of complications post-PCI. However, the overall sensitivity of all these markers for the detection of complications was considerably lower than that of cTnI.

Cardiac markers may play a significant role in detecting and quantifying myocardial damage during angioplasty and may have important clinical applications. As compared with CK-MB mass, myoglobin, H-FABP, and cTnT, cTnI has emerged as the most sensitive marker for the detection of major complications (major dissection, SBO, TVC, and AMI) in patients undergoing PCI. This reflects superior sensitivity of cTnI assays used by Stratus CS. Cardiac-TnI was also abnormally elevated in patients with minor complications and in patients without reported complications post-PCI. The use of cTnI post-PCI offers a reliable detection of myocardial damage that is sometime not obvious by visual contrast angiographic assessment alone. The adjunctive measurements of cardiac markers post-PCI also provides important prognostic informations and could help identify certain groups with elevated cardiac markers concentrations that might benefit from long-term treatment with newer antiplatelets therapy. The ability of cTnI to detect subtle and subclinical damage to the myocardium may provide a sensitive tool for evaluating the impact of future antiplatelet therapy, newer angioplasty devices and stents during PCI. Measurements of cTnI 16 - 24 hours post-PCI should be part of the routine management of patients following elective PCI.

5.7.2 LIMITATIONS OF THE STUDY

(1) The study had no additional confirmatory test e.g. pathological examination or intravascular ultrasound that can verify independently the magnitude of irreversible ischaemic damage as a result of angioplasty in patients with elevated cardiac markers concentrations and in those with and without reported complications. (2) The interpretation of sensitivity results are also influenced to a greater extent by the

diagnostic threshold selected for each marker and it is possible that the most sensitive threshold for troponins may have detected cytosolic release not necessarily irreversibly injured myocyte. At present we have no reliable method that will define the ischaemic threshold for reversible and irreversible ischaemic injury.

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CHAPTER 6

THE DIAGNOSTIC VALUES OF SERIAL CARDIAC MARKERS HEART FATTY ACID BINDING PROTEIN, CREATINE KINASE MUSCLE BRAIN MASS, CARDIAC TROPONIN I, CARDIAC TROPONIN T, AND MYOGLOBIN IN THE MANAGEMENT OF PATIENTS WITH UNSTABLE ANGINA AND NON-Q WAVE MYOCARDIAL INFARCTION

6.1 INTRODUCTION

Acute coronary syndromes encompass a spectrum of conditions with sudden cardiac death at one end of the spectrum and silent ischaemia at the other end. In between it ranges successively through AMI, non-Q wave MI, UA, and stable angina. Unstable angina, which is at the centre of this spectrum, describes a heterogeneous population with single or multiple vessel coronary artery disease, with or without prior myocardial infarction, and with uncertain outcome. Historically, UA is identified in the presence of one or more of the following features in the absence of clear-cut ECG changes and enzyme rise diagnostic of AMI; (1) Angina at rest or minimal exertion; (2) A relatively new onset of angina brought on by minimal exertion; and (3) Crescendo angina i.e. more severe/prolonged/frequent episodes of angina superimposed on pre-existing pattern of stable effort-related angina.[1]

Non ST elevation non-Q wave MI is a condition that is interposed between ST elevation MI and UA. It is defined clinically when there is an increase in cardiac markers proteins consistent with infarction and ischaemic ECG changes, in the absence of the characteristic persistent ST segment elevation or new Q waves.[2] The term 'non-Q wave MI' will be used throughout the remaining of this chapter to refer to this condition. Unstable angina and non-Q wave MI represent unstable coronary artery disease and are usually classified together under non-ST elevation ACS. In the early stages, the identification and distinction between these two conditions is difficult, based on clinical history and ECG changes alone. There are also no distinguishing features at angiography.[3] However, this distinction is less important therapeutically, because the two conditions are usually treated in a similar manner,

although the risks of coronary events differ.[4] These conditions are associated with initial lower incidence of death, but the long-term (1 year) outlook is similar to patients with Q wave MI.[5]

6.1.1 EPIDEMIOLOGY AND PATHOPHYSIOLOGY

Unstable angina accounts for about 130,000 admissions annually in the United Kingdom.[6] The rate of death or non-fatal myocardial infarction at 6 months for UA and myocardial infarction without ST elevation is 12.2%.[7] It is also estimated that 55% of CCU beds are occupied by patients with UA.[8] The incidence of non-Q wave MI (compared to Q wave MI) over the past 20 years is also rising.[9] Most patients (85%) with UA and non-Q wave MI have obstructive atherosclerotic coronary artery lesions as the underlying disease process. Episodes of angina are believed to follow atherosclerotic plaque rupture or erosion with subsequent platelet activation and aggregation to form platelet thrombus or white thrombus.[12]

Platelet aggregation causes the release of thromboxane A_2, which is a platelet pro-aggregator and vasoconstrictor leading to further reduction of coronary blood flow. Exposure of thrombogenic materials beneath the intima (lipid, collagen, and tissue factor) to circulating blood stimulates the coagulation process and the deposition of fibrin to form red thrombus. Activation of platelets and subsequent formation of platelet thrombus is thought to play a greater component in the pathogenesis of UA and non-Q wave MI.[14] The presence of thrombi in these conditions had been demonstrated at autopsy, angioscopy and angiography examinations.[10-14] Intraluminal thrombus can be detected by angiography in 70% of patients with UA.[15]

6.1.2 TREATMENT

There is evidence that aspirin and unfractionated heparin (UFH) are beneficial in the treatment of patients with UA and non-Q wave MI, and are associated with a significant reduction in subsequent rate of death and re-infarction.[16;17] The introduction and subsequent evaluation of low molecular weight heparin in controlled clinical trials, had showed better efficacy and safety profile compared to UFH.[18;19] Low molecular weight heparin have better bioavailability, predicted efficacy, lower incidence of side effects (e.g. thrombocytopenia, major bleeding), can be self-injected by patients, and does not require blood monitoring. The use of LMWH was associated with 20% reduction in the incidence of death, MI, and recurrent angina as compared with UFH.[20]

With better understanding of the biochemistry and pharmacology of platelet functions, it is now well understood that aspirin alone has limitations as the sole antiplatelet treatment in UA and non-Q wave MI. Platelet activation can occur via several different other receptors or metabolic pathways that are not affected by aspirin (Figure 1). The activation of glycoprotein GP IIb/IIIa receptor on the platelet is the final common pathway of platelet activation, which leads to the binding of fibrinogen and cross linking of platelets to form thrombus.[4] The development of new and more potent antiplatelets (e.g. GP IIb/IIIa inhibitors, clopidogrel), that block different receptors in the platelet activation pathways had been a major advance in the potential armament of agents used in the treatment of patients with UA and non-Q wave MI, and had contributed significantly to further risk reduction in these groups.[21-23]

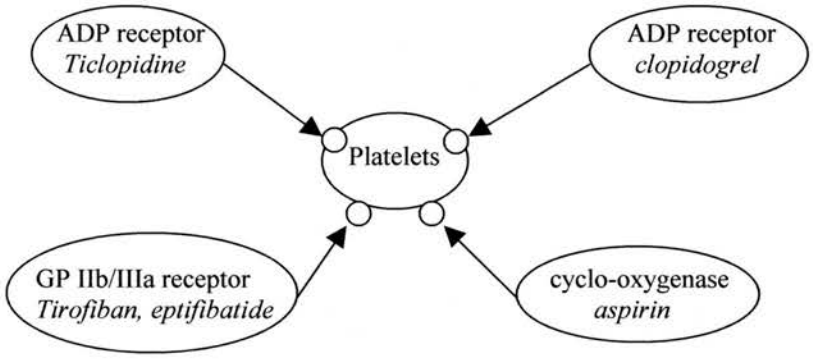


Figure 1. This schematic diagram shows the different receptors and metabolic pathways that activate platelets and the antiplatelet sites of action.

Since the only differentiating factor between UA and non-Q wave MI is positive cardiac markers concentrations consistent with infarction. The availability of cardiac markers that are sensitive and specific for the early identification of myocardial injury is very important and will help distinguish these two groups in the early phase from other causes of chest pain. When this information is combined with the clinical history and ECG changes at admission, this may help guide further management in these high-risk groups. Heart fatty acid binding protein is a novel marker protein with potential for the very early identifications of patients with AMI.[24] Heart-FABP and myoglobin are both very early markers of myocardial injury within the first 3 hours. Heart-FABP is considered more cardio-specific than myoglobin. This advantage may be utilised for the early identifications of patients with UA and non-Q wave MI. The diagnostic value of H-FABP in patients with non-ST elevation ACS has not been studied before.

6.1.3 AIMS OF THE STUDY:

1. Compare and contrast the sensitivity and the predictive value of the new cardiac marker H-FABP for the early diagnosis of myocardial injury within 7 hours in patients with suspected UA and non-Q wave MI, with standard markers CK-MB mass, cTnT, cTnI, and myoglobin and determine whether H-FABP has an advantage in terms of early release kinetics in this group of patients.

2. Examine the clinical benefits from implementations of serial cardiac markers testing in the acute setting in patients with chest pain and their impact on patients' management. Several examples will be used to illustrate the clinical benefits of these markers in each group.

3. Study the additional diagnostic value of serial cardiac markers testing for the early diagnosis of MI in patients without the classical ST segment elevation. Whether their selective use in such cases can influence further decisions on reperfusion therapy. Several examples will be used to illustrate this.

6.2 PATIENTS AND METHODS

6.2.1 PATIENTS AND TREATMENT

The recruitment of patients in this group, the inclusion and exclusion criteria, and the diagnostic criteria are all described in details in Chapter 4 materials and methods pages 114 - 117. Patients were treated in a conventional manner for UA and non-Q wave MI using bed rest, oxygen, and antianginal treatment with or without β-Blockers, nitrates, and calcium antagonists. Intravenous nitroglycerin was given to

control ongoing chest pain. All patients were given 300 mg of aspirin at admission followed by a daily dose of 75mg. Patients were also given enoxaparin, which is the standard LMWH used in our unit in a specified protocol adjusted to body weight. Coronary angiography was not part of the protocol and it was done at the discretion of the responsible cardiologist. The results of angiography were used to support the discharge diagnosis. The discharge diagnosis was made by the consultant cardiologists in charge of these patients.

6.2.2 PATIENTS' CLASSIFICATION

Based on clinical history, admission ECG and routine cardiac markers and other investigations during admission, patients were divided into three main groups. Group 1, included patients with non-Q wave MI. Group 2, included patients with UA. Group 3, included a heterogeneous population of patients with mixed medical diagnoses other than UA and non-Q wave MI. Patients in group 3 were referred to as 'atypical/anginal chest pain'. Five serial blood samples were collected from each patient at 0 hour (presentation), and at 2 hours, 4 hours, 8 - 10 hours, and 16 - 24 hours after presentation. Creatine kinase-MB mass, cTnI, cTnT, myoglobin, and H-FABP were measured in each sample. The sensitivity of each cardiac marker using two different cut-off concentrations were measured at each time after presentation and compared. These selected cut-off concentrations were based on receiver operator characteristic curve analysis (Chapter 4 pages 120 - 122), that best discriminate patients with myocardial injury (UA and NSTEMI) and patients without myocardial injury (normal controls and atypical/anginal chest pain), and those that discriminate patients with UA from patients with NSTEMI.

6.2.3 ANALYSIS OF CARDIAC MARKERS

The analysis of CK-MB mass, myoglobin, and cTnI was done on Stratus CS. The Stratus CS, from Dade-Behring-Germany, is a flourometric enzyme immunoassay analyser for quantitative determination of CK-MB mass, cTnI, and myoglobin.[25] The test system is designed to analyse closed routine samples tubes containing anticoagulated whole blood (lithium-heparin). Alternatively, this system can analyse pre-processed plasma or serum specimens placed into sample cups. For separation of the plasma from whole blood samples, the centrifugation step is incorporated into the test system. Tests to be performed are selected by introducing bar-coded test packs. Up to four test packs can be introduced for each sample. All required reagents are enclosed within the test packs, which are transferred by disposable pipette tips. Dilution can be performed automatically by adding test-specific dilution packs. The test system utilises radial partition immunoassays technology, which have been enhanced through the use of monoclonal capture antibody coupled to Starburst® dendrimers. The cTnI specific antibodies used are identical to those used on the Stratus II, and likewise are capable of detecting both free and complexed cTnI.[26] The dendrimer technology provides for better presentation and functionality of the capture antibody on the glass fibre solid phase surface used in the assay. This in turn leads to more efficient capture of the target antigen.

The assay process is initiated by applying the dendrimer-antibody reagent onto the glass fibre matrix to form a reaction zone, which serves to capture the analyte of interest. Centrifuged plasma/serum is then added, followed by the first incubation period. Thereafter, the alkaline phosphatase-labelled second antibody is applied to

the matrix, followed by a second incubation period. The unbound labelled antibody reaction is removed from the reaction zone by radial illusion using the substrate wash reagent. Captured phosphate-labelled antibodies convert the included enzyme substrate into a fluorescent product that permits quantitative measurement of the cardiac markers by front surface fluorescent measurement. The result of all three cardiac markers is available within 18 minutes. The coefficients of variations for cTnI were 4.5%, and 6.5% at 0.1 μg/l, and 0.82 μg/l respectively. The detection limit was 0.01 μg/l. The 97.5% of a control healthy population was 0.08 μg/l.[27] The normal base line concentration of cardiac markers had been explained previously in Chapter 4 pages 109 - 110.

6.2.4 STATISTICAL ANALYSIS

Values were expressed as mean ± SD. Chi-square tests were used to explore the group differences with respect to categorical variables. Kruskal Wallis H test was conducted to compare continuous variables and mean cardiac markers concentrations differences in the three groups (non-Q wave MI, UA and atypical/anginal groups). Friedman tests were used to analyse variations of cardiac markers concentrations over time (0 hour, 2 hours, 4 hours, 8 – 10 hours, and 16 - 24 hours). Significant results are indicated by probability values less than or equal to 0.05. The best cut-off concentrations were established from ROC curve analysis.

6.3 RESULTS

Out of 55 consecutive patients presenting with acute chest pain, 14 patients (26%) had non-ST elevation non-Q wave MI (group 1), 20 patients (36%) had UA (group 2)

and 5 patients (9%) had stable angina. In the remaining 16 patients (29%), 3 were diagnosed with chest pain, 3 had ischaemic heart disease, 4 patients had muskeloskeletal or gastrointestinal related chest pain and in 6 patients the discharge diagnoses were atypical/possible angina in two patients, congestive cardiac failure in one patient and in the remaining three patients the exact diagnoses of chest pain were not clear. The last 21 patients constituted group 3. The demographic data of the patients in these three groups is shown in Table 1. The study group consisted of 37 males (67%) and 18 females (33%). The non-Q wave MI group included 9 males and 5 females. The mean age and time to presentation in group 1 was 67.36 ± 11.34 years and 5.3 ± 1.28 hours respectively. The time to presentation and the mean age in groups 2 and 3 was similar to patients in group 1. All patients in these three groups presented with chest pain ≤ 7 hours after symptom onset.

The prevalence of cardiac disease (CABG, PCI) and risk factors (high blood pressure and high cholesterol) was slightly increased in the non-Q wave MI group but not statistically significant. The systolic blood pressure at presentation was different between the three groups ($p < 0.024$). Patients with non-Q wave MI and UA had more typical presentations with ischaemic chest pain ($p < 0.027$). The type and distribution of ECG changes on admission are also shown in the table. The commonest ECG changes at presentation were ST segment depression and T wave inversion (47%). Six patients (11%) had transient ST segment elevation noted at some stage on the ECG. In 33% there were no acute ECG changes and in 7% there were other ECG changes e.g. arrhythmias and left bundle branch block. The distributions of ischaemic changes were in the anterior, inferior, and lateral leads

39.5%, 39.5%, and 18% respectively. Both non-Q wave MI and UA groups received similar treatment with pharmacological agents e.g. aspirin, LMWH, and antianginal medications. One patient was initially given tissue plasminogen activator (tPA) for suspected AMI, which was later discontinued because of insufficient grounds for thrombolysis.

There was more frequent use of PCI in the UA and non-Q wave MI groups compared to atypical/anginal group (46% vs 5%). Seventeen patients (31%) were investigated by angiography, and in nine patients (53%) angiography was followed by PCI, because of severe stenosis in one of the coronary arteries or vein grafts. Coronary angiography was undertaken mostly in patients with repeated admissions for angina, for ongoing chest pain ± ECG changes of ischaemia, and in patients with elevated cardiac markers concentrations. In group 1, four patients had angiography followed by angioplasty in 3 patients (75%). In group 2, eight patients had angiography followed by angioplasty in 5 patients (62.5%). In group 3, five patients had angiography followed by angioplasty in one patient only (20%). Seventy-eight per cent (78%) of patients who had angioplasty had significant cardiac markers elevations. In patients who had coronary angiography without angioplasty, 75% had normal cardiac markers concentrations.

Table 1 (opposite). Shows the demographic data of patients with non-Q wave MI, UA and anginal/atypical chest pain. Abbreviations: UA, unstable angina; MI, myocardial infarction; SD, standard deviation; CABG, coronary artery bypass grafting; PCI, percutaneous coronary intervention; DM, diabetes mellitus; HTN, hypertension; FHX of IHD, family history of ischaemic heart disease; HR, heart rate; BP, blood pressure; ST, T, Q, are ST segment, T wave segment and Q wave segment of the electrocardiogram (ECG); NS, not significant. See text for explanation.

Demographic data	*Non-Q wave MI Group 1*	*UA Group 2*	*Angina/atypical Group 3*	*P value*
No. Patients	14	20	21	
Age (years)	67.36 ± 11.34	67.45 ± 12.31	64.55 ± 9.54	NS
Sex:				
Male	9 (64)	16 (80)	12 (57)	NS
Female	5 (36)	4 (20)	9 (43)	NS
Time to presentation (hours)	5.3 ± 1.28	5.63 ± 1.17	5.0 ± 1.95	NS
Previous cardiac history:				
Angina	9 (64)	10 (50)	15 (71)	NS
UA	3 (21)	3 (15)	4 (19)	NS
AMI	6 (43)	10 (50)	8 (38)	NS
CABG	5 (36)	2 (10)	5 (23)	NS
PCI	4 (29)	2 (10)	3 (14)	NS
Risk factors:				
DM	2 (14)	3 (15)	2 (10)	NS
HTN	5 (36)	4 (20)	3 (14)	NS
Smoking:				
Smoker	5 (36)	3 (15)	9 (43)	NS
Ex-smoker	1 (7)	8 (40)	2 (10)	NS
Non-smoker	8 (57)	9 (45)	10 (48)	NS
High cholesterol	10 (71)	9 (45)	13 (62)	NS
FHx of IHD	3 (21)	1 (5)	6 (29)	NS
Haemodynamics at admission:				
HR	75.56 ± 17.99	66.92 ± 11.18	77.0 ± 16.35	NS
Systolic BP	133.22 ± 34.26	151.92 ± 17.93	134.67 ± 10.49	0.024
Diastolic BP	71.44 ± 18.44	74.92 ± 11.7	79.0 ± 13.99	NS
Chest pain:				
Typical ischaemic pain	13 (93)	17 (85)	13 (62)	0.027
Atypical chest pain	1 (7)	3 (15)	8 (38)	
ECG changes at admission:				
Transient ST elevation	3 (21)	2 (10)	1 (5)*	
ST depression	7 (50)	7 (37)	0	
T wave changes	2 (14)	7 (37)	3 (14)	
Other ECG changes	1 (7)	1 (5)	2 (10)	
No acute changes	1 (7)	2 (10)	15 (71)	
Old Q waves present	1 (7)	5 (25)	6 (29)	
ECG leads involved:				
Anterior	1 (7)	8 (40)	1 (5)	
Inferior	4 (29)	5 (25)	1 (5)	
Anterior-lateral	3 (21)	0	0	
Inferior-lateral	1 (7)	1 (5)	1 (5)	
Posterior	1 (7)	0	0	
Lateral	2 (14)	3 (15)	1 (5)	
Treatment:				
Pharmacologic + PCI	3 (21)	5 (25)	1 (5)	
Pharmacologic - PCI	11 (79)	15 (75)	20 (95)	
Hospital stay:				
Average admission (days)	8.57±3.69	5.21±2.95	3.95±2.16	0.0005
Total duration (days)	120	102	80	

Table 1. Shows the demographic data of patients in this group.

The serial concentrations of CK-MB mass, cTnI, cTnT, H-FABP, and myoglobin in the non-Q wave MI group are shown in Table 2, and are represented graphically in Figure 2. The concentrations of these markers were markedly elevated and showed significant concentration changes over time. Heart-FABP and myoglobin peak concentrations were achieved at 2 hours and the concentrations of these markers had decreased to normal levels at 16 - 24 hours after presentation. For CK-MB mass, the peak concentration occurred at 8 - 10 hours. The maximum increase in cTnI and cTnT occurred late at 16 - 24 hours (Table 2). The concentrations of CK-MB mass, cTnI, and cTnT were still present in significant levels at 16 - 24 hours. Most of the patients in this group had typical history of ischaemic chest pain (93%), and positive ECG changes of ischaemia (ST segment shift or T wave inversion) were present in 86% at or during admission.

Of the 20 patients in the UA group, 85% presented with typical history of ischaemic chest pain, and 84% had ST-T segment changes of ischaemia on the ECG at or during admission (Table 1). The concentrations (mean ± SD) of cardiac markers in the UA group are shown in Table 3 and are represented graphically in Figure 3. The release patterns of cardiac markers in patients with UA followed closely those seen in patients with non-Q wave MI. However, the maximum concentration rise of these markers was only a small fraction of that seen in patients with non-Q wave MI (compare Table 2 and Table 3). There were significant concentration changes over time for the groups of patients that had elevated cardiac markers concentration as reflected by low probability values (Table 3). The concentrations of cardiac markers

in UA and non-Q wave MI groups were not directly related to either the severity of chest pain or to the types of ischaemic ECG changes at admission.

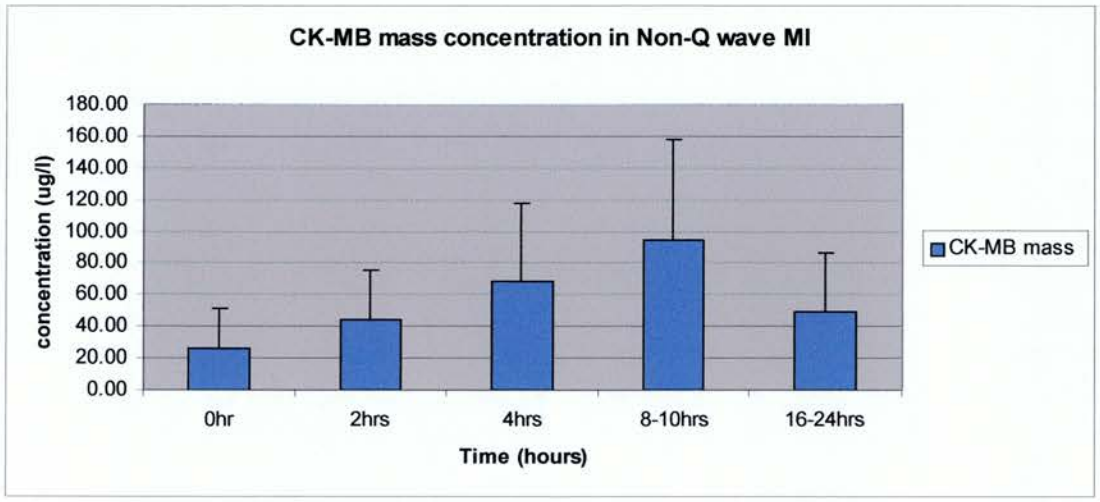
Marker(s)	0hr	2hrs	4hrs	8-10hrs	16-24hrs	P value
CK-MB mass (mean)	26.16	43.82	68.48	94.41	48.85	0.0005
± SD (μg/l)	24.89	31.55	49.09	63.55	37.67	
CTnI (mean)	4.53	8.09	10.27	17.66	19.66	0.0005
± SD (μg/l)	4.78	7.37	6.69	15.62	17.07	
CTnT (mean)	0.56	0.89	1.21	1.69	2.41	0.0001
± SD (μg/l)	0.55	0.85	1.19	1.31	1.53	
Myoglobin (mean)	291.71	347.57	338.79	284.29	173.79	0.001
± SD (μg/l)	208.03	212.00	214.95	254.62	145.52	
H-FABP (mean)	46.79	124.36	110.86	93.21	33.41	0.007
± SD (μg/l)	35.94	117.77	99.39	79.71	29.87	

Table 2. This table shows cardiac markers concentrations (mean ± SD) in the non-Q wave MI group at 0, 2, 4, 8 - 10, and 16 - 24 hours after presentation. These values are represented graphically in Figure 2. There were significant cardiac marker concentration changes over time as reflected by the very low probability values.

Early exclusion of acute myocardial infarction

The importance of concomitant serial cardiac markers testing in this group is illustrated in the following example: This was a 60 years old man who had had previous MI. He had a transurethral resection of the prostate 3 weeks prior to admission. The admission ECG showed Q waves, with ST elevation < 2 mm anteriorly. The diagnosis was thought to be STEMI and he was considered a candidate for thrombolytic therapy. Due to some concerns regarding recent surgery and the absence of reciprocal ST segment depression, he was treated with non-thrombolytic pharmacologic agents instead. An echocardiogram revealed a large anterior apical aneurysm. The ST elevation in this patient could be due to UA, non-Q

wave MI, evolving ST elevation MI, or a misleading sign due to the aneurysm itself. He presented within 6 hours after symptom onset, which was sufficient time for some of the early markers to appear in blood. Normal H-FABP or myoglobin concentration at admission would rule out AMI reliably.



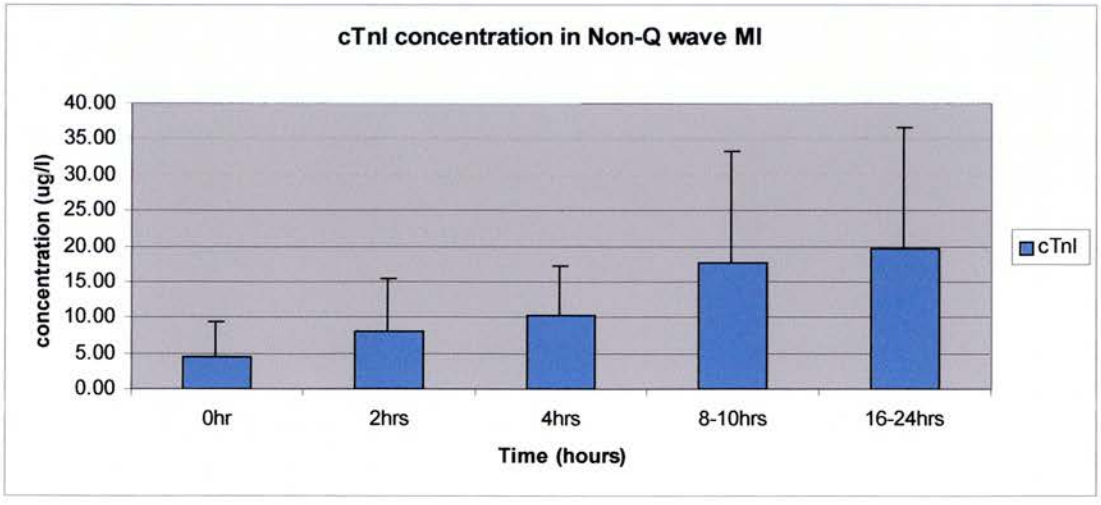
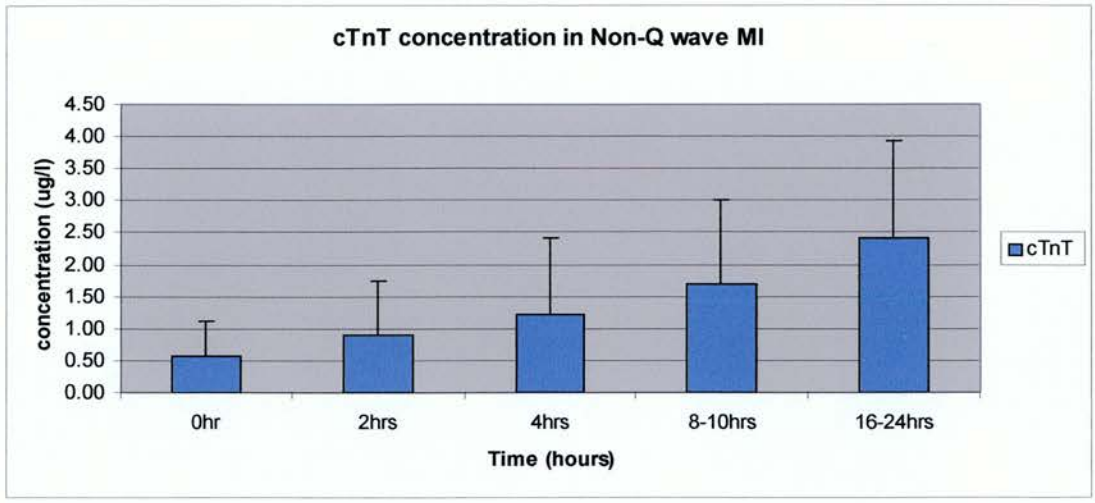
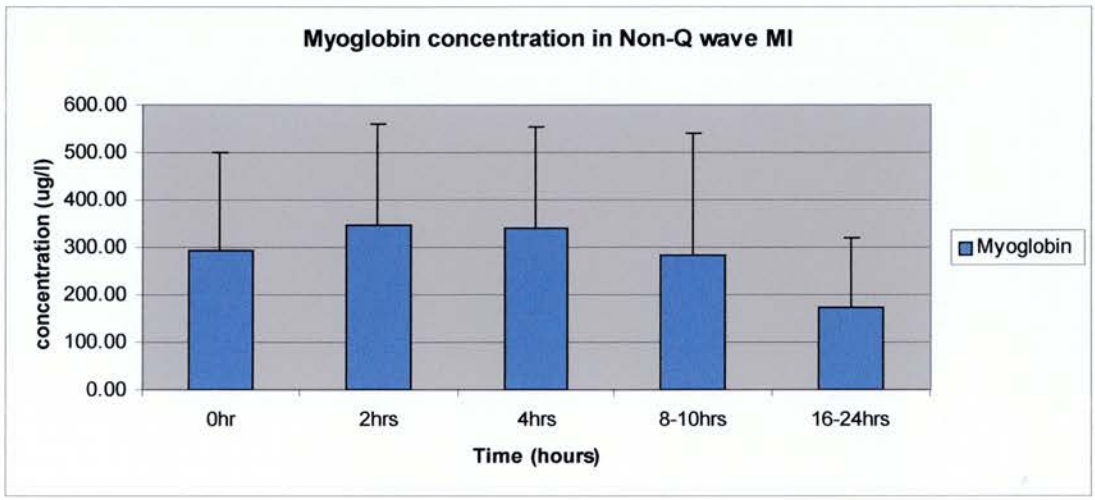


Figure 2 continued on page 197.





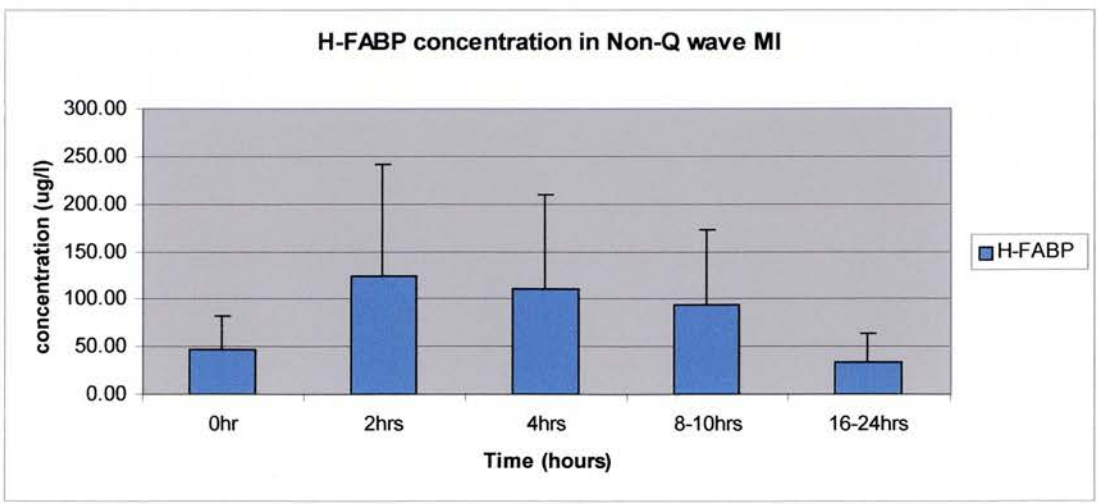
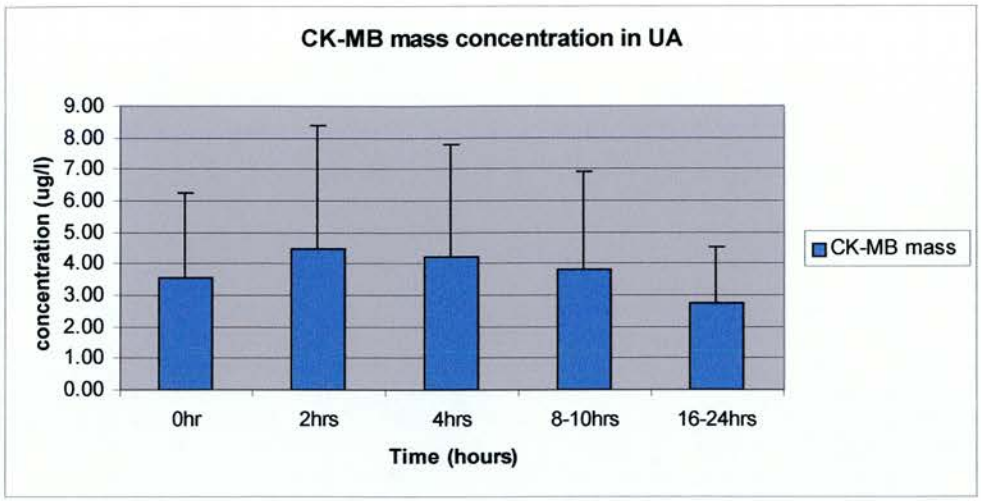


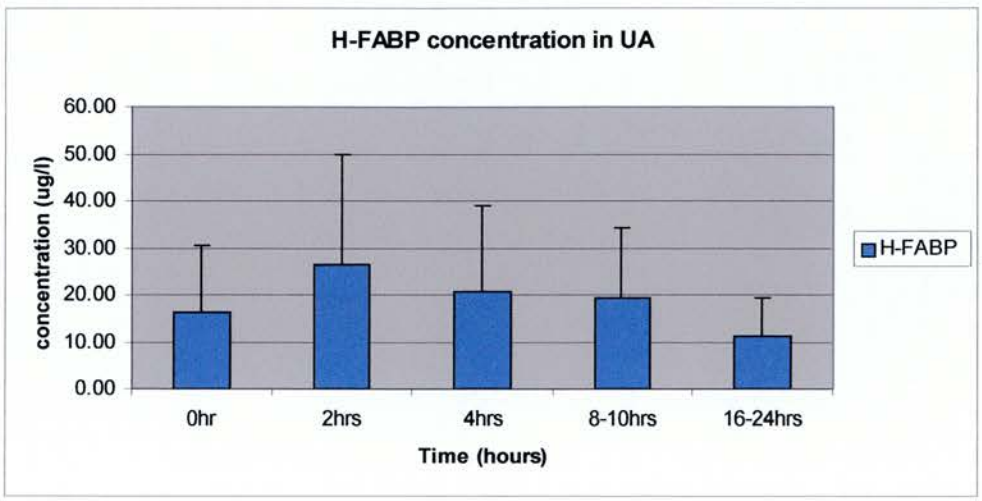
Figure 2. These graphs illustrate the release pattern of CK-MB mass, cTnI, cTnT, H-FABP, and myoglobin in patients with non-Q wave MI.

All five cardiac markers measured at admission were normal. By doing serial cardiac markers testing, it was clear that this patient's symptoms were most probably due to UA rather than AMI (cTnI = 0.3 μg/l, cTnT = 0.14 μg/l, myoglobin, H-FABP and CK-MB mass were all normal). Thus, the more serious diagnoses could be ruled out early, and this would avoid any confusion of treatment. This patient requires antithrombotic and antiplatelet treatment and not thrombolytic therapy. This example also illustrates the superior sensitivity of cardiac troponins for the diagnosis of UA compared to the less specific markers like CK-MB mass, myoglobin, and H-FABP.

Marker(s)	0hr	2hrs	4hrs	8-10hrs	16-24hrs	P value
CK-MB mass	3.57	4.48	4.20	3.82	3.24	0.001
± SD (μg/l)	2.86	3.92	3.60	3.09	1.82	
CTnI	0.14	0.22	0.29	0.30	0.26	0.015
± SD (μg/l)	0.12	0.19	0.25	0.24	0.23	
CTnT	0.07	0.07	0.11	0.12	0.14	0.001
± SD (μg/l)	0.06	0.07	0.09	0.10	0.13	
Myoglobin	78.70	94.20	91.70	76.45	65.60	0.001
± SD (μg/l)	53.65	79.62	74.65	49.53	31.94	
H-FABP	16.25	26.50	20.55	19.27	11.33	0.007
± SD (μg/l)	14.33	23.50	18.51	15.07	8.06	

Table 3. This table shows cardiac markers concentrations (mean ± SD) in the UA group at 0, 2, 4, 8 - 10, and 16 - 24 hours after presentation. These values are represented graphically in Figure 3. There were significant concentration changes over time for all markers in the groups with elevated concentration.





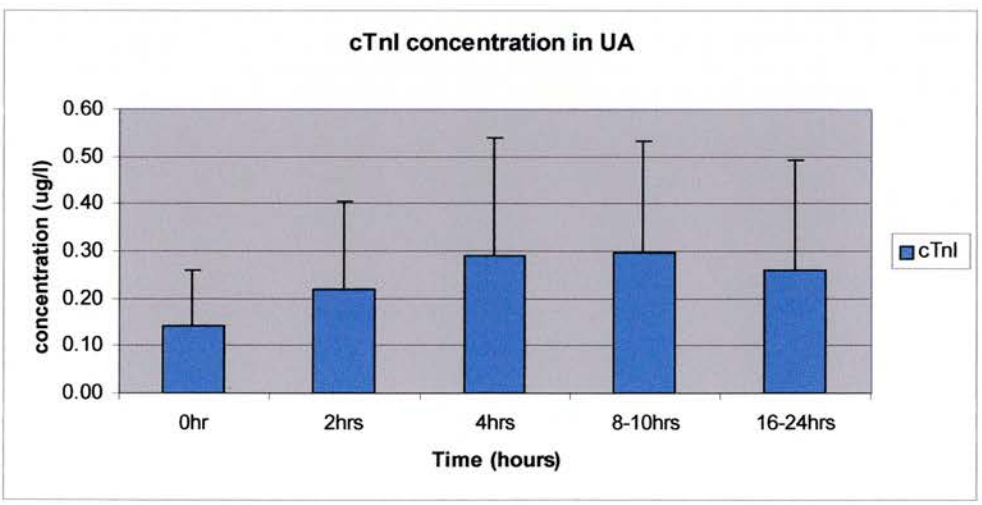
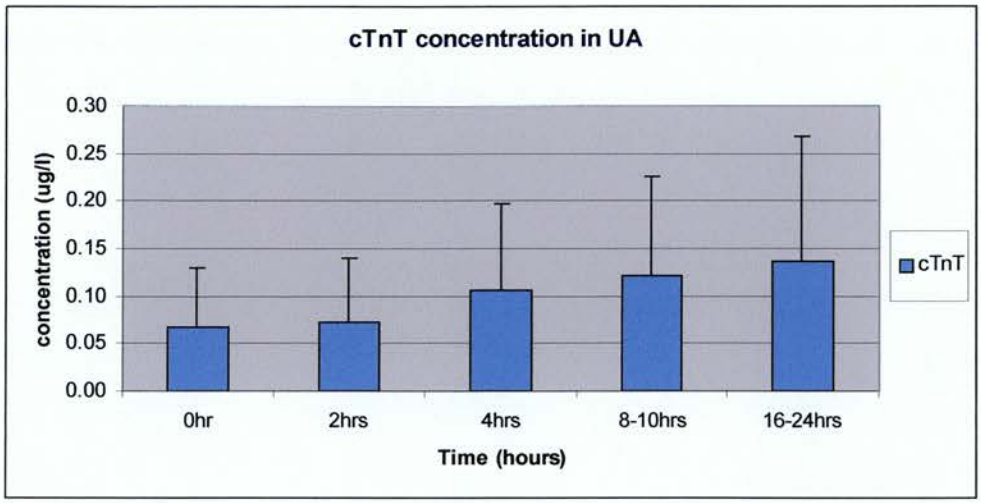


Figure 3 continued on page 200.



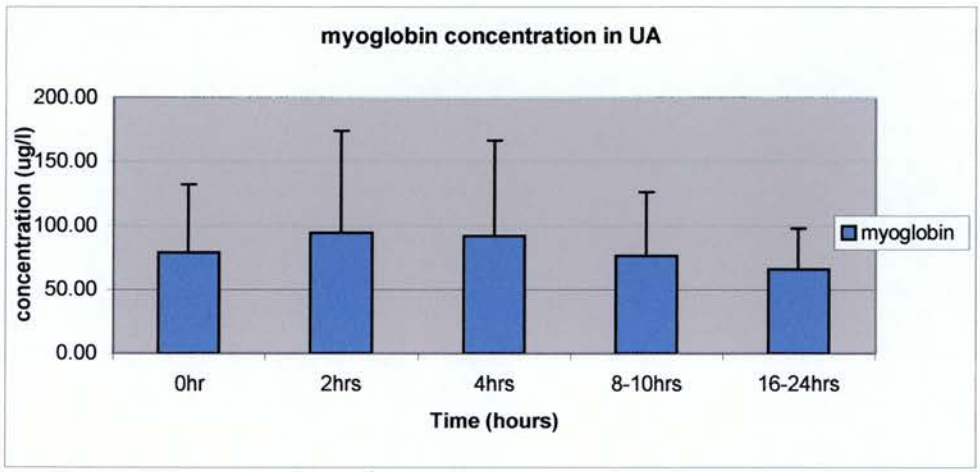


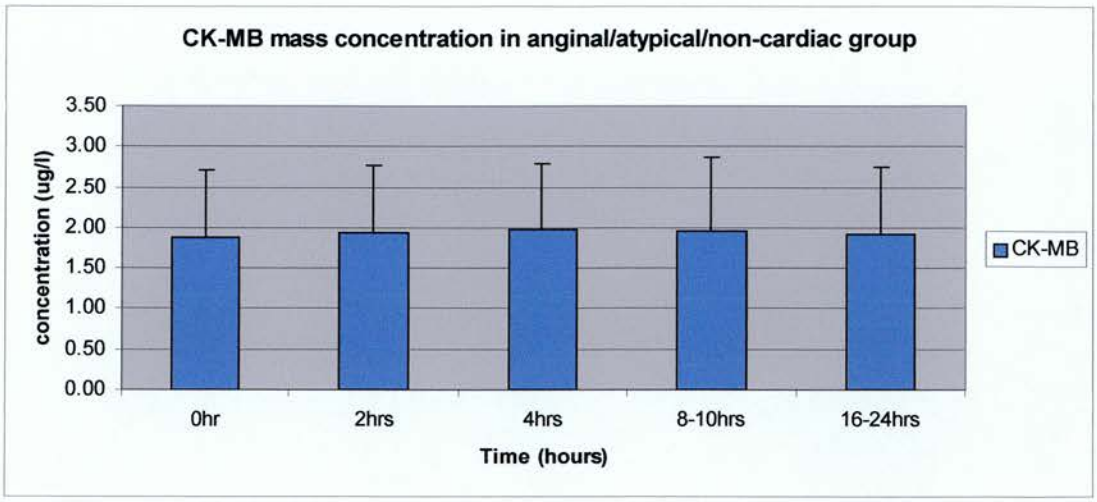
Figure 3. These graphs illustrate the release pattern of CK-MB mass, cTnI, cTnT, H-FABP, and myoglobin in patients with UA.

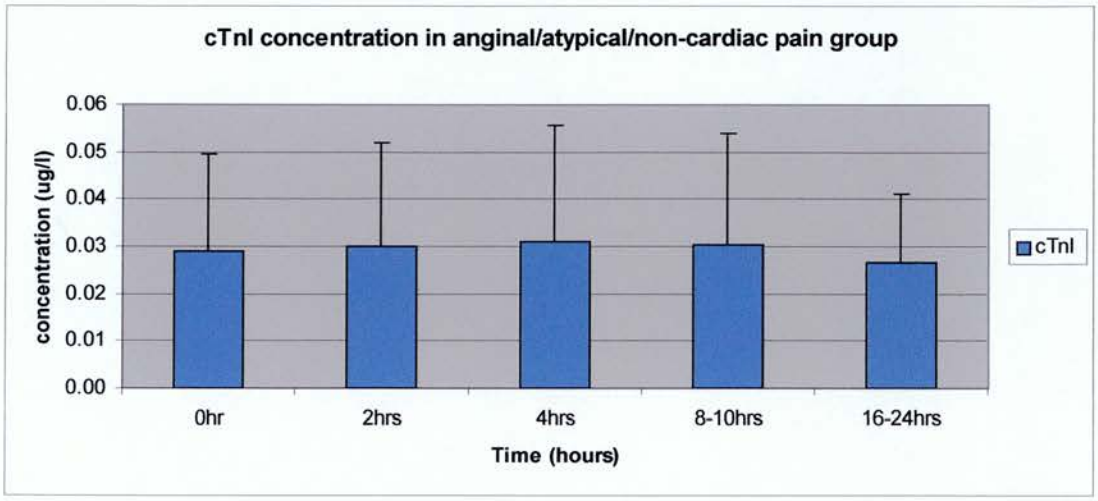
Twenty-one patients were included in the anginal/atypical chest pain group. The majority of patients had previous cardiac events. From a clinical point of view, these patients will be in a high clinical category of suspicion for ACS if they present with chest pain. Sixty-two per cent had typical ischaemic chest pain at presentation, but 38% of patients had atypical chest pain. Comparatively, there were fewer ECG changes in this group compared to patients in the UA and non-Q wave MI groups.

Only 28% had mild ECG changes of ischaemia (three patients had mild T wave flattening or T wave inversion < 1 mm, one patient had old ST elevation, and two patients had transient conduction abnormalities). Table 4 and Figure 4 illustrate the release patterns of the different cardiac markers in this group. The concentrations of these markers remained below the diagnostic thresholds throughout the sampling period. Only one patient had mildly elevated H-FABP concentration (24 μg/l). It can be seen from Table 4 that irrespective of the clinical history or the minor ECG changes, cardiac markers testing can reliably rule out serious myocardial damage very early after admission.

Marker(s)	0hr	2hrs	4hrs	8-10hrs	16-24hrs	P value
CK-MB mass	1.89	1.95	1.97	1.97	1.91	0.204
± SD (μg/l)	0.83	0.81	0.82	0.91	0.84	
CTnI	0.03	0.03	0.03	0.03	0.03	0.664
± SD (μg/l)	0.02	0.02	0.02	0.02	0.01	
CTnT	0.01	0.01	0.01	0.01	0.01	0.077
± SD (μg/l)	0.01	0.01	0.01	0.01	0.01	
Myoglobin	46.90	45.38	45.67	43.80	45.19	0.525
± SD (μg/l)	14.38	13.60	15.64	13.92	14.73	
H-FABP	7.33	6.67	8.22	7.78	5.89	0.837
± SD (μg/l)	5.00	4.53	6.53	7.14	5.35	

Table 4. This table shows cardiac markers concentrations (mean ± SD) in the anginal/atypical chest pain group at 0, 2, 4, 8 – 10, and 16 - 24 hours after presentation. These values are represented graphically in Figure 4. There were no significant concentration changes over time as reflected by probability values > 0.05.





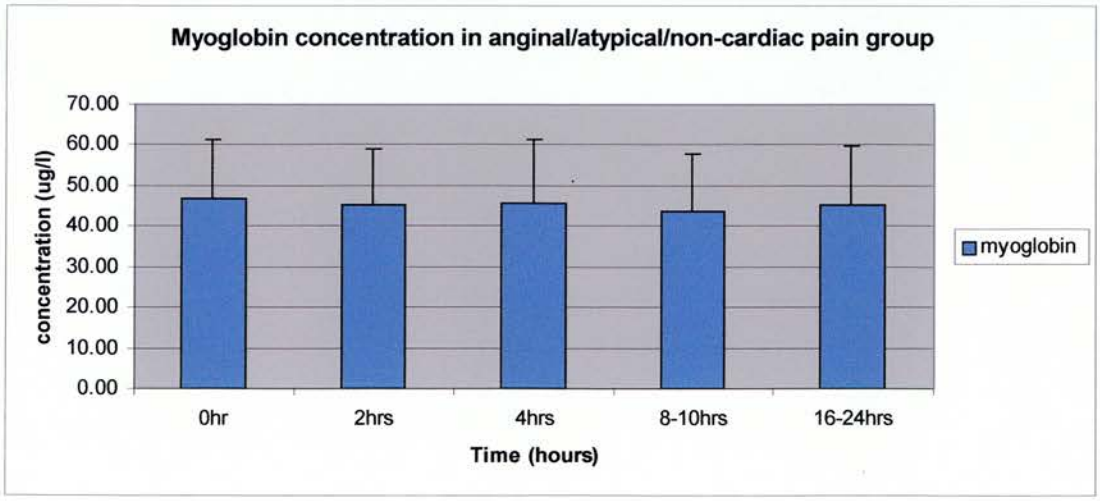
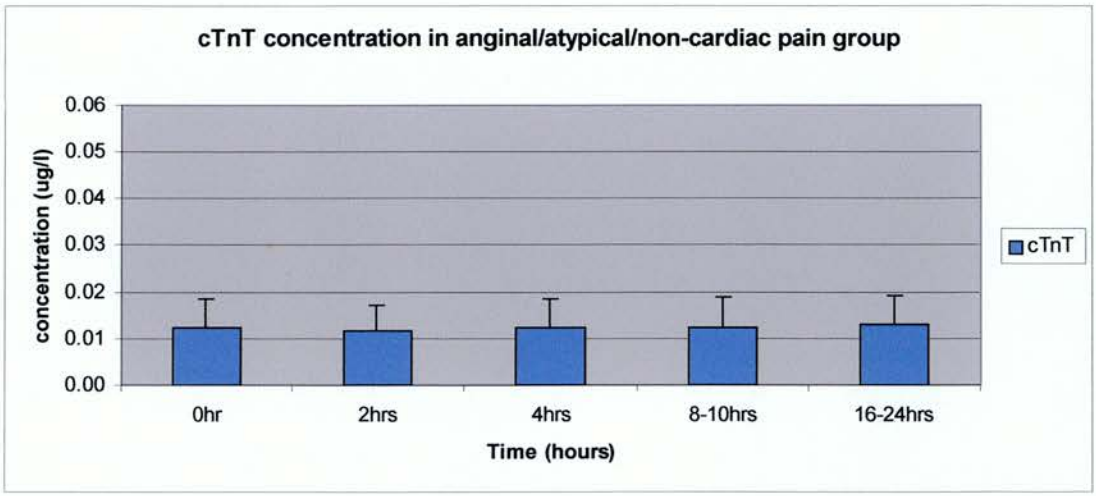


Figure 4 continued on page 203.



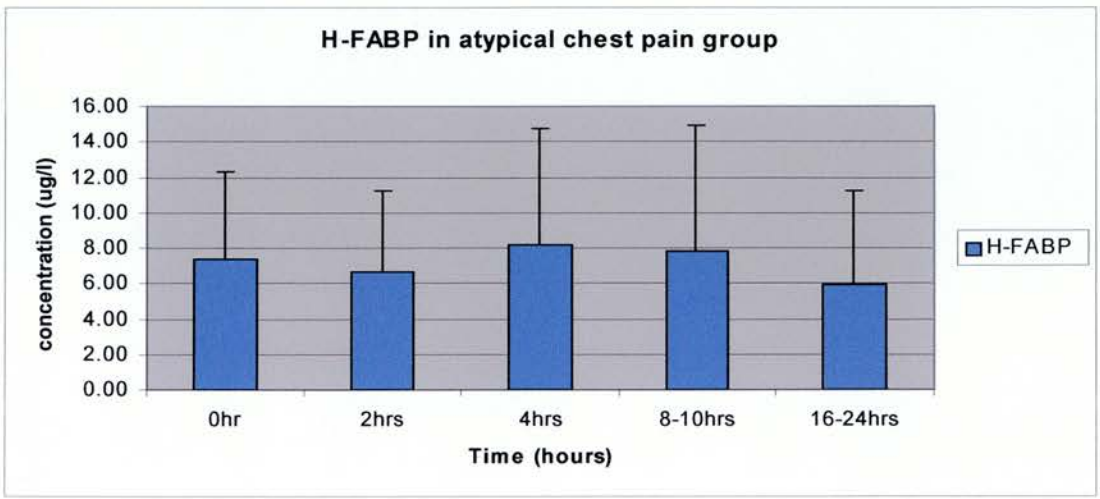


Figure 4. These graphs illustrate the release pattern of CK-MB mass, cTnI, cTnT, H-FABP, and myoglobin in patients in patients with anginal/atypical chest pain.

The values of serial cardiac markers testing in decision-making regarding patients' management will be highlighted by describing two examples from the atypical/anginal chest pain group.

Mild ECG changes and early pre-discharge exercise tolerance test

This was a 58 years old lady with multiple risk factors. She had typical presentation with ischaemic chest pain, which lasted more than five hours. The ECG showed mild T wave inversion in V1, III, and aVf. Initially she was treated as a case of UA with pharmacologic agents. The next day of admission when all the informations including cardiac markers results were available, it became clear that she did not suffer any serious cardiac insult. The repeat ECGs revealed that the T wave inversion was an old finding and remained unchanged and the routine cardiac markers were normal. She underwent an exercise tolerance test that was normal as well. She was discharged following all these reassuring investigations. Cardiac markers concentrations were normal in all samples, which would have ruled out any underlying serious cardiac damage soon after presentation. This information may be utilised to plan management. An exercise tolerance test could have been arranged earlier and this would have shortened the duration of admission (5 days).

Avoiding unnecessary invasive investigations

Another example of mild T wave changes on admission was a lady with a clear history of crescendo angina, which lasted for more than one hour. She was treated as UA with pharmacologic agents. Routine cardiac markers results the next day were normal including CK and cTnI. She underwent coronary angiography, which showed normal coronaries. Serial cardiac markers testing in this patient were completely normal throughout the 24 hours sampling period. In this patient who is considered at low-risk of further ischaemic events, investigation by invasive coronary angiography in the first instance is not warranted. Less invasive investigations e.g. exercise

tolerance test during admission or as an outpatient may be more appropriate. Serial testing at admission may help guide the use of coronary angiography to the most appropriate group of patients at high-risk from further ischaemic events.

The sensitivities of cardiac markers for the detection of high-risk patients with non-Q wave MI and UA at 0 hour, 2 hours, 4 hours, 8 – 10 hours, and 16 - 24 hours after presentation, using two different cut-off concentrations (as described in Chapter 4 pages 120 - 122) are shown in Table 5. Table 5A and 5B illustrate the sensitivities of cardiac markers using the following cut-off concentrations, CK-MB mass $\geq$ 5.0 μg/l, cTnI $\geq$ 0.18 μg/l, cTnT $\geq$ 0.1 μg/l, H-FABP $\geq$ 16 μg/l, and myoglobin $\geq$ 95 μg/l. These cut-off concentrations discriminate (based on ROC curve analysis) patients with myocardial injury (UA and NSTEMI) and patients without myocardial injury (healthy blood donors, normal controls and atypical/anginal chest pain group).

Table 5A shows the sensitivities of cardiac markers for the diagnosis of patients with non-Q wave MI during the first 24 hours after presentation. Heart-FABP was the most sensitive early marker with almost 80% of patients showing elevated concentrations at presentation. The sensitivity had increased to 93% at 2 hours, and remained elevated at this level for the next 8 - 10 hours. The sensitivity had decreased to 64% at 16 - 24 hours. Myoglobin was the second most sensitive early marker with 71% of patients in the non-Q wave MI group showing elevated concentrations ($\geq$ 95 μg/l) at presentation. The sensitivity increased to 93% at 2 hours, and then gradually dropped to 71% over 16 - 24 hours. The sensitivity of myoglobin and H-FABP were similar between 2 and 4 hours (93%).

The sensitivity of cTnI at presentation was 67%. However, cTnI reached 100% sensitivity 2 hours later, and remained at this high level throughout the 16 - 24 hours sampling period. Cardiac-TnT and CK-MB mass sensitivities were similar at presentation (64%), but the maximum sensitivity for CK-MB mass (93%) and cTnT (100%) were reached at 2 hours and 16 - 24 hours respectively (Table 5A). The sensitivities of these markers for the diagnosis of myocardial injury in patients with UA are shown in Table 5B. Cardiac-TnI was the marker that showed the most abnormal values. Fifty-five per cent of patients had elevated cTnI $\geq$ 0.18 μg/l. Cardiac-TnT ($\geq$ 0.1 μg/l) and H-FABP ($\geq$ 16 μg/l) detected myocardial injury in 40% of patients with UA. The sensitivity of CK-MB mass and myoglobin was 30% and 25% respectively. The peak sensitivity of H-FABP for the diagnosis of UA was achieved early at 2 hours, whereas the peak sensitivity of all other markers occurred more than 4 hours after presentation (Table 5B).

Table 5C and 5D show the sensitivity using different cut-off concentrations that best discriminate (based on ROC curve analysis) patients with UA from patients with NSTEMI. These cut-off concentrations were, CK-MB mass $\geq$ 8 μg/l [sensitivity = 81%, specificity = 81.5%], cTnI $\geq$ 0.6 μg/l [sensitivity = 89.9%, specificity = 87.6%], cTnT $\geq$ 0.4 μg/l [sensitivity = 80%, specificity = 80%], myoglobin $\geq$ 107.5 μg/l [sensitivity = 80%, specificity = 80.4%], and H-FABP $\geq$ 21.5 μg/l [sensitivity = 72.5%, specificity = 70.7%]. The sensitivities of these markers for the diagnosis of non-Q wave MI are shown in Table 5C. As shown in Table 5C and 5A, the overall sensitivity pattern of myoglobin ($\geq$ 107.5 μg/l) was not much different with respect to these two different cut-off concentrations.

The overall sensitivity of CK-MB mass (≥ 8 μg/l) and cTnI (≥ 0.6 μg/l) remained unchanged compared with Table 5A. There was two hours delay in the maximum sensitivities reached by CK-MB mass and cTnI (4 hours vs 2 hours) and a drop in sensitivity towards 16 - 24 hours (86% and 93% respectively). There was some drop in the overall sensitivity of cTnT for the diagnosis of non-Q wave MI with cut-off concentration ≥ 0.4 μg/l (93% vs 100%). The sensitivity of H-FABP at presentation and 8 - 10 hours was reduced with cut-off concentrations exceeding 21.5 μg/l (71% vs 79% and 64% vs 93%), but the early sensitivity at 2 hours was relatively unchanged.

Table 5D, shows the percentages of patients with UA that would be misclassified as non-Q wave MI (false positive rate), using these cut-off concentrations. Twenty-five per cent (25%) of patients admitted with UA would fulfil the diagnostic criteria to be classified as non-Q wave MI based on CK-MB mass and cTnI. Only a small number of patients (10%) would be classified as non-Q wave MI using cTnT. There was little change in the sensitivity of H-FABP in the range 16 - 21.5 μg/l with forty per cent of patients with UA having concentration ≥ 21.5 μg/l i.e. high number of 'false positive' classification for non-Q wave MI. There was also little improvement on the specificity of H-FABP from using concentrations more than three times the upper limit of normal (36 μg/l). Based on ROC curve analysis, a discriminatory cut-off concentrations of H-FABP ≥ 21.5 μg/l had a sensitivity of 72.5% and specificity of 70.7% only, for the diagnosis of non-Q wave MI. Selecting a higher cut-off concentrations (e.g. 36 μg/l) decreased the sensitivity significantly (62.5%).

The small numbers of patients in this group may have partly contributed to this problem. Despite the small overlap, there was still a significant difference between the concentrations of H-FABP in patients with UA and patients with non-ST elevation MI and ST elevation MI, which reflects an increase in myocardial injury in these conditions. The mean concentration (± SD) of H-FABP in patients with UA, non-ST elevation MI and ST elevation MI was 28.27 ± 19.62 μg/l [range 19 - 62 μg/l], 165.36 ± 128.74 μg/l [range 17 - 431 μg/l], and 373.66 ± 244 μg/l [range 20 - 1184 μg/l] respectively. Animal studies provide evidence to support large releases of H-FABP with larger areas of myocardial ischaemia.[59] As compared with H-FABP, myoglobin (≥ 107.5 μg/l) was equally sensitive (71%) but more specific (20% false positive rate) for the diagnosis of non-Q wave MI, but its sensitivity for the diagnosis of UA was less than H-FABP (25% vs 40%).

Table 5A. Non-Q wave MI group

Marker(s)	0 hr	2 hrs	4 hrs	8-10 hrs	16-24 hrs
CK-MB	64	93	93	93	93
CTnI	67	100	100	100	100
CTnT	64	79	79	86	100
Myoglobin	71	93	93	79	71
H-F ABP	79	93	93	93	64

Table 5B. UA group

Marker(s)	0 hr	2 hrs	4 hrs	8-10 hrs	16-24 hrs
CK-MB	20	25	30	25	20
CTnI	35	40	55	55	45
CTnT	15	20	35	40	30
Myoglobin	20	20	20	25	25
H-FABP	30	40	30	30	17

Table 5A and 5B. Shows the sensitivities of the various cardiac markers for the diagnosis of non-Q wave MI and UA at 0, 2, 4, 8 - 10, and 16 - 24 hours after presentation, using the following concentrations CK-MB mass ≥ 5 μg/l, cTnI ≥ 0.18 μg/l, cTnT ≥ 0.1 μg/l, H-FABP ≥ 16 μg/l, and myoglobin ≥ 95 μg/l.

Table 5C. Non-Q wave MI group

Marker(s)	0 hr	2 hrs	4 hrs	8-10 hrs	16-24 hrs
CK-MB	64	79	93	93	86
CTnI	64	86	100	100	93
CTnT	50	71	71	86	93
Myoglobin	71	93	86	79	71
H-FABP	71	93	79	64	57

Table 5D. UA group

Marker(s)	0 hr	2 hrs	4 hrs	8-10 hrs	16-24 hrs
CK-MB	15	20	25	20	20
CTnI	10	15	25	20	15
CTnT	0	0	5	10	10
Myoglobin	20	20	20	20	20
H-FABP	25	40	30	30	10

Table 5C and 5D. Table 5C shows the sensitivities of the various cardiac markers for the diagnosis non-Q wave MI, at 0, 2, 4, 8 - 10, and 16 - 24 hours after presentation, using the following concentrations CK-MB mass ≥ 8 μg/l, cTnI ≥ 0.60 μg/l, cTnT ≥ 0.40 μg/l, H-FABP ≥ 21.5 μg/l, and myoglobin ≥ 107.5 μg/l. Table 5D shows the number of patients with UA that would fulfil the diagnostic criteria for non-Q wave MI (false positive results).

The value of serial cardiac markers testing for the diagnosis of myocardial infarction in patients without the classical ST segment elevations on the ECG will be illustrated by several examples. It will be made clear from these examples the importance that rapid (within 15 minutes) cardiac markers testing availability will have on clarifying the diagnosis and influencing appropriate decisions in the management of these patients. These examples will concentrate on common situations in the A&E department, where either the clinical history is atypical or the admission ECG shows ST-T segment changes that are inconclusive or difficult to interpret.

Non-diagnostic initial ECG

In one patient, a 61 years old lady with extensive cardiac history who presented with atypical chest pain, the admission ECG showed 1.5 mm ST segment elevation in lead III only, with ST depression in lead I and AVL. Initially she was admitted to CCU and treated as a case of AMI with streptokinase. Later, it was thought that she did not sustain an AMI and instead this was a significant episode of UA and the treatment was discontinued. Cardiac markers results suggested that this patient had suffered an AMI [CK-MB mass = 27 μg/l, cTnI = 13.37 μg/l, cTnT = 1.05 μg/l, H-FABP = 40 μg/l and myoglobin = 354 μg/l]. At 2 hours after presentation the concentration of cTnI was 1.26 μg/l, H-FABP was 24 μg/l and myoglobin was 137 μg/l, and at 4 hours cTnI had risen to 3.64 μg/l, H-FABP to 40 μg/l, myoglobin to 307 μg/l and CK-MB mass to 13 μg/l. This patient was kept in hospital for 7days.

This case highlights a relatively common situation and a diagnostic dilemma. That is when significant ST segment elevation is present in one lead only. The significance of this elevation is even more difficult to interpret when the patient presents for the first time and had no prior ECGs recording. This ECG change could represent a large AMI in evolution, a small MI involving limited territories, UA or an old finding. The availability of rapid cardiac markers results would help resolve this confusion and influence management decisions. The following ECG changes at admission were also other examples from this study, where concomitant serial cardiac markers testing have contributed significantly towards clarifying the early diagnosis of MI in these patients; (1) Transient ST segment elevation; (2) Mild ST segment elevation <

1 mm; (3) Marked ST segment depression; (4) Deep T wave inversion; and (5) Poor R wave progression.

Presentation prior to release of diagnostic levels of cardiac markers

Another example of equivocal ST segment elevation was a 65 years old man with multiple risk factors who presented with two weeks history of worsening chest pain. The last episode of chest pain was one hour before presentation. The ECG revealed 1 mm ST segment elevation anteriorly, ST segment depression laterally, ECG voltage criteria for left ventricular hypertrophy with strain pattern, and T wave inversion inferiorly. He also had mild pulmonary oedema. The interpretation of the ECG was difficult because of the changes due to left ventricular hypertrophy. The interpretation of subtle ST elevation was also difficult. These ECG changes were considered insufficient criteria for thrombolytic therapy and the patient was treated with pharmacological agents without percutaneous coronary intervention.

He developed acute shortness of breath shortly after admission and proceeded to coronary angiography. Coronary angiography showed occlusion of the left anterior descending artery, with two further severe lesions in the right coronary artery and the left circumflex artery. Cardiac markers concentrations in this patient on arrival were almost normal. At 2 hours, cardiac markers concentrations had risen considerably CK-MB mass = 50.7 μg/l, cTnI = 8.72 μg/l, cTnT = 0.82 μg/l, H-FABP (peak concentration) = 431 μg/l and myoglobin > 900 μg/l. Four hours later, CK-MB mass increased to 80.2 μg/l, cTnI > 50 μg/l, cTnT = 1.9 μg/l, H-FABP = ↓344 μg/l and myoglobin = 1843 μg/l. The absence of abnormal concentrations at presentation is

consistent with the very early presentation of this case (one hour). Heart-FABP showed a significant early peak (within 3 hours) compared with other markers including myoglobin. The ECG was unhelpful for the diagnosis myocardial infarction in this patient because of the presence of confounding changes due to left ventricular hypertrophy. Serial cardiac markers testing would have established the diagnosis shortly after admission and permitted appropriate therapy to be implemented within 3 hours after symptoms onset.

Patients with major conduction disorders on the ECG

One patient with acute chest pain had left bundle branch block that was not a new finding and was present in old ECGs. He had poor left ventricular function and severe three vessel disease with occluded left anterior descending artery and right coronary artery that were considered unsuitable for any form of intervention. This old conduction abnormality made interpretation of the admission ECG very difficult. Cardiac markers were significantly increased at presentation [CK-MB mass = 150 μg/l, cTnI > 50 μg/l, cTnT = 4.18 μg/l, H-FABP = 26 μg/l, and myoglobin = 527 μg/l]. The measurements of cardiac markers in this situation had clarified the diagnosis. The cardiac markers concentrations profile at presentation (i.e. high cTnI and low H-FABP) is consistent with a diagnosis of an earlier AMI event (> 12 hours old).

Previously documented coronary artery disease but uncertain recent events

This was a 63 years old man with extensive previous cardiac history (CABG, redo CABG, PCI, three previous MI's, and left ventricular failure) who presented with

typical history of ischaemic pain unresponsive to usual medications. Electrocardiogram showed an old partial conduction abnormality. He was admitted and treated for UA with pharmacologic agents. The ECG changes later evolved to lateral T wave inversion. He experienced recurrent chest pains and proceeded to coronary angiography, which showed re-stenosis of saphenous vein grafts to obtuse marginal and left circumflex artery branches. He was treated with PCI. Routine CK and cTnI were markedly elevated at 494 IU/L and 14.7 μg/l respectively. The value of serial cardiac markers testing in clarifying the significance of these ECG changes can be demonstrated in this patient.

At presentation, cardiac markers concentrations in this patient were normal. At 2 hours myoglobin and H-FABP were significantly elevated at 285 μg/l and 49 μg/l respectively but cTnI and cTnT were only slightly elevated at 0.33 μg/l and 0.103 μg/l respectively. At 4 hours, significant rises had occurred in CK-MB mass, H-FABP, and myoglobin 26.2 μg/l, 99 μg/l, and 587 μg/l respectively, whereas cTnI was 0.95 μg/l and cTnT was 0.169 μg/l. Eight hours after presentation, most cardiac markers concentrations were significantly elevated (CK-MB mass = 81.7 μg/l, cTnI = 4.38 μg/l, H-FABP = 123 μg/l and myoglobin = 585 μg/l). At 19 hours after presentation, there were even more significant increases in these markers [CK-MB mass = 131.4 μg/l, cTnI = 21.99 μg/l, cTnT = 2.22 μg/l] and myoglobin and H-FABP concentrations had decreased to 169 μg/l and 24 μg/l respectively. This example clearly illustrates the early release and clearance characteristics features of H-FABP (and myoglobin) compared to other markers.

Patients with posterior myocardial infarction

The accurate early diagnosis of posterior MI is sometime difficult to identify from the 12-lead ECG. The changes may be subtle and sometimes more investigations are needed to clarify this diagnosis e.g. V7 - V8 leads or echocardiogram. In the next example an eighty-three years old lady was admitted with ischaemic chest pain but the ECG changes on admission were thought to reflect UA and she was treated with pharmacologic agents without PCI. The retrospective interpretation of these ECG changes in light of routine cardiac markers results was considered as possible posterior infarct (CK = 645 IU/L and cTnI = 20 μg/l). Serial cardiac marker concentrations in this patient were significantly elevated at presentation (CK-MB mass = 36.6 μg/l, cTnI = 20.84 μg/l, cTnT = 3.41 μg/l, H-FABP = 17 μg/l and myoglobin = 190 μg/l). It is worth noting that apart from cTnT that continued to increase in subsequent samples (3.41→ 4.33 μg/l), the maximum value for the other markers occurred at presentation. The low H-FABP concentrations and elevated cTnI suggested delayed presentation or ongoing subclinical ischaemic events prior to presentation. This case illustrates the difficulty of diagnosing possible posterior MI and the additional diagnostic benefit of using serial cardiac marker testing in this situation.

Detection of re-infarction

This was a 75 years old man with extensive previous cardiac history who was admitted with mild atypical chest pain and severe congestive cardiac failure (CCF). He was treated with continuous positive airways pressure and inotropic support. Initially, he made some clinical improvement, but he deteriorated a few days later,

became acidotic and developed acute mesenteric infarction. He died 18 days after admission. The cardiac markers concentration profiles in this patient were very informative. Although he initially presented with chest pain as well as CCF, the chest pain was only a minor complaint and was overshadowed by CCF, which was the more dominant presenting feature. The ECG at presentation was normal. He was treated as a case of severe CCF. As early as 2 hours after presentation, the cardiac markers concentration profile in this patients were suggestive of MI with CK-MB mass concentration of 27.7 μg/l rising to > 150 μg/l, cTnI 3.25 μg/l rising to 32.47 μg/l, cTnT 0.649 μg/l rising to 5.04 μg/l, H-FABP of 122 μg/l rising to 441 μg/l, and myoglobin 861 μg/l rising to 1815 μg/l.

Further analysis of the time-concentration profile of cardiac markers H-FABP and myoglobin suggests that this patient may have suffered re-infarction shortly after the first infarct. This was reflected by a significant drop in concentrations in the third sample following two initial successive increases and was followed by two further increases. Such drop in concentration was not found with CK-MB mass and troponins, which showed progressive increases in all samples. Congestive cardiac failure may contribute some of the increases in cardiac markers concentrations. However, with concentrations as high as this, it most probably indicates underlying severe ischaemic damage, and the CCF may be a consequence of this initial ischaemic insult.

All these cases highlight the importance of concomitant serial cardiac markers testing for clarifying the diagnoses and influencing further management in situations where

there is a high clinical suspicion of ACS in the presence of unhelpful ECG changes.

A preliminary cost effective analysis of serial cardiac markers testing in evaluating patients with acute chest pain was performed. In this study, fifty-five patients were admitted to hospital and in every patient five serial blood samples were collected during the first 24 hours. In each sample five cardiac markers were analysed. The total cost of analyses for the whole group (1375 tests) was approximately £4500. This figure includes assays and all the quality controls and calibrator reagents required to run these analyses. In clinical practice, the analysis can be reduced to two markers at two intervals (or a maximum of three, depending on the duration between symptoms onset and presentation) during the first 8 hours of presentation. This will result in a further substantial reduction of the cost of these analyses by approximately 80%. Serial cardiac markers testing are reliable and will identify high-risk patients with UA and non-Q wave MI and rule out myocardial damage within 8 hours after presentation.

Based on informations obtained from the hospital financial department, the estimated consumable cost of hospital stay for the whole group was approximately £29,596.[29] This was only a conservative estimate, because it excludes the cost of specialised investigations such as coronary angiography, thallium scan, computerised tomography scan, ventilation - perfusion scan and also excludes specialised treatment e.g. antiplatelet drugs and angioplasty procedures. Table 6 shows the average cost of analyses in each group and the estimated cost reduction. Implementation of cardiac markers testing will result in a reduced cost of in-hospital care by approximately

34%. This reduction in cost is mostly due to a reduction in hospital stay, as a result of early identification and management. The greatest reduction in cost (60%) is expected in group 3 as a result of early rule out of ACS, reduction in the number of investigations, and early discharge of low-risk patients [maximum hospital stay 1 - 2 day]. Early identification and better management may reduce the average hospital stay for uncomplicated patients with UA [3 - 4 days]. Early diagnosis, treatment, and implementation of post-MI care in patients with NSTEMI may also reduce hospital stay. The cost reduction in the non-Q wave MI group was based on an average admission of 7 days. [See section 6.4.2 later]

Group(s)	Average stay (days)	Total stay (days)	Average cost of care (£)	Estimated analytical Cost (£)	Estimated cost reduction (%)
Group 1	8.57 ± 3.69	120	11,760	229	2156 (18)
Group 2	5.21 ± 2.95	102	9,996	327	3136 (31)
Group 3	3.95 ± 2.16	80	7,840	275	4753 (60)
Total	-	302	29,596	831	10,045 (34)

Table 6. Shows the estimated cost reduction for the whole groups based on serial cardiac markers analyses using an early marker and cardiac specific marker.

6.4 DISCUSSION

As shown in this study, and depending on threshold concentration used, the incorporation of serial cardiac markers testing, using H-FABP, myoglobin, CK-MB mass or cTnI in patients who present with non-Q wave MI within 7 hours after symptom onset, can diagnose 93 - 100% of patients (Table 5A), or 79 - 93% (Table

5C) within 2 hours of presentation. Heart-FABP was the most sensitive indicator of myocardial injury and was increased in a substantial numbers of patients (79%) at presentation. The maximum sensitivity achieved for myoglobin at presentation was 71%. However, the sensitivity of both markers at 2 hours was similar (93%). Heart-FABP was either equally sensitive or superior to myoglobin for the early diagnosis of non-Q wave MI (Table 5A and 5C).

The sensitivity of all other markers including cTnI, CK-MB mass, and cTnT at presentation was low and ranged from 50 - 67%. Their value for the early rule in of patients with non-Q wave MI was limited. Their sensitivity was considerably lower than that of H-FABP. These differences are related to the early release characteristic feature of H-FABP and the short time between symptom onset and presentation. The sensitivity and the early release characteristics of cTnI were found in this study to be superior to that of cTnT and CK-MB mass. Cardiac-TnI was highly reliable [100% sensitive] for the diagnosis of non-Q wave MI after four hours of presentation. These variations may be due to the superior sensitivity of cTnI on Stratus CS, which allows earlier detection of small amounts of this mass protein in serum following release from the myocardial cells. This early release feature of cTnI supports the presence of cytosolic pools for cTnI within myocardial cells.

The major influences found from using low diagnostic cut-off concentrations that were based on normal controls groups (a common method used in many clinical studies) to diagnose patients with non-Q wave MI, were an improvement in the early and overall sensitivity (5A), on the expense of specificity. Some patients with UA

will be misclassified as non-Q wave MI (5B). This problem was reduced considerably by using two different threshold concentrations that best define myocardial injury for UA and non-Q wave MI groups (5C and 5D).

Cardiac-TnI and cTnT were the two most sensitive markers [55% and 40% respectively] for the detection of myocardial injury in patients with UA. Heart-FABP showed a high positive value in UA (40%) as well, and was low in normal coronary patients having chest pain (10%). Its sensitivity was comparable to that of cTnT, but was superior to CK-MB mass and myoglobin. There was however, some overlap between the concentrations of H-FABP in non-Q wave MI and UA groups. It was difficult to draw a clear distinctive cut-off concentration that will reliably discriminate both conditions without severely affecting the sensitivity as well.

When early differentiation of non-Q wave MI from UA is required, concentrations of H-FABP equivalent to five times the upper limit of normal (51.5 μg/l) can be used. This high cut-off concentration was associated with more than 90% specificity, albeit at a rather reduced sensitivity (57%) for the diagnosis of non-Q wave MI. The use of this high threshold does not compromise the early value of H-FABP, because these diagnostic concentrations were reached within 2 hours after presentations (8 hours after symptom onset). Alternatively, H-FABP concentrations (> 16 μg/l - < 21.5 μg/l) and (≥ 21.5 μg/l) may be combined with cardiac troponins to increase its specificity for the diagnosis of UA or non-Q wave MI respectively. Concentrations between 0.18 - 0.6 μg/l for cTnI and 0.1 - 0.4 μg/l for cTnT are sensitive (> 80 - 85%) and specific (> 80 - 83%) for the diagnosis of UA in these patients.

The frequency of H-FABP elevations in patients presenting with UA and atypical/anginal chest pain were similar to that reported by Tsuij et al (1993). They reported that 56% and 17.8% of patients with UA and chest pain syndrome respectively had elevated H-FABP concentrations. The concentration of H-FABP in patients with UA was also lower than the concentration in patients with AMI.[49] The sensitivity of myoglobin for the early diagnosis of non-Q wave MI in this study (93%) was similar to the sensitivity reported by Gornall et al (1996). They reported the sensitivity and specificity of myoglobin within eight hours after symptom onset for the diagnosis of NSTEMI to be 93% and 100% respectively.[52]

Subtle changes of ischaemia on the admission ECG (such as minimal ST elevation < 1 mm, transient ST elevation, significant ST elevation in one lead only, suspected but not evident changes of posterior MI, marked and deep T waves inversion, poor R wave progression and conduction abnormalities e.g. left bundle branch block) were common. The diagnostic power of the ECG alone in these circumstances was limited. These ischaemic ECG changes may be considered non-specific and insufficient to influence definitive diagnosis or treatment. However, when these ECG changes are present in combination with positive cardiac markers concentrations consistent with very early infarction, the evidence is shifted in favour of MI and may be towards an early implementation of reperfusion therapy in some of these patients.

It is worth noting that most of these patients were initially diagnosed and treated as severe case of UA with non-thrombolytic pharmacological agents. The diagnosis of non-Q wave MI in these patients was retrospective and was evident 12 - 24 hours

after admission, from routine cardiac markers results. The hospital stay for this group of patients was considerably long.

The reliance on clinical judgment and ECG changes alone is not sufficient to diagnose MI in some groups of patients without the classical ST segment elevation MI.[30;31;50;56] These patients are put at risk from being misdiagnosed and subsequently given inappropriate treatment and care.[57] There was no consistent relation between the types of ST-T segment changes on the admission ECG and the degree of cardiac markers elevations. This adds to the poor specificity of the ECG for predicting the significance of myocardial damage. Serial testing of two cardiac markers at two intervals within 8 hours of presentation can identify most patients with early myocardial infarction, irrespective of the subtlety of ischaemic changes on the admission ECG. The earliest evidence of myocardial damage that can be detected using serial markers testing was present in 93 - 100% of patients within 2 hours after presentation. This early identification of myocardial damage will have important implications for the triage and management of these patients.

'Q wave MI' and 'non-Q wave MI' are considered old terminology. These two terms are not thought to represent two distinct medical conditions.[32-34] Phibbs et al (1999) had recently reviewed nine major studies. They concluded that from a clinical, pathological and electrocardiographic point of view, these two terms are meaningless and should be an obsolete. They ascribed the continuous use of the term ''non-Q wave MI'' as a separate clinical entity to improper study protocols and misdiagnosis of Q wave equivalent.[35] These protocols fail to differentiate between first and

subsequent infarcts, because the frequency of new Q wave appearance decreases by 50% in subsequent infarcts. Also, the presence of Q wave equivalent on the ECG (tall R waves in right precordial leads, poor R wave progression, low voltage QRS, R/S pattern, QRS notching) is often ignored or missed, hence many patients in these two groups will be classified as non-Q wave MI.[35]

These arguments may in turn call for a review of the current treatment strategy of patients who are classified as non-Q wave MI. Unstable angina and non-Q wave MI share similar pathophysiology with STEMI i.e. plaque rupture, fissure or erosion with subsequent platelet aggregation and thrombus formation. With STEMI there is more fibrin deposition leading to complete and prolonged coronary artery occlusion.[14] In UA and non-Q wave MI the disease process may be more dynamic, with active thrombosis and fibrinolysis and the occlusion is either incomplete or less severe or not prolonged, and platelet deposition is more of a component than fibrin deposition.[10] Patients with non-Q wave MI have a higher incidence of total coronary artery occlusion (21%), and a higher incidence of a characteristic type II eccentric lesion (65%) i.e. an eccentric stenosis with overhanging edges or irregular borders.[3] Elevated cardiac markers were correlated with the presence of underlying complex pathologies in these conditions.[58]

The routine use of thrombolytic therapy in NSTEMI (UA and non-Q wave MI) within 24 hours of admission in the study by TIMI IIIB investigators, had not been shown to be beneficial and was even associated with more adverse outcome e.g. AMI and intracranial haemorrhage.[28] This view is also supported by several small

studies.[36-39] On the other hand, there are some studies that reported benefits of thrombolytic therapy in refractory UA, especially when this treatment was used in low doses and over a prolonged period of time in selected patients.[40-42] Prolonged administration of low dose recombinant tPA and heparin in patients with refractory UA was associated with decreased rate of MI, need for revascularisation (PCI and CABG), and a decrease in the number of episodes and total duration of ischaemia as compared with heparin treatment only. These treatment benefits were evident over 14 ± 6 months of follow-up, with more patients in the treatment group pain-free, and had a lower incidence of re-admissions.[41]

The adverse outcomes associated with the use of thrombolytic therapy in UA and non-Q wave MI in earlier studies could be due to patient's selection bias attributed to by several factors. First, the use of thrombolytic therapy in UA and non-Q wave MI was guided more by ECG changes rather than by ECG changes and cardiac markers concentrations at admission. As mentioned earlier, elevations of cardiac markers concentrations were not consistently or directly related to the types of ECG changes at admission. When most of these studies were conducted, the development of the most sensitive and specific cardiac markers e.g. troponins, CK-MB mass and H-FABP was still in its early stages and they were not part of the protocol used for patients selection.[39;41]

Second, the greatest benefits of thrombolytic therapy in AMI were observed if this treatment was given within the first 12 hours after the start of symptoms. Beyond this interval generally the risks outweigh the benefits. The inclusion of patients in some

of these studies was late and sometimes within 25 hours after symptom onset.[28;39] The inclusion of patients beyond 12 hours may have offset the benefits that may be gained from early enrolment and treatment within 12 hours after symptom onset. Sometimes the failure of treatment was attributed to small sample size that does not provide sufficient power to rule out treatment effect.[36]

It is understandable that without the sensitive and specific cardiac markers that are available today, proper early diagnosis and differentiation between patients with minimal myocardial injury in UA and patients with non-Q wave MI was not possible using ECG changes alone. The judicious use of thrombolytic therapy on individualised bases, in selected high-risk patients with early positive cardiac markers concentrations of infarction, and subtle ECG changes of infarction such as 'Q-wave equivalent', posterior MI, and non-classical ST elevations < 1 - 2 mm, who present within 12 hours after symptom onset has not yet been evaluated in clinical trials. These are patients who fulfil the criteria for AMI based on clinical history and positive cardiac markers concentrations but in the absence of the classical ST segment elevations in the admission ECG. The potential benefits and risks of thrombolytic therapy in patients without the classical ST segment elevations should be examined and monitored closely in a pilot study. The result of such study will determine whether further trials should be conducted.

The availability of point of care instruments that can offer simple, rapid (< 20 minutes), and reliable round the clock measurements of cardiac markers will help with the management of high-risk patients with acute chest pain in several ways; (1)

Clarifying atypical clinical history and equivocal or ambiguous ischaemic ECG changes at presentation. The overall sensitivity of cardiac markers, clinical history and ECGs for the diagnosis of non-Q wave MI was 100%, 93% and 86% respectively; (2) Early stratification of high-risk patients; (3) Appropriate and speedy triage of high-risk patients to CCU or dependent units.

(4) Where appropriate, some of these high-risk patients with non-Q wave MI could be considered for early with PCI. Alternatively, many of these high-risk patients can be scheduled for early treatment with aspirin, LMWH, and glycoprotein GPIIb/IIIa receptor inhibitor; (5) Early identifications will lead to proper instigation of post-MI management and care e.g. aspirin, β-blockers and lipid lowering therapy; (6) Early identifications of patients and prompt therapy may reduce complications and the time of hospital stay. This may in turn reduce the consumable running cost; (7) Most importantly early diagnosis will prevent inadvertent discharge of these high-risk patients.

The development of new and more potent antiplatelet agents that act on different platelet receptors had been a major advance in the treatment of patients with UA and non-Q wave MI (Figure 1). Many studies had shown benefits of treatment in terms of reducing the incidence of AMI and death when agents like tirofiban or clopidogrel were used in the treatment of patients with ACS.[21-23] The selection of the most appropriate groups of patients for these treatments is an important issue, in terms of maximising the benefits and minimising the cost and side effects. The ECG changes do not necessarily reflect the significance of myocardial damage in vivo. As shown

here and elsewhere, cardiac markers concentrations can be either normal or elevated in similar ECG patterns.[51] Nevertheless, the presence of significant ECG changes of ischaemia e.g. ST segment depression are considered high-risk prognostic signs, and are associated with the presence of three vessel disease, and a five-fold greater risk of death and new myocardial infarction compared to those with normal ECG.[48;7]

The diagnostic and prognostic value of elevated cardiac troponins in patients with ACS is supported by many studies.[44-47;53-55] Patients with ACS and positive cTnI are at increased risk of death and MI (13%) compared to patients with negative cTnI (4.9%), [13.7% and 3.5% for cTnT positive and cTnT negative respectively].[43] Stratifying patients by troponins concentrations at admission was associated with the greatest reduction of death and MI in the groups treated with tirofiban medically and for 48 hours before revascularisation.[43] Furthermore, in a substudy by the fragmin in unstable coronary artery disease investigators, long-term (five weeks) treatment with dalteparin was most beneficial in reducing the risk of death and AMI compared to placebo in patients with cTnT $\geq$ 0.1 μg/l [7.4% vs 14.2% at 40 days, $p < 0.01$] compared to cTnT < 0.1 μg/l [5.7% vs 4.7%].[46] The combination of serial cardiac markers testing at admission with ECG changes provide simple and reliable methods that will help select patients most at risk of further ischaemic events. This group can be considered for further treatment with LMWH, clopidogrel, and GP IIb/IIIa inhibitors, and other new antiplatelet compounds.

Coronary angiography was followed by angioplasty in a higher percentage of patients with elevated cardiac markers at admission compared to those with normal

cardiac markers (67% vs 20%). Stratifying patients by cardiac markers concentrations at admission may help guide the application of this invasive procedure. Patients who demonstrate significantly elevated cardiac markers concentrations at admission are started on aspirin, LMWH and GP IIb/IIIa and allocated to early cardiac catheterisation ± angioplasty. Patients who are clinically stable and with no significant elevations in cardiac markers are treated conservatively. These patients can be investigated by angiography if they develop further episodes of prolonged angina at rest, recurrent angina ± ST segment changes on ECG, increased troponins, haemodynamic instability, or positive exercise tolerance test or thallium scan. Patients with UA and no release of troponins have less severe coronary artery disease and have excellent prognosis. It is suggested that these patients may be managed more conservatively and without invasive evaluation before discharge.[58]

6.4.1 ATYPICAL/ANGINAL CHEST PAIN GROUP

Chest pain is a common complaint in the A&E department. Excluding patients without serious cardiac chest pain is not possible by reliance on clinical history and ECG alone. Sixty-two per cent (62%) of patients in this group had typical ischaemic chest pain, and there were minor ECG changes of ischaemia in 28%. Most of these patients end up in hospital for several days, and are usually treated in a similar manner to patients with UA with pharmacologic agents, and some of them may have invasive investigations. Serial cardiac markers testing at admission could help put the clinical conditions in perspective, and avoid unnecessary prolonged hospitalisation and invasive investigations in patients considered (based on their clinical condition,

ECG, and cardiac markers concentrations) at low-risk of further acute ischaemic events.[47] Cardiac markers concentrations were all entirely normal in this group and would have classified these patients into a low-risk group for whom admission to a regular bed or early discharge would be appropriate.

It is possible that some of the patients classified in this group may well represent UA patients with normal ECG and normal cardiac markers concentrations. However, the fact that serial cardiac markers concentrations are normal is reassuring. Furthermore, there are no major benefits of treatment with new antiplatelets or antithrombotic in patients with normal ECG and troponins.[43;46] Investigations in this group should be proportionately scaled down especially the use of invasive investigations. The duration of hospital stay should also be cut down to the minimum. If clinical doubt exists and further risk stratifications were deemed necessary, these patients may be subjected safely to an early exercise tolerance test before discharge.

Cardiac markers testing will optimise the triage and management of patients with atypical/anginal pain from A&E department. Measurement of cardiac markers in patients with chest pain can help with; (1) Proper identification of low-risk patients with chest pain; (2) Triage of patients to less dependant units e.g. regular bed; (3) Avoiding unnecessary invasive investigations; (4) Early discharge of low-risk patients may reduce the consumable running cost substantially; and (5) In certain cases where further risk assessment e.g. exercise tolerance test or stress echocardiography is required, these patients may be subjected to this assessment early before discharge or planned as an outpatients tests.

6.4.2 LIMITATIONS OF THE STUDY

(1) The cost effective analysis was only a preliminary study and was based mainly on the cost reduction expected from early identification using biochemical markers and subsequent management based on these results in patients with uncomplicated ACS. Such as early discharge and avoiding invasive investigations and newer antiplatelet treatment in low-risk patients; hence a reduction of overall cost and in-hospital stay. This analysis was based primarily on the cost of occupying a hospital bed per day and includes the cost of essential basic investigations and treatment. However, the cost of specialised investigations [such as coronary angiography, thallium scan, and computerised tomography scam] and specialised treatment [such as newer antiplatelet and PCI] had been excluded from the analysis. It is therefore possible that the savings made by doing an additional test may be offset by the increased cost of identifying patients who need earlier treatment with more sophisticated and costly intervention. The results of this preliminary study need to be verified with more formal cost effective analysis.

(2) Although the sample size in the chest pain group has a high level of statistical power (95%) to detect an early diagnostic benefit for H-FABP, the number of patients in each subgroup (non-Q wave MI, UA, and atypical/anginal chest pain) was small, to allow definitive recommendations to be made regarding the application of these cut-off concentrations to clinical practice. (3) These cut-off concentrations are also assay dependent, and in the absence of general standardisation of cardiac markers assays, different cut-off concentrations that differentiate patients with UA from patients with myocardial infarction will needs to be worked out for that

particular assay used by each institution. This should be based on ROC curve analysis in a larger number of patients in these three subgroups.

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CHAPTER 7

THE ROLE OF HEART FATTY ACID BINDING PROTEIN IN THE EARLY DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION AND THE POTENTIAL INFLUENCE ON PATIENTS' MANAGEMENT

7.1.1 ACUTE CORONARY SYNDROMES

The new classification by the ESC/ACC identifies five main conditions that can be classified as ACS.[1] These five conditions are: (1) ST elevation Q wave MI; (2) ST elevation Non-Q wave MI. These two conditions are referred to as ST elevation ACS; (3) Non-ST elevation Q wave MI; (4) Non-ST elevation Non-Q wave AMI. These two conditions are referred to as Non-ST elevation MI and are associated with cardiac enzymes or proteins elevations diagnostic of AMI in the absence of the classical ST segment elevation on the ECG. Most of the patients with NSTEMI have prolonged ST segment depression and/or T wave inversion and a bout 20% of these patients develop Q waves on subsequent ECGs;[2] and (5) The last condition that is classified as ACS is unstable angina. Patients with UA do not have classical ST segment elevation, new Q waves on the admission ECG, or cardiac enzymes or proteins elevation diagnostic of AMI. The last three conditions (3, 4, and 5) are referred to as non-ST elevation ACS (Figure 1 page 16). Non-ST elevation ACS (Unstable angina and NSTEMI) have been discussed in Chapter 6 and in this chapter ST elevation ACS will be discussed.

From a first impression one might think that all these five conditions are separate clinical entities and each one has its own presenting clinical features, ECG diagnostic criteria, and specific treatments. The clinical reality is that all these conditions are seen in a continuous spectrum with STEMI at one end and UA at the other end, and NSTEMI in between without a clear-cut distinctive boundaries between them. The only arbitrary distinction between these conditions exists in research protocols and not in clinical practice. The only valid distinguishing criterion used to differentiate

these conditions in clinical practice is the presence of significant ST segment elevation in the ECG. The importance of ST segment elevation is to help decide whether or not to administer thrombolytic therapy. The rest of the patients without significant ST segment elevations are called non-ST elevation ACS and are usually treated in a similar manner with pharmacologic agents including antiplatelets, antithrombotic, and anti-ischaemic medications. The best treatment strategy for this group is still controversial, not least because of the difficulty to differentiate the various conditions that make up non-ST elevation ACS early after presentation.

In an attempt to provide some form of valid clinical distinction between conditions referred to as non-ST elevation ACS, the ESC/ACC has established cardiac markers concentrations above the 99th percentile of control group as the main distinguishing factor between UA and the rest of conditions classified as non-ST elevation ACS. According to this definition, myocardial infarction should be diagnosed in any patient with cTnT or cTnI > the 99th percentile of control value on at least one occasion or a maximum value of CK-MB mass exceeding the 99th percentile control group on two successive samples or exceeding twice the upper limit of normal on one occasion.[1] Further classifications of these conditions by the presence or absence of Q waves is probably academic since classifying patients into Q wave and non-Q wave MI has no additional prognostic or therapeutic implications.[3]

7.1.2 PATHOPHYSIOLOGY OF ACUTE MYOCARDIAL INFARCTION

In more than 90% of affected patients atherosclerosis is the major underlying disease process resulting from combinations of inflammation, smooth muscle cell

proliferation, and fibrosis in response to vascular injury.[4-8] The 'response to injury hypothesis' implicates endothelial dysfunction or injury as the fundamental step in the development of atherosclerosis.[9;13] Many risk factors acting singly or in unison (hyperlipidaemia, smoking, hypertension, and diabetes), along with local factors such as (shear stress, endothelin, and abnormal vasomotion), contribute to the development and progression of endothelial atherosclerotic lesions.[7;10] The altered endothelial barrier allows circulating monocytes and plasma lipids into the intima of the vessel.[6;11;12]

Simultaneous aggregation and adherence of platelets to the endothelial cell causes the release of platelet-derived growth factor and other mitogenic factors.[6;13] These in turn stimulate the migration and proliferation of vascular smooth muscle cells and production of connective tissue matrix (e.g. collagen, elastin, protein, and proteoglycan) and angiogenesis.[14] Accumulated monocytes mature to macrophages and ingest lipid products through scavenger receptors and become large cells loaded with lipid products known as 'foam cells'.[6;15] The ensuing inflammatory processes involving cells like monocytes, macrophages, and lymphocytes, that result from low density lipoprotein oxidation, cell necrosis, and the release of cytokines and inflammatory mediators. In addition, episodes of plaque rupture and thrombosis occur followed by healing and volume expansion, and the continuous production and breakdown of the tissue matrix. All these processes lead to the formation of the typical atherosclerotic plaque over several years or decades.[8]

Many cases of AMI and UA result from lesion fissure, rupture or erosion with subsequent thrombosis and occlusion of the lumen.[6;16;17] The process of plaque rupture represent imbalance between matrix production and breakdown. Plaques that rupture are of the advanced or raised fibro-lipid type. The core of the plaque consists of extracellular lipid, inflammatory cells, foam cells, necrotic materials, and often deposits of calcification separated from the lumen by thin fibrous tissue cap.[6;18;19] The plaque characteristically ruptures at its margins the so-called 'shoulder area'.[25] This area is rich in macrophages.[20] These macrophages are capable of releasing inflammatory mediators and enzymes like matrix metalloproteinases (gelatinases, collagenases, and stromelysin), which are capable of destroying virtually all the structural components of the vascular matrix.[21;22;23;24] The other factors that may also contribute to plaque progression and destabilisation are infectious agents, and mechanical stress on the weak plaque.[41;25]

Cardiac markers are important tools for the diagnosis of AMI. This topic had been covered extensively in Chapter 2. So far this role had been mainly in the retrospective confirmation of AMI within 12 - 24 hours after admission. The utilisations of cardiac markers for the early diagnosis and treatment of AMI (within 6 - 12 hours) had not been possible before due to the lack of sensitive and specific early markers of myocardial damage. Myoglobin is an early marker of myocardial damage. However, the utilisation of myoglobin for the early diagnosis of AMI has been hampered by poor specificity for myocardial tissues.[26;27] Heart-FABP is a novel marker that is released very early following myocardial tissue damage and had been postulated as a marker with potential for the early diagnosis of AMI within 6 hours

after symptoms onset.[28] Its sensitivity for the diagnosis of AMI had been equated with that of myoglobin. However, the specificity of H-FABP for myocardial tissue is significantly better than myoglobin because its concentration in serum of healthy populations is about fifteen-fold lower than myoglobin.[19] This early release feature coupled with relative cardiac tissue specificity are considered superior advantages of H-FABP that can be utilised for the early diagnosis of AMI. The ratio of myoglobin and H-FABP has also been claimed to have superior sensitivity and specificity for the diagnosis of myocardial injury compared to the measurement of either of these markers alone.[36] So far there had been few studies comparing the value of these two markers for the early diagnosis of AMI. Also there had been no studies comparing these two markers in a standardised setting with other cardiac markers such as CK-MB mass, cTnI, and cTnT.

7.1.3 AIMS OF THE STUDY:

1. Compare and contrast the sensitivity, specificity, positive predictive value, and negative predictive value of the new cardiac marker H-FABP for the early diagnosis of AMI within 6 hours after onset of symptoms in patients who present with AMI, with standard cardiac markers (CK-MB mass, cTnI, cTnT, and myoglobin) and whether H-FABP has an advantage release characteristics.

2. To combine myoglobin and H-FABP in a ratio and to study the usefulness of this ratio for the early differentiation between cardiac and non-cardiac injury in patients who present with chest pain and the effect this ratio has on sensitivity and specificity compared to the concentration of either marker alone.

3. Study the clinical benefits from the implementations of serial cardiac markers testing in the early management of patients with AMI. Several examples from this group will be used to illustrate this.

7.2 PATIENTS AND METHODS

7.2.1 PATIENTS AND TREATMENT

The recruitment of patients with AMI, the inclusion and exclusion criteria, and the diagnostic criteria for AMI are described in details in Chapter 4 materials and methods pages 112 - 114. The methods used for analysis of cardiac markers and the diagnostic cut-off concentrations for AMI are described under the methods section in Chapter 4 pages 106 – 109, and Chapter 6 pages 189 - 190. Patients who had ST segment elevations suggestive of AMI on the admission ECG and chest pain were given intravenous thrombolytic therapy (if they have no contraindications) and fast-tracked to CCU. The aim was a door to needle fast-track time less than 20 minutes.

Patients received intravenous streptokinase [1.5 million units for 60 minutes] or tPA [a bolus of 15 mg, then 50mg (0.75 mg kg^{-1}) during the first 30 minutes and 15 mg (0.5 mg kg^{-1}) over 60 minutes]. The latter regime used concomitant intravenous heparin [a bolus of 5000 units followed by intravenous infusion 1000 units per hour] adjusted to achieve activated partial thromboplastin time of 60 - 85 seconds. The allocations of patients to either of these thrombolytic protocols were based on standard local selection criteria such as age, type and severity of myocardial infarction, and previous use of thrombolytic therapy. Patients received a conventional treatment before CCU admission with sublingual administration of

glyceryl trinitrate (GTN) ± intravenous diamorphine (2.5 - 5 mg) to relieve pain, aspirin (300 mg initially to chow and swallow) and oxygen inhalation if necessary. Additionally, all patients received routine coronary care and were treated with aspirin (75 mg daily), nitrates, β-blockers, angiotensin converting enzyme inhibitors and anti-arrhythmic drugs as required.

7.2.2 STATISTICAL ANALYSIS

Variables were expressed as mean ± SD. The sensitivity, specificity, PPV, and NPV were measured for each marker at each time point after presentation and compared. The changes in serum concentrations of cardiac markers cTnI, cTnT, CK-MB mass, myoglobin, and H-FABP at 0, 2, 4, 8 - 10, and 16 - 24 hours were analysed with one way analysis of variance for repeated measures (Greenhouse-Geisser correction) with time as dependent factor. Post-hoc comparisons between times were performed with the Bonferroni's test for multiple comparisons. Significant values were defined as P value ≤ 0.05. The best cut-off concentrations between patients with definite AMI and those without AMI (UA, atypical/anginal chest pain, and normal controls) were based on ROC analysis (Figure 2, A and B). The study used the following concentrations to define the presence of AMI [CK-MB mass $\geq$ 8 μg/l, cTnI $\geq$ 0.6 μg/l. cTnT $\geq$ 0.4 μg/l, H-FABP $\geq$ 16 μg/l, and myoglobin $\geq$ 107.5 μg/l].

7.3 RESULTS

The study group consisted of 45 patients (16 females and 29 males) who were admitted with AMI within 6 hours after symptom onset. The mean age of the group was 66.58 ± 11.74 years [range 38 - 82 years]. The demographic data of all patients

in this group are shown in Table 1. The time from onset of symptoms to presentation was 3.57 ± 2.33 hours (median 3 hours [range 1 - 6 hours]). Thirteen per cent of patients in this group had previous history of angina and MI. Of interest was the high number of patients who had no prior clinical history of ischaemic heart disease. Thirty-three per cent (15 patients) had their first presentation with AMI without any previous documentations of ischaemic heart disease. The commonest risk factor in this group was hypercholestrolaemia (69%). The second most common risk factor was smoking with 42% of patients are either current or ex-smokers. The prevalence of other risk factors such as diabetes mellitus, hypertension, and family history of ischaemic heart disease in this high-risk group was moderate (18 - 24%).

Table 2 shows the clinical details concerning the type of chest pain at admission, types and leads distribution of ECG changes, and methods of reperfusion therapy given at admission. Thirty-four patients (76%) presented with typical history of ischaemic chest pain. In 11 patients (24%), the description of chest pain was not typical of ischaemic heart disease. The admission ECG was immediately diagnostic of AMI [significant ST segment elevations $\geq$ 2 mm in more than two leads] in 37 patients (82%). Eighteen per cent (18%) had no thrombolytic eligible ECG changes at admission. The implementation of reperfusion therapy and the subsequent diagnosis of AMI in these patients were based on the evolution of new Q waves, the development of further ST segment elevations on subsequent ECG's or the presence of routine cardiac enzymes or proteins elevation consistent with the diagnosis of AMI.

Demographic data of patients with AMI

Demographic data	Number(s)
Total number of patients	45 patients
Age (years)	66.58 ± 11.74
Sex: Female Male	 16 (36) 29 (64)
Time to presentation (hours)	3.57 ± 2.33
Previous cardiac history: Previous angina Previous AMI Previous PCI	 6 (13) 6 (13) 1 (2)
First presentation with IHD	15 (33.3)
Prevalence of risk factors: Diabetes mellitus Hypertension Smokers (ex-smokers = 8.7%) Hypercholestrolaemia Positive family history of IHD	 8 (18) 11 (24) 15 (33.3) 31 (69) 10 (22.2)

Table 1. Shows the demographic data of patients with AMI. Continuous variables are presented as mean ± SD and categorical variables are presented as percentages. Abbreviations: PCI, percutaneous coronary intervention; SD, standard deviation; AMI, acute myocardial infarction; IHD, ischaemic heart disease.

The commonest ECG changes were ST segment elevation. Eighty-seven per cent of patients who were admitted with chest pain evolved ST segment elevation at or during admission. Six patients (13%) had other forms of ischaemia on the admission ECG such as ST segment depression ± T wave inversion. In the latter group, new Q waves were either present or cardiac markers were abnormally elevated. Overall 29% evolved new Q waves during admission 'Q wave MI' and in 71% there were no new Q waves seen on the ECG 'Non-Q wave MI'.

Clinical data of patients with AMI

Clinical data	Number(s)
Haemodynamic parameters at admission	
Heart rate (BPM)	72.97 ± 17.14
Systolic blood pressure	136.4 ± 25.93
Diastolic blood pressure	71.83 ± 16.86
Clinical history at presentation:	
Typical history of ischaemic chest pain	34 (76)
Atypical history or equivocal history	11 (24)
First ECG diagnostic of AMI	37 (82)
First ECG not diagnostic of AMI	8 (18)
ECG changes of ischaemia:	
ST elevation	39 (87)
ST depression (± T wave inversion)	6 (13)
Q waves MI	13 (29)
Non-Q waves MI	32 (71)
Leads distributions:	
Anterior MI	17 (38)
Inferior MI	23 (51)
Anterior lateral MI	1 (2)
Inferior lateral MI	3 (7)
Posterior MI	1 (2)
Routine cardiac marker concentration:	
CK (IU/L)	1310.4 ± 1008.9
Type of thrombolytic therapy:	
SK	32 (71)
TPA	11 (24.5)
Thrombolytic therapy + Rescue PCI	2 (4.5)

Table 2. Shows the clinical data of patients admitted with AMI. Continuous variables are presented as mean ± SD and categorical variables are presented as percentages. Abbreviations; BPM, beats per minute; ECG, electrocardiogram; ST, Q are ST segment and Q wave of the ECG; CK, creatine kinase; SK, streptokinase; tPA, tissue plasminogen activator; PCI, percutaneous coronary intervention; AMI, acute myocardial infarction.

The distributions of ECG changes are also shown in Table 2. Seventeen patients (38%) had anterior MI and 23 patients (51%) had inferior MI. In a very small number of patients the ECG changes were in the anterior lateral, inferior lateral, and posterior leads 2%, 7%, and 2% respectively. The mean concentration of the enzyme CK in routine samples was significantly elevated in these patients 1310.3 ± 1008.9 IU/L. Most patients received standard treatment with streptokinase infusion (71%)

and most of the remaining patients received tPA. Two patients had persistent chest pain and failure of ST segment resolution after thrombolytic therapy and they were treated by rescue angioplasty. Emergency coronary angiography followed by angioplasty and stenting was undertaken in three more patients who developed episodes of recurrent chest pain several days after admission.

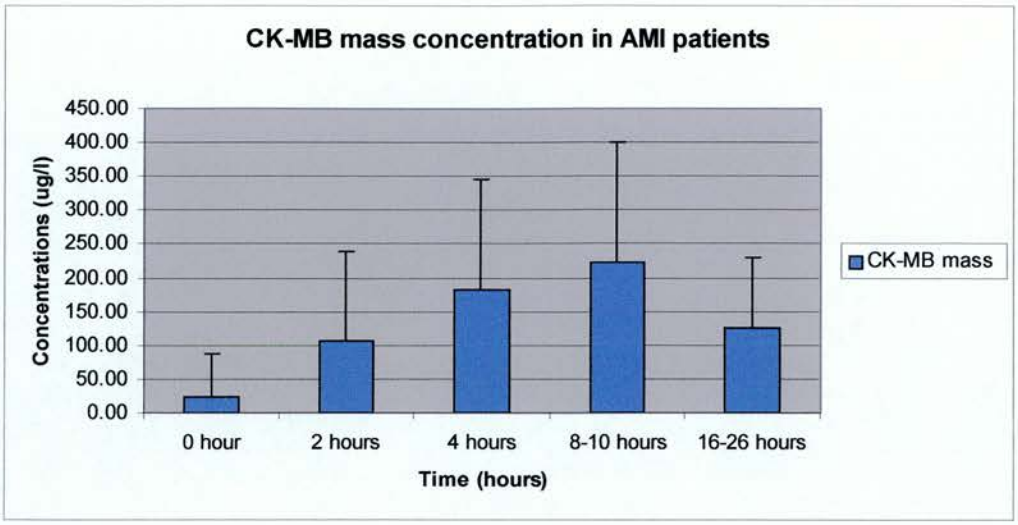
The serial concentrations of cardiac markers (CK-MB mass, cTnI, cTnT, myoglobin, and H-FABP) are shown in Table 3. There was a significant difference between cardiac markers mean concentrations at each time point of sampling after admission and the proceeding concentrations. The exception to this was cTnT concentration between 8 - 10 and 16 - 24 hours; H-FABP and myoglobin mean concentrations between 2 and 4 hours, where the mean concentration difference between these time periods was not significant. The average concentrations of myoglobin, H-FABP, CK-MB mass, and cTnI were above the diagnostic cut-off concentrations in all samples taken after admission.

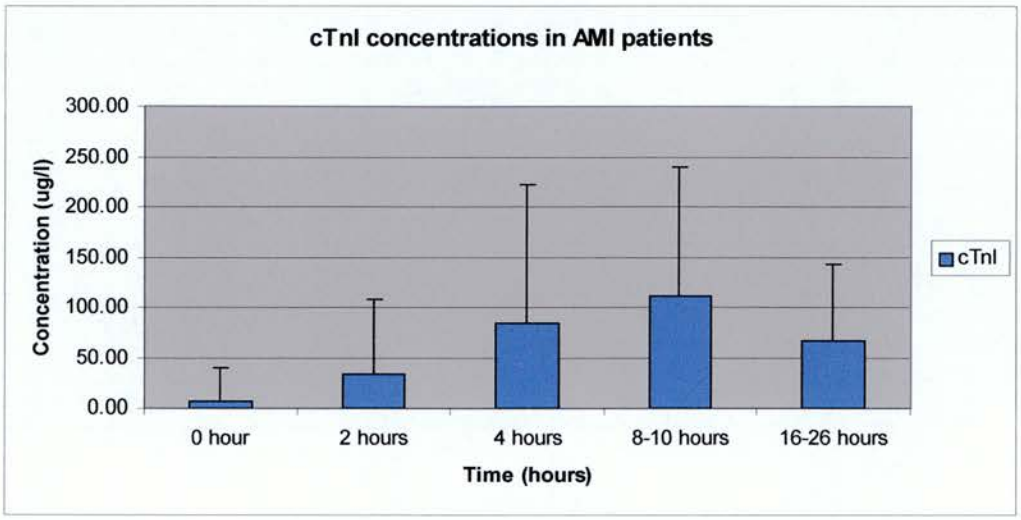
Figure 1 shows graphic representations of cardiac markers concentrations in Table 3. Myoglobin and H-FABP were the earliest makers of myocardial injury. The peak concentrations of myoglobin and H-FABP were reached in samples taken 2 hours after presentation (8 hours after symptoms onset). The peak concentrations of CK-MB mass and cTnI occurred at 8 - 10 hours after presentation (14 - 16 hours after the onset of chest pain). The peak concentrations of cTnT could not be estimated precisely because cTnT maximum concentration was present in the last sample taken at 16 - 24 hours after presentation (24 - 30 hours after onset of symptoms). The changes in cTnT concentration beyond this interval were not known because no

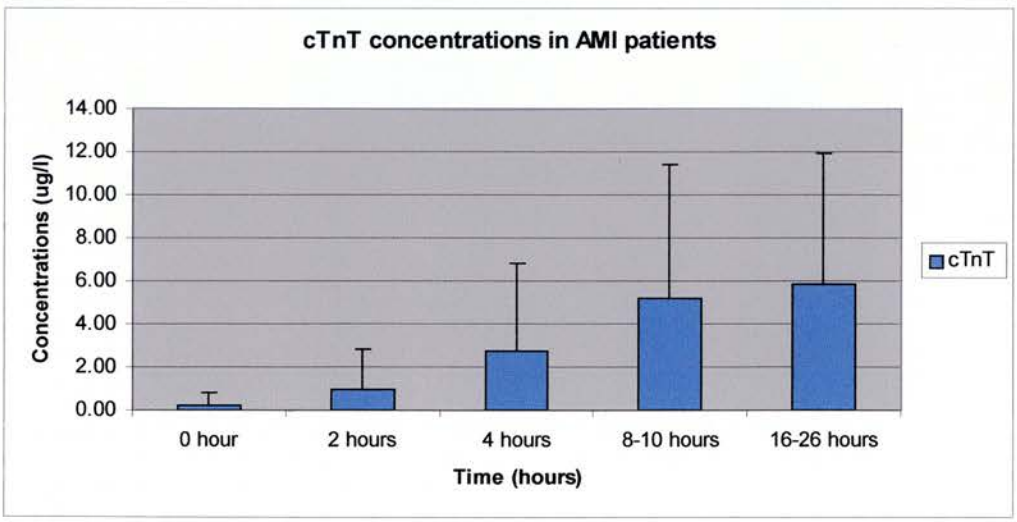
further blood sampling was undertaken. Heart-FABP and myoglobin concentrations had decreased significantly at 16 - 24 hours, whereas CK-MB mass, cTnI, and cTnT were still present in high concentration. These two features, early concentration peak following myocardial injury and rapid return to normal base line concentration of H-FABP and myoglobin demonstrate the superb qualities of these two proteins as potential markers for early detection of myocardial injury and that they are also the most suited markers for the detection of complications e.g. re-infarction in the immediate post-MI period.

Marker(s)	0 hr	2 hrs	4 hrs	8-10 hrs	16-24 hrs	P value
CK-MB	24.38	106.46	182.75	223.19	126.32	0.0005
SD	62.79	133.51	163.31	176.51	103.62	
CTnI	6.53	33.48	83.44	111.11	66.33	0.038
SD	32.53	74.12	138.08	128.48	75.95	
CTnT	0.19	0.94	2.72	5.16	5.84	0.004
SD	0.64	1.90	4.12	6.27	6.12	
Myoglobin	331.82	1897.69	1379.62	597.07	253.36	0.0005
SD	411.57	2504.51	1463.81	713.16	300.78	
H-FABP	56.89	251.23	235.64	103.98	45.68	0.0005
SD	72.05	218.8	186.23	95.86	51.06	

Table 3. Shows the serial concentrations (mean ± SD) of cardiac markers CK-MB mass, cTnI, cTnT, myoglobin, and H-FABP in patients with AMI at 0, 2, 4, 8 - 10, and 16 - 24 hours after presentation. The p value refers to the differences between the mean concentration at each time and the proceeding mean concentration.







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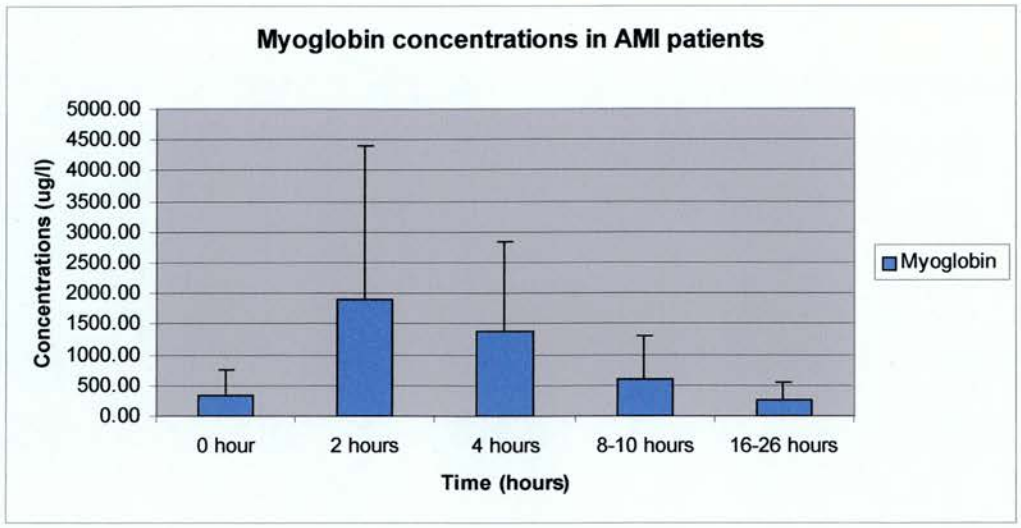

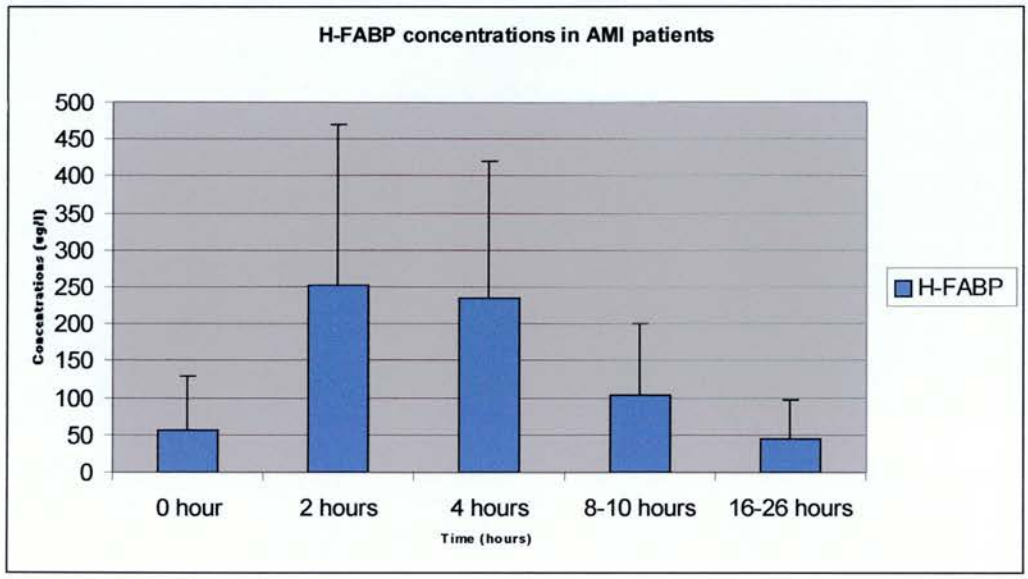

Figure 1. These graphs illustrate the release pattern (time-concentration profile) of the different cardiac markers (CK-MB mass, cTnI, cTnT, myoglobin, and H-FABP) at each time after presentation in patients with AMI.

The sensitivity of cardiac markers CK-MB mass, cTnI, cTnT, myoglobin, and H-FABP for the diagnosis of AMI in patients who presented within 6 hours after symptom onset is shown in Table 4. Heart-FABP was the most significant early marker with 60% of patients with AMI correctly diagnosed at presentation. The sensitivity had risen to 100% two hours after presentation and remained elevated for

the next 2 - 8 hours after presentation (93 - 100%). The diagnostic window of H-FABP was relatively prolonged up to 8 hours after presentation (14 hours after symptom onset). The optimum sensitivity for the diagnosis of AMI was present between 2 - 8 hours after presentation. Myoglobin was the second most sensitive early marker shortly after presentation. At 2 hours after presentation, 93% of patients with chest pain suggestive of AMI can be correctly identified using myoglobin concentrations.

The optimum diagnostic sensitivity of myoglobin was present between 2 - 4 hours after presentation. The sensitivity of myoglobin had decreased significantly at 16 - 24 hours after presentation. Cardiac-TnI was the third best sensitive early marker of AMI. Ninety-three per cent of patients had positive concentrations at 2 hours. The sensitivity had risen to 100% in subsequent samples taken at 4, 8 - 10, and 16 - 24 hours.

The sensitivity of CK-MB mass was low at presentation (31%) and significantly increased 2 hours after presentation (88.8%). Between 4 - 16 hours after presentation most patients had positive CK-MB mass concentrations that were diagnostic of AMI (97.7 - 100%). The sensitivity of cTnT was very low at and shortly after presentation (0 hour, 2 hours, and 4 hours) and it increased to a reasonable level (91%) only at 8 - 10 hours after presentation (Table 4), making cTnT a late marker that is unsuitable for the early diagnosis of AMI within the first 12 hours after symptoms onset.

The effects of altering the diagnostic cut-off concentrations on cardiac markers sensitivity for the early diagnosis of AMI are shown in Table 5. These selected diagnostic cut-off concentrations were based on ROC curve analysis (Figure 2, C and D) between this group of patients with definite AMI and in patients in whom myocardial injury had been excluded (normal controls, UA patients excluded).

These cut-off concentrations were CK-MB mass $\geq$ 5 μg/l [AUC = 0.950, SE = 0.01, P < 0.0005, 95% CI = 0.931 – 0.970, sensitivity = 86.3%, specificity = 99%], cTnI $\geq$ 0.18 μg/l [AUC = 0.993, SE = 0.003, p < 0.0005, 95% CI = 0.988 – 0.999, sensitivity = 90.8%, specificity = 99%], cTnT $\geq$ 0.1 μg/l [AUC = 0.931, SE = 0.012, p < 0.0005, 95% CI = 0.907 – 0.955, sensitivity = 76.4%, specificity = 99%], H-FABP $\geq$ 12.5 μg/l [AUC = 0.932, SE = 0.019, p < 0.0005, 95% CI = 0.895 – 0.968, sensitivity = 91.4%, specificity = 86%], and myoglobin $\geq$ 95 μg/l [AUC = 0.945, SE = 0.012, p < 0.0005, 95% CI = 0.921 – 0.969, sensitivity = 81.2%, specificity = 99%]. Figure 2 shows the ROC curves that were used for the selection of these cut-off concentrations.

There were different effects of these modifications on cardiac markers sensitivity. In general, there was a noticeable improvement in the sensitivity of all markers (with the exception of cTnT) for the diagnosis of AMI at presentation. The sensitivity at presentation had increased to 51%, 60%, and 62% for cTnI, CK-MB mass, and myoglobin respectively. The overall sensitivity of these three markers remained relatively unchanged. There was a significant improvement in the overall sensitivity of cTnT at 8 - 10 hours [100% vs 91%] (Table 5).

Marker(s)	0 hr	2 hrs	4 hrs	8-10 hrs	16-24 hrs
CK-MB mass	31	88.8	97.7	100	100
CTnI	26	93	100	100	100
CTnT	11	46.6	68.8	91	95.5
Myoglobin	57.7	93	93	88.8	66.6
H-FABP	60	100	100	93	77.7

Table 4. Shows the percentage sensitivity of cardiac markers CK-MB mass, cTnI, cTnT, H-FABP, and myoglobin for the early diagnosis of AMI at 0, 2, 4, 8 - 10, and 16 - 24 hours after presentation in patients with AMI within 6 hours after symptom onset. The cut-off concentrations were CK-MB mass ≥ 8 μg/l, myoglobin ≥ 107.5 μg/l, cTnI ≥ 0.6 μg/l, cTnT ≥ 0.4 μg/l, and H-FABP ≥ 16 μg/l.

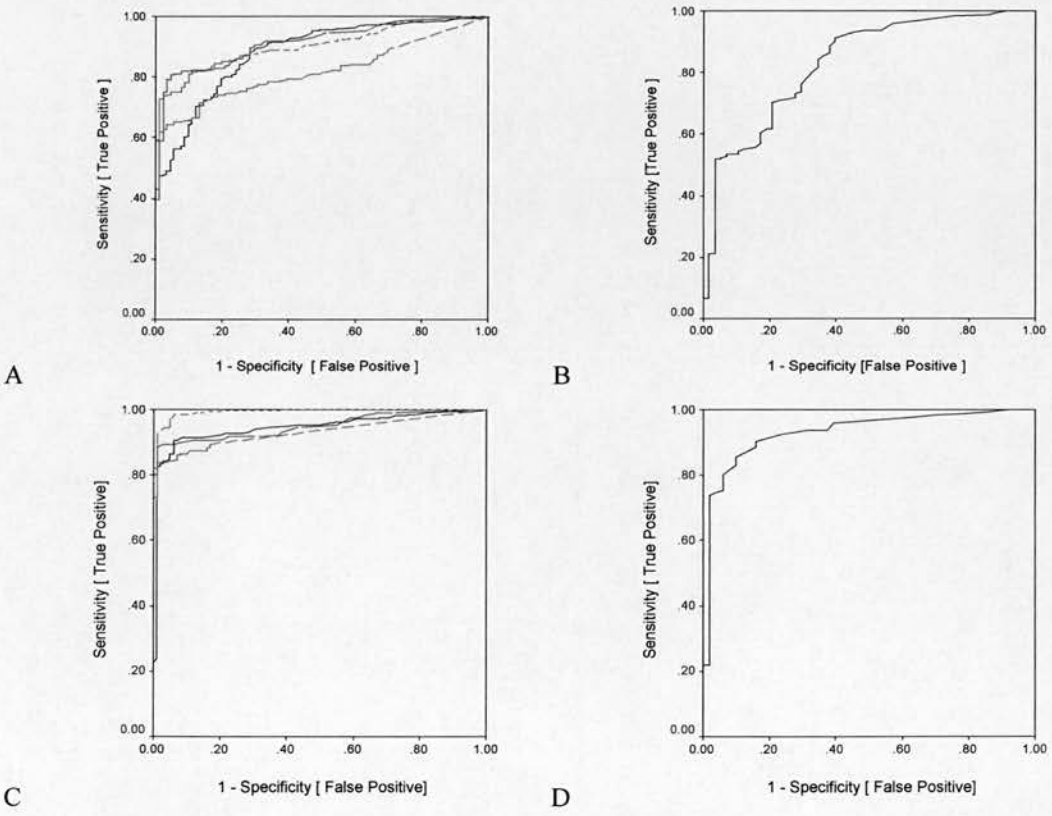

Figure 2. Shows four receiver operator characteristic curves that were used to derive the cut-off concentrations of the various cardiac markers in this study. A and C represent the improvement in cut-off concentration (sensitivity and specificity) with respect to the two different cut-off concentrations used in Table 4 and 5 respectively. B and D show equivalent cut-off concentrations of H-FABP. Blue, CK-MB mass concentrations; green, cTnI concentrations; red, cTnT concentrations; black, myoglobin concentrations. See text for explanation.

Figure 3 shows simple line chart representation of the sensitivity values in Table 5. Heart-FABP was the most sensitive marker at presentations (75.5%), and it remained elevated at 100% for the next 2 - 8 hours. Heart-FABP sensitivity was also superior to other markers within the first two hours of presentation. Myoglobin and CK-MB mass sensitivity were similar in the first 2 hours (93%). However, the peak sensitivity of myoglobin (93%) and CK-MB mass (100%) were reached at 2 hours, and 8 - 10 hours respectively. Cardiac-TnI reached higher sensitivity (97.7%) earlier (2 hours) than myoglobin, CK-MB mass, and cTnT. The sensitivity of cTnT gradually increased from a low level at presentation (24%) to the highest level (100%) at 8 - 10 hours after presentation. There was no evidence that cTnT had reached peak concentrations during the sampling interval (Figure 3).

Marker(s)	0 hr	2 hrs	4 hrs	8-10 hrs	16-24 hrs
CK-MB mass	60	93	97.7	100	100
CTnI	51	97.7	100	100	100
CTnT	24	68.8	86.6	100	100
Myoglobin	62	93	93	91	71
H-FABP	75.5	100	100	100	84.4

Table 5. Shows the percentage sensitivity of cardiac markers CK-MB mass, cTnI, cTnT, and myoglobin for the early diagnosis of AMI at 0, 2, 4, 8 - 10, and 16 - 24 hours after presentation in patients with AMI within 6 hours after symptom onset. The cut-off concentrations were CK-MB mass ≥ 5 μg/l, myoglobin ≥ 95 μg/l, cTnI ≥ 0.18 μg/l, cTnT ≥ 0.1 μg/l, and H-FABP ≥ 12.5 μg/l.

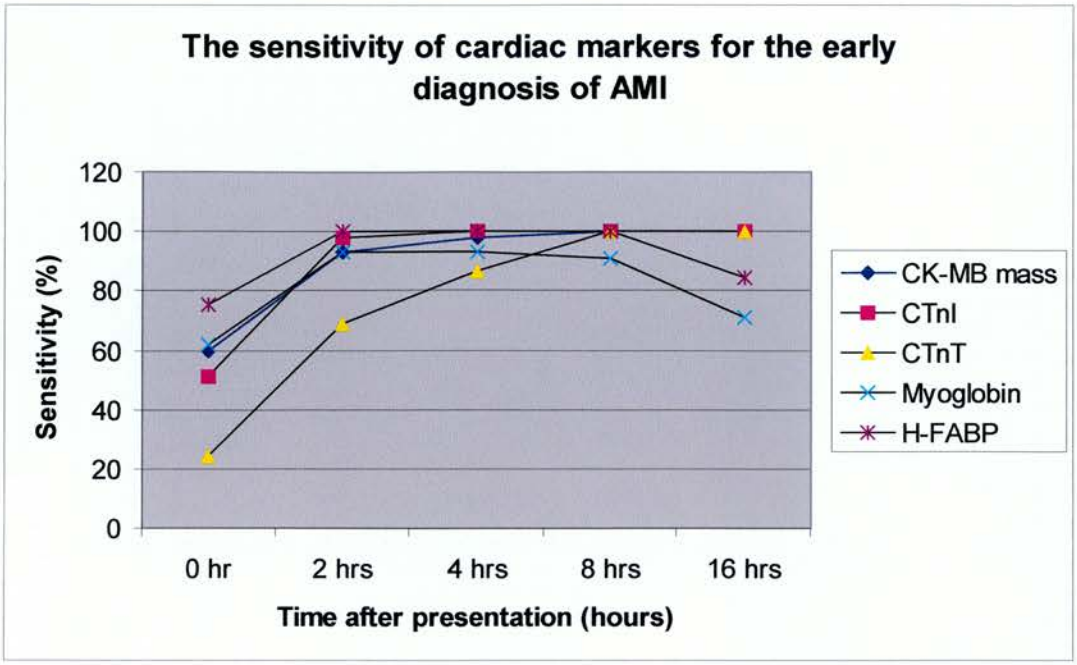

Figure 3. Shows a line chart representation of the sensitivity values in Table 5. Each mark represents the percentage sensitivity of the cardiac marker at that time point.

In a separate subgroup analysis a series of possible diagnostic cut-off concentrations for H-FABP were evaluated to decide the best discriminate range of values. Table 6 shows the sensitivities of H-FABP for the early diagnosis of AMI based on a series of increasing cut-off concentrations in the range 9 to 21.5 μg/l. There was a significant but gradual reduction in the early sensitivity of H-FABP at presentation (0 hour) as a result of increasing the diagnostic cut-off concentrations from 9 μg/l to 21.5 μg/l. There was little effect from increasing the diagnostic threshold to values ≤ 18 μg/l on the sensitivity between 2 and 4 hours with 97.7 - 100% of patients correctly diagnosed (i.e. within 8 - 10 hours after symptoms onset). The sensitivity (early and late) had decreased significantly with concentration ≥ 21.5 μg/l. Cut-off concentrations between 9 μg/l and 12.5 μg/l offered the best discriminate values,

which included both, better early sensitivity at presentation combined with very high sensitivity between 2 - 8 hours after presentation (Table 6).

— Increasing levels of diagnostic cut-off concentrations→

Time (hrs)	≥ 9 µg/l	≥10 µg/l	≥12.5 µg/l	≥14 µg/l	≥16 µg/l	≥18 µg/l	≥21.5 µg/l
0	81.8	79.5	75.5	68.2	59.1	56.9	42
2	100	100	100	100	100	100	93.3
4	100	100	100	100	100	97.7	95.5
8-10	97.7	97.7	100	97.7	93.2	90.9	88.8
16-24	88.6	88.6	84.4	81.8	77.3	72.7	57.7

Table 6. Shows the various sensitivities of H-FABP at 0, 2, 4, 8 - 10, and 16 - 24 hours after presentation in patients admitted with AMI. The analysis was based on a series of increasing cut-off concentrations ranging from 9 µg/l to 21.5 µg/l.

The overall sensitivity, specificity, PPV, and NPV of CK-MB mass, cTnI, cTnT, myoglobin, and H-FABP for the diagnosis of AMI was analysed in 100 patients who were admitted to hospital with chest pain suggestive of ischaemia within 6 - 7 hours after symptom onset. The total numbers of patients who had confirmed myocardial infarction were 59 patients. Fourty-one patients had no MI (20 patients had UA and 21 patients had no myocardial damage). The cut-off concentrations used are shown in Table 5 page 254. The study had chosen an overall decision threshold for sensitivity, specificity, PPV, and NPV to be equal or greater than 90% to indicate the required reliability of cardiac markers for the early rule in or rule out of patients with AMI. Table 7 shows the percentages of these values for the different cardiac markers at each time after presentation.

Marker(s)	Feature(s)	0 hr	2 hrs	4 hrs	8-10 hrs	16-24 hrs
CK-MB	Sensitivity	38.9	86.4	96.6	98.3	96.6
	Specificity	92.7	90.2	87.8	90.2	90.2
	PPV	88.5	92.7	91.9	93.5	93.4
	NPV	51.4	82.2	94.7	97.4	94.8
CTnI	Sensitivity	35.5	91.5	96.6	96.6	96.6
	Specificity	95.1	92.7	87.8	90.2	92.7
	PPV	93.1	94.7	91.9	93.4	95
	NPV	50.6	88.4	94.7	94.8	95
CTnT	Sensitivity	20.3	52.5	69.5	89.8	94.9
	Specificity	100	100	97.6	95.1	95.1
	PPV	100	100	97.6	96.4	96.6
	NPV	46.6	59.4	83.3	86.7	92.8
H-FABP	Sensitivity	65.5	98.3	96.6	89.6	75.8
	Specificity	81.8	72.7	77.3	77.3	86.4
	PPV	90.4	90.4	91.8	91.2	93.6
	NPV	47.4	94	89.5	74	57.5
Myoglobin	Sensitivity	61	93.2	91.5	86.4	67.8
	Specificity	90.2	90.2	90.2	90.2	90.2
	PPV	90	93.2	93.1	92.7	90.9
	NPV	61.6	90.2	88.1	82.2	66.1

Table 7. This table shows the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of cardiac markers CK-MB mass, cTnI, cTnT, H-FABP, and myoglobin for the early diagnosis of AMI within 6 - 7 hours after symptoms onset at each time (0, 2, 4, 8 - 10, and 16 - 24 hrs) after presentation.

Heart-FABP had the highest sensitivity at presentation (65.5%). Almost all patients (98.3%) with MI were diagnosed within 2 hours after admission (8 hours after onset of symptoms). The specificity of H-FABP at 2 hours was only 72.7%. This is largely because of the inclusion of patients with UA in the AMI negative group, who had H-FABP concentration rise consistent with myocardial injury. These patients were inaccurately referred to as 'false positive' results. Exclusion of these patients from the analysis improves the specificity to 93.75%. As mentioned earlier in Chapter 6, H-FABP detected a substantial numbers of patients with UA (40%). However, there was some overlap between the concentrations of H-FABP in patients with UA and in those with NSTEMI and STEMI.

Myoglobin had the second highest sensitivity at 0 hour (61%) rising to 93.2% two hours after presentation. The corresponding specificity, PPV, and NPV were 90.2%, 93.2%, and 90.2% respectively. There was a similar performance by CK-MB mass and cTnI. The sensitivity and NPV of CK-MB mass and cTnI were both low at presentation. Reliable sensitivity, specificity, PPV, and NPV were observed at 4 - 10 hours after presentation. There was a relatively low NPV (i.e. high false negative rate) of CK-MB mass within the first 2 hours of presentation (82.2%). Using a threshold above 0.1 μg/l, the sensitivity of cTnT at presentation was very low (20.3%). However, the specificity was 100%. Reliable sensitivity (94.9%), specificity (95.1%), PPV (96.6%), and NPV (92.8%) were evident at 16 - 24 hours after presentation.

The value of myoglobin to H-FABP ratio for the early differentiations between cardiac injury and non-cardiac injury was assessed. It has been suggested that a myoglobin to H-FABP ratio around 5 is highly specific for myocardial injury, whereas a ratio ≥ 21 - 70 is consistent with non-cardiac injury.[36] In this group of patients with confirmed AMI, 7.7% had myoglobin to H-FABP ratio ≥ 21. In the remaining patients the ratio (mean ± SD) of myoglobin to H-FABP was 5.6 ± 4.0 [range 0.6 - 14.7].[38] The overall sensitivity of myoglobin to H-FABP ratio for the diagnosis of AMI during admission was 92.3%. The diagnostic sensitivity of this combination ratio was lower than either H-FABP or myoglobin alone (Table 7). In patients presenting with acute chest pain in whom AMI and skeletal muscle injury had been excluded, 93% had myoglobin to H-FABP ratio similar to that seen in patients with AMI [mean ± SD = 7.8 ± 4.64]. Only 7% of patients in this group had myoglobin to H-FABP > 21.

The other potential values that serial cardiac marker testing could have in the management of patients with acute myocardial infarction will be highlighted by considering several clinical examples from this group.

Timing the onset of acute myocardial infarction event

One patient who was later excluded from the study, presented 11 hours after symptom onset with anterior MI. It was decided that this presentation was late and contraindicated thrombolytic therapy. He was treated with LMWH and other adjunctive pharmacological agents. Cardiac markers concentrations in this patient at presentation were high [CK-MB mass = 389 μg/l, cTnI = 50 μg/l, cTnT = 4.23 μg/l,

H-FABP = 209 μg/l, and myoglobin = 1623 μg/l]. The concentration of CK-MB mass, cTnI, and cTnT continued to rise in the next two samples (at 2 and 4 hours), before levelling off and declining. For myoglobin and H-FABP, after a peak concentration rise at presentation, it continued to decline gradually towards normal. At 16 - 24 hours after presentation the concentrations of myoglobin and H-FABP has dropped to 212 μg/l and 6 μg/l respectively.

The early release and peak concentration features of H-FABP as well as the rapid return to normal base line concentration makes it an excellent marker to use to time the approximate onset of AMI event. Raising concentration of H-FABP as well as cTnI may suggest a recent onset of AMI within the last 12 hours. Declining concentration of H-FABP and raising concentrations of other markers suggest presentation more than 12 hours after symptom onset. Normal concentration of H-FABP suggests delayed presentations more than 24 hours. This biochemical time-concentration profile of H-FABP might help with decision-making on reperfusion therapy in those patients with chest pain and ST elevation but with a presumed late presentation or in whom the onset of symptom could not be determined precisely.

Detection of reperfusion

This study was not designed to examine in detail the value of cardiac markers for the detection of successful reperfusion after the initiation of intravenous thrombolytic therapy. This section briefly presents analytical comparison between the usefulness of cardiac markers ratios for the detection of successful reperfusion. These were based on the ratios of cardiac markers obtained by dividing the concentrations at two

hours by the basal concentration i.e. the concentration before thrombolysis. The diagnosis of reperfusion in this group was based on clinical criteria alone such as resolutions of chest pain and a significant decrease ≥ 50% (or resolution) of ST segment elevation. The diagnosis of reperfusion was not based on coronary angiography. The ratios of cardiac markers at two hours following reperfusion were CK-MB mass [range 0.95 - 257], cTnI [range 0.89 - 337], cTnT [range 0.23 - 352], H-FABP [range 0.72 - 240], and myoglobin [range 0.6 - 151].

Based on ROC curve analysis between patients who received reperfusion treatment (STEMI group) and those who were not thrombolysed (NSTEMI group), the following were found to be the best cut-off ratios for the discrimination between successful and failed reperfusion at two hours after the initiation of thrombolysis, CK-MB mass ≥ 2.6 [sensitivity = 72.7%, specificity = 75%], cTnI ≥ 3.4 [sensitivity = 63.6%, specificity = 75%], cTnT ≥ 2.3 [sensitivity = 61.4%, specificity = 91.7%], myoglobin ≥ 2.2 [sensitivity = 75.5%, specificity = 83.3%], and H-FABP ≥ 1.7 [sensitivity = 77.3%, specificity = 75%].

Detection of re-infarction

A seventy-seven years old lady with previous history of MI and angina was admitted with AMI 6 hours after symptom onset. She had ST segment elevations in the anterior and inferior leads of the admission ECG. She was treated with streptokinase with good resolution of ECG changes. Four hours after presentation (10 hours after the onset of chest pain), she had recurrent episodes of chest pain with further ST segment elevation. The diagnosis of re-infarction was entertained and she was treated

with another course of thrombolytic therapy (tissue plasminogen activator). She had no further routine CK concentration rise. The temporal profiles of cardiac markers concentrations in this patient are shown in Figure 4. Myoglobin was the most significantly elevated cardiac marker at presentation. CK-MB mass, H-FABP, and cTnI were also diagnostic of AMI at presentation. Re-infarction was reflected by a prominent second peak rise in concentrations of myoglobin. Creatine kinase-MB mass had a small second peak but it was not marked. Heart-FABP showed small increases in concentrations after re-infarction (33, 35, and 38 μg/l). For cTnI and cTnT, such peak rise was absent and their concentrations continued to rise slowly after the first infarct (Figure 4).

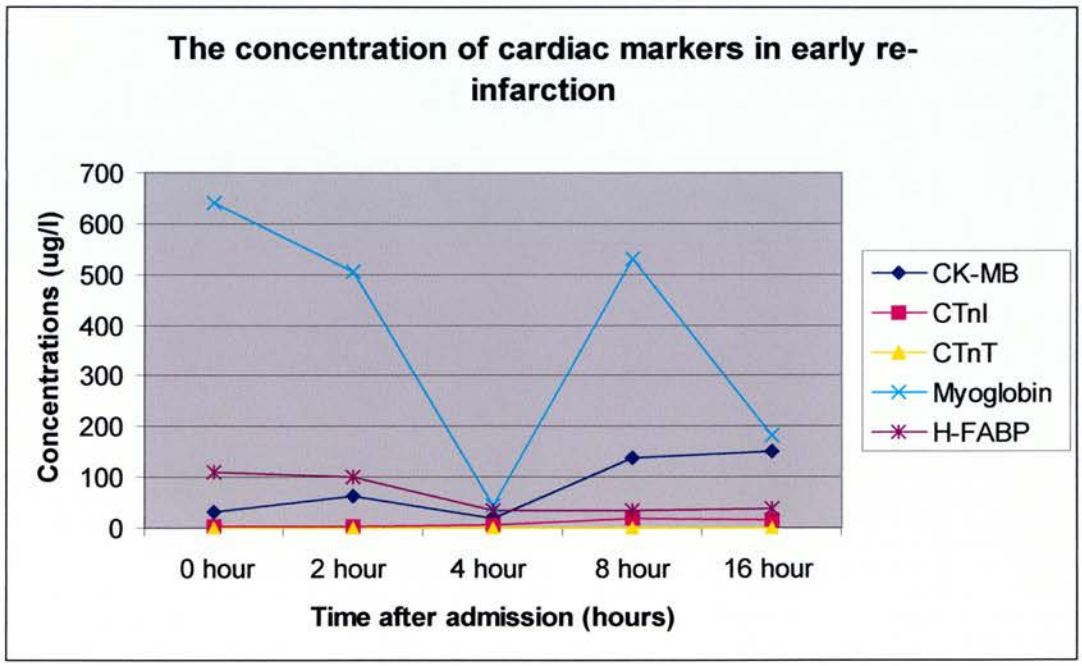

Figure 4. This graph shows the time-concentrations profile of cardiac markers CK-MB mass, cTnI, cTnT, H-FABP, and myoglobin at 0, 2, 4, 8 - 10, and 16 - 24 hours, in a patient who had re-infarction ten hours after the first infarct. There was a second peak rise in concentrations of myoglobin after re-infarction. There was a small but less marked second peak for CK-MB mass. Heart-FABP had small rises in concentrations, but there was no prominent peak during the sampling interval. Cardiac-TnI and cTnT concentrations continued to increase steadily in serum.

A marker that can detect re-infarction must be released early and cleared rapidly from the circulation i.e. has a short diagnostic window. In the event of re-infarction it will show another peak rise in concentrations. There was some discrepancy between H-FABP and myoglobin for the detection of re-infarction. Myoglobin was useful for detecting re-infarction but H-FABP did not show a second prominent peak rise in concentration in this patient who was a presumed case of re-infarction. However, after an initial decline in concentration, H-FABP was staring to show a slight upward trend in concentration in the last two samples taken after re-infarction. It might be possible that the second peak occurred late outwith the sampling interval.

Value of cardiac markers for detection of infarct extension

One patient was admitted with inferior MI and treated with streptokinase. The second day of admission she developed episodes of chest pain with further elevations of ST segment in the inferior leads. It was suspected that she was developing infarct extension and was given another course of tPA. No serial testing of cardiac markers were performed in this patient beyond 16 - 24 hours after presentation. However, it is important to consider the changes in cardiac markers concentration in the last sample taken at 16 - 24 hours and draw some assumptions on their possible usefulness for the detection of infarct extension. The concentration of CK-MB mass continued to rise from a normal concentration at presentation (2.4 μg/l) to a maximum concentration (665.4 μg/l) at 16 - 24 hours. Similarly, cTnI also rose from 0.1 μg/l to a maximum concentration of 327 μg/l at 16 - 24 hours. Cardiac-TnT rose from undetectable concentrations (0.01 μg/l) to a maximum concentration of 11.65 μg/l at

8 - 10 hours and then started to decrease reaching a concentration of 6.15 μg/l at 16 - 24 hours.

The concentration of myoglobin was normal (63 μg/l) at presentation and increased to a maximum concentration at 4 hours (1113 μg/l), and had decreased significantly (110 μg/l) at 16 - 24 hours. Heart-FABP concentration increased from 11 μg/l at admission to a maximum concentration at 4 hours (280 μg/l), and had decreased significantly (20 μg/l) at 16 - 24 hours. The diagnosis of infarct extension was made several hours after the last sample was taken. It would be expected that myoglobin and H-FABP concentrations would have decreased back to normal by this time. In the event of significant myocardial extension there may be further rise in myoglobin and H-FABP concentration. The other cardiac markers were still present in high concentrations and they probably would not display a second prominent peak rise in concentrations as a result of infarct extension.

Decisions with implementation of thrombolytic therapy

This was a 75 years old man who was admitted with chest pain and tall R wave on V2 and ST segment depression on the ECG. He also had congestive cardiac failure with raised jugular venous pressure and pulmonary crepitation. The diagnosis was assumed to be posterior myocardial infarction and he was admitted to CCU and treated with tPA. During the course of treatment it was decided to stop tPA because of insufficient ground for thrombolysis. Echocardiogram showed global and severe impairment of left ventricular function. This patient's condition later deteriorated and

despite inotropic support he developed acute renal failure and died 5 days after admission.

At presentation myoglobin and H-FABP where diagnostic of ischaemic event, the other cardiac markers concentrations in this patient were normal or slightly raised above the upper limit of normal, CK-MB mass = 7.4 μg/l, cTnI = 0.27 μg/l, cTnT = 0.01 μg/l, H-FABP = 45 μg/l, and myoglobin = 580 μg/l. The cardiac markers concentrations in this patient were diagnostic of AMI at 2 hours after presentation, CK-MB mass = 42 μg/l, cTnI = 24.47 μg/l, myoglobin = 3120 μg/l, and H-FABP = 260 μg/l. There was a significant change in cardiac markers concentrations 2 hours after presentation. This massive cardiac markers concentrations change most probably reflects underlying posterior MI. These measurements were completed before the onset of renal impairment (normal urea and creatinine). The availability of this information sooner may have affected the decision on thrombolytic therapy.

The influence of several factors like infarct size, time to presentation, and reperfusion on the rate of cardiac markers appearance in blood following the index event and the effects that these might have on the sensitivity were studied. The peak concentrations of cardiac markers were used as a qualitative measure of infarct size. This peak concentration of cardiac markers in patients with STEMI was compared with the peak concentration of cardiac markers in patients with NSTEMI (compare Figure 1 pages 249 - 250 with Figure 2 pages 196 - 197). There were two to five-fold increases in cardiac markers concentrations in patients with STEMI reflecting larger myocardial tissue damage in this group compared to patients with NSTEMI. The

sensitivity of cardiac markers for the diagnosis of AMI was also higher in this group compared to patients with NSTEMI (compare sensitivity in Table 5A page 208 with sensitivity in Table 5 page 254). This would support that infarct size contributes to the rate at which cardiac markers appear in blood and that large infracts are associated with more significant release of these markers after the index event.

The duration between onset of symptoms and presentation was also an important factor in determining the rate of appearance of these markers in blood. This was demonstrated by the time-concentration profile of cardiac markers in the several clinical examples quoted in these studies. The release of cardiac markers after AMI was also affected by the administration of thrombolytic agents. There was a significant increase in concentrations (and an improvement in sensitivity) of most cardiac markers several hours after successful thrombolysis compared with sensitivity at presentation or in the group of patients with NSTEMI who were not thrombolysed.

7.4 DISCUSSION

This study investigated the role of H-FABP in the early diagnosis of AMI in 45 patients who were admitted to CCU with AMI within 6 hours after symptom onset and before the administration of thrombolytic therapy. The diagnostic power of H-FABP for the early detection of AMI was compared with standard cardiac markers of myocardial injury like CK-MB mass, cTnI, cTnT, and myoglobin. Heart-FABP appeared in reliable concentrations 6 hours after symptom onset, peaked at 8 hours and had decreased significantly towards normal concentrations by 16 - 24 hours after

presentation.[37] This early peak of H-FABP is consistent with previous reports, which suggested that H-FABP peak concentration occurs within 5 - 10 hours after symptom onset.[37]

The sensitivity of H-FABP for the detection of AMI within the first 6 hours after symptoms onset was 75.5%. Our results were in agreement with those published previously. Glatz et al (1998) reported a sensitivity of 78% for H-FABP, 53% for myoglobin in 83 patients with confirmed myocardial infarction who were admitted less than 6 hours after symptom onset.[19] Kleine et al (1992) using a threshold concentration $\geq$ 19 μg/l, found in 18 out of 22 patients with established AMI, the H-FABP concentration was increased above the threshold in blood samples taken at 3.5 hours after symptom onset of AMI. Only 40% of patients had elevated CK-MB concentration.[31] Ishii et al (1997) using cut-off concentrations $\geq$ 12 μg/l, the sensitivity of H-FABP for the diagnosis of AMI within 6 hours after symptom onset was 81.8%. The cut-off concentration in their study was based on ROC analysis between patients with AMI and normal healthy volunteers (similar to the method used to obtain the cut-off concentrations in Table 5). These investigators also reported superior sensitivity of H-FABP over myoglobin.[38]

The sensitivity of cardiac markers was mostly dependent on the threshold concentrations. Overall, H-FABP was the most sensitive marker at presentation and within the first two hours after presentation. It also demonstrated the highest NPV (94%). The high sensitivity is essential for the early rule in of patients with AMI, and the high NPV is very important to rule out AMI, since more than 90% of patients

who present with acute chest pain to A&E department do not have AMI. Most of the standard cardiac markers had limited diagnostic value at presentation. The sensitivity of these markers for the early diagnosis of AMI was < 62%. This low sensitivity of cardiac markers at presentation is consistent with previously published data.[36] Bakker et al (1993) reported low sensitivity (< 64%) and low negative predictive value of routine cardiac markers CK-MB mass, cTnT, myoglobin, CK-MB activity, and CK to allow early exclusion of AMI.[39]

This low early sensitivity may be attributed to; (1) the short time between symptoms onset and presentations. As shown in Figure 1, cardiac markers need sufficient time after onset of symptoms to accumulate in blood in reliable concentrations. The time delay in cardiac markers appearance is different for each marker. At presentation, H-FABP was the most sensitive early marker (75.5%), followed by myoglobin (62%), CK-MB mass (60%) and cTnI (51%). From two hours onwards after presentation most cardiac markers (except cTnT) were present in significant concentrations in most patients with AMI; (2) the use of high diagnostic cut-off concentrations. Myocardial injury in our study was defined as cTnI concentration $\geq$ 0.18 μg/l (Table 5). This was very similar to the cut-off concentrations used by Heeschen et al (1999) to define AMI in their group of patients. These investigators used Stratus CS and based on ROC curve analysis, AMI was defined as cTnI $\geq$ 0.15 μg/l.[46]

The usefulness of myoglobin and H-FABP for the early diagnosis of AMI has been supported by other studies.[29;30;31] However, the performance of H-FABP in this study was significantly better than myoglobin (sensitivity at presentation, 75.5% vs 62%).

Heart-FABP also had higher NPV (94%) for the early exclusion of AMI compared to myoglobin (90.2%). The superiority of H-FABP over myoglobin (both are cytosolic protein and have similar molecular weight) has been suggested before.[19;38] This advantage of H-FABP may be attributable to more cardiac specificity and low base line concentrations in normal patients. The distribution of H-FABP in the heart and skeletal muscle is comparable to that of CK-MB and is inverse to the distribution of myoglobin.[48] These features allow the use of lower but discriminate cut-off concentration thus improving the sensitivity.

The range of H-FABP in the control group ranged from 1 - 14.5 μg/l, median = 6.9 μg/l. The concentrations of H-FABP in normal healthy controls in previous studies ranged from 1 - 11.4 μg/l, and 9 - 14 μg/l, which is consistent with the normal range in our group of controls.[31;38] Based on the subgroup analysis, H-FABP in the concentration range 9 - 12.5 μg/l was the most appropriate threshold to define myocardial injury, both in terms of early and late sensitivity. Haastrup et al (1999) has found that H-FABP between 8 - 12 μg/l detected 90 - 95% with a specificity of 81 - 94%, and PPV 47 - 73% of patients with NSTEMI within 6 hours after symptom onset. The low PPV was related to the low prevalence of AMI in the study group.[50]

Some of the earlier studies have supported the additional beneficial role of myoglobin to H-FABP ratio over and above the measurements of either myoglobin or H-FABP alone (page 76).[36] The value of myoglobin to H-FABP ratio as a discriminator between cardiac and non-cardiac injury is not supported by the findings of this study. This ratio was falsely negative (i.e. > 21) in a small number of patients

with confirmed AMI and had a high false positive rate in normal patients with chest pain, and does not offer any advantage over the measurement of H-FABP alone. Furthermore, the measurement of both markers to obtain the ratio does not improve the sensitivity and is not cost effective. Instead one of these markers (e.g. H-FABP) should be combined with cTnI to allow risk stratification of patients with UA and improve the specificity for definitive diagnosis of AMI. Ishii et al (1997) reported no clear advantage of myoglobin to H-FABP ratio over the measurements of H-FABP alone.[38] The comparison of the area under the ROC curve for H-FABP (0.946), and myoglobin (0.895) in patients with AMI was significantly greater than the area under the ROC curve for myoglobin to H-FABP ratio (0.823). In addition, the myoglobin to H-FABP ratio is not reliable following physical exercise, in the presence of renal failure, and when there is a combined cardiac and skeletal muscle damage e.g. coronary bypass surgery.[36;43;44]

The maximum concentrations of cTnT in these studies was achieved very late (about 24 - 30 hours after symptom onset) compared to other markers e.g. cTnI.[34] This late peak may be due to genuine delay in the release of this marker from damaged myocardial tissue, or could be related to matrix effects of cTnT and cTnI assays. There is some supportive evidence for the existence of free cytosolic cTnI pool, as well as cTnI attached to myofibril proteins. For cTnT, protein turnover studies do not support the existence of such cytosolic pool for cTnT.[32;33] The existence of cTnI freely in the cytosol of myocardial cell, may explain the rapid release of cTnI following myocardial tissue damage (within 8 - 10 hours after symptom onset) that had been noticed consistently throughout these studies. This delay may also be

related to different release kinetics as a result of differences in molecular weight between CTnI (22.5 Kilodalton) and cTnT (37 Kilodalton). Cytosolic localisation or matrix effect as opposed to molecular size of troponins (compare CK-MB mass [80 Kilodalton] and cTnT [37 Kilodalton]) is the most likely explanation for the rapid appearance following myocardial injury. The continuous increase of cTnT concentrations during the sampling interval will support the prolonged diagnostic window (5 days), which had been observed for this marker following its release from damaged myocardium.[34]

Cardiac markers can play an important role in the diagnosis and management of patients with chest pain suggestive of AMI. Clarifications of non-diagnostic initial ECG and the detection of posterior MI are just some of the useful aspects of adjunctive serial cardiac markers testing in the diagnosis of AMI. As shown in this study, about 20% of patients in this selected high-risk group had no diagnostic ECG changes of AMI at admission and a similar number had atypical history of chest pain. Cardiac markers (cTnI and H-FABP) were diagnostic of AMI in 93 - 100% of patients within the first 2 hours of admission. Early serial ECGs and serial cardiac markers testing should both be an integral part of the investigation of patients with suspected AMI in the presence of non-diagnostic ECG or when the clinical history is atypical.

Other aspects where serial cardiac markers testing might be useful are in the detection of re-infarctions and infarct extension. The frequency of re-infarction in the early phase of AMI is 2.4 - 13%, and the frequency of re-occlusion after successful

thrombolytic therapy is about 17%.[35] Clinical features of re-infarction or infarct extension e.g. chest pain or new ECG changes may or may not accompany these complications. The clinical suspicion of re-infarction or infarct extension may necessitate the use of invasive investigations e.g. coronary angiography to ascertain the diagnosis. These complications will also require further interventions to open the occluded artery (thrombolytic agents or PCI). The availability of simple, safe and reliable non-invasive alternative methods like cardiac markers for the detection of early re-infarction or infarct extension will help with the management of some of these patients. Most of the cardiac markers like CK-MB mass, cTnI, and cTnT take longer time to return to the pre-infarction level, and thus are not reliable for the detection of re-infarction if it occurs early.

The value of H-FABP and myoglobin for the detection of re-infarction has been suggested by other studies.[31;36] Due to the unpredictable time of occurrence of these complications, it is suggested that serum should be collected at the same times when other conventional samples are obtained and stored. The analysis is performed if in retrospect there is a significant doubt regarding the diagnosis of re-infarction or infarct extension. Further studies that include large numbers of patients with these complications are needed to examine in much more details the value of myoglobin and H-FABP for the detection of re-infarctions and infarct extension.

Thrombolytic therapy has become the standard therapeutic approach in patients with AMI. The detection of successful reperfusion after the administration of thrombolytic therapy is mandatory in the management of patients with AMI. Unsuccessful

reperfusion is associated with a low ejection fraction and increased one year mortality.[42] it is estimated that between 15 - 50% of patients fail to achieve early infarct artery patency following intravenous thrombolytic therapy[49] and only 50% of patients treated with thrombolytic therapy achieve TIMI grade 3 flow (unrestricted flow) within 90 minutes of admission.[51] The clinical markers of reperfusion e.g. resolution of chest pain and ST segment elevation, and reperfusion arrhythmias have 70% sensitivity for the detection of reperfusion.[43]

The early assessment of reperfusion using cardiac markers ratios after the initiation of treatment may be more sensitive than clinical markers. The reason for the low sensitivity of these ratios in this study may be related to the difference between the timing of post-thrombolytic samples. This study predicted the success of reperfusion two hours after initiation of thrombolytic therapy, whereas most of the other studies examined this effect within one hour. The sensitivity of these ratios may be inversely related to the timing of post-thrombolytic samples and decreases with delayed sampling after thrombolysis.

Abe et al (1991) has showed that a rise of H-FABP ratio $\geq$ 1.5, 30 minutes after thrombolysis was associated with 100% accuracy for the detection of reperfusion. This sensitivity drops to 94% at 60 minutes after thrombolytic treatment. Creatine kinase-MB ratio > 2 and myoglobin ratio > 2.4 were found to be associated with 95 - 100% sensitivity for the detection of reperfusion 60 minutes after the initiation of thrombolysis.[40;47] Measurement of cardiac markers ratios in addition to clinical markers of reperfusion may offer a simple but efficient alternative to the use of

coronary angiography for the detection of successful reperfusion, especially in institutions that do not have access to coronary angiography facilities.

The administration of thrombolytic therapy in this group of patients may have contributed to some extent towards the earlier release of cardiac markers in blood samples taken beyond 0 hours, the so-called 'wash-out' phenomenon. This 'iatrogenic effect' may have influenced the sensitivity of some of the cardiac markers in this study. This study did not include patients with STEMI who were admitted within 6 hours but were not given thrombolytic agents for comparison. Hence, it is difficult to draw firm conclusions regarding the size of contribution of successful reperfusion towards sensitivity.

There are however, several arguments for the robustness of these results, and that such improved sensitivity was not explained entirely by the effect of reperfusion; (1) At 0 hour i.e. before the administration of thrombolytic therapy, the concentration of H-FABP in the blood had significantly increased compared to other markers, reflecting its genuine role as a sensitive early marker of AMI; (2) The sensitivity of these markers over the first 24 hours was assessed in a group of patients with NSTEMI, who were not given thrombolytic therapy. Although the proportion of infarct size in this group of patients was less than in patients with STEMI. Nevertheless, there were similar cardiac markers release characteristics patterns between NSTEMI and STEMI (compare Figure 1 pages 249 - 250 with Figure 2 pages 196 - 197), and the peak concentrations of cardiac markers occurred at similar times. Heart-FABP demonstrated superior sensitivity in this group of patients as

well, compared with other markers over the first two hours of presentation; (3) The effects of reperfusion on cardiac markers release were distributed across all markers, thus excluding specific bias.

7.4.1 SUMMARY

Overall 76% of patients in this group had typical history of ischaemic chest pain at admission. The overall diagnostic power of the ECG in this highly selected group of patients was 86%. Heart-FABP was the most sensitive marker of myocardial injury with 75.5% and 100% of patients with AMI identified at presentation and 2 hours after presentation. It also had the highest NPV (94%) for the early exclusion of AMI. The early diagnostic power of the other cardiac markers (CK-MB mass, cTnI, cTnT, and myoglobin) at presentation was low (< 62%). However, within 4 hours of presentation most cardiac markers (CK-MB mass, cTnI, and myoglobin) had 90 - 100% sensitivity, specificity, PPV, and NPV for the diagnosis and exclusion of AMI. The adjunctive value of serial cardiac markers testing using H-FABP in combination with cTnI in AMI diagnosis and management in the presence of non-diagnostic ECG is invaluable. Serial cardiac markers testing may also be helpful in timing the onset of MI event, detection of re-infarction, infarct extension, and reperfusion.

7.4.2 LIMITATIONS OF THE STUDY

(1) Patients admitted to the CCU constitute a highly selected group of patients at high-risk for myocardial injury compared for example to patients with chest pain seen at the A&E department. This factor is a disadvantage and may have affected positively the sensitivity and specificity of the different cardiac markers in the study.

(2) The role of H-FABP and other markers for the detection of complications such as early re-infarction and infarct extension could not be assessed properly due to the relative absence of these complications in the study group.

(3) This study had no control group (i.e. patients with STEMI who were not thrombolysed) for comparison. Therefore the measurements of the ratios for detecting successful reperfusion and the release pattern of the different cardiac markers were compared with a group of patients with NSTEMI who were not thrombolysed. The timing of the first post-thrombolytic sample at two hours had limited our ability to examine the role of these markers for the detection of successful reperfusion. In the future, such studies should be based on ratios obtained from ROC curve analysis in patients with STEMI who were thrombolysed and patients with STEMI who were not thrombolysed. These ratios should also be based on samples obtained within one hour after intravenous thrombolytic therapy.

7.5 REFERENCES

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CHAPTER 8

IMPLICATIONS OF STUDIES, LIMITATIONS, AND FUTURE HORIZONS FOR IMPROVING THE EARLY IDENTIFICATIONS AND MANAGEMENT OF PATIENTS WITH ACUTE CORONARY SYNDROMES USING HEART FATTY ACID BINDING PROTEIN

8.1 CARDIAC MARKERS AND PERCUTANEOUS CORONARY INTERVENTION

Percutaneous coronary intervention is an invasive interventional procedure that is becoming increasingly used in the treatment of a wide range of patients with ACS. The introduction of PCI into interventional cardiology is relatively recent. However, the remarkable successes in symptom relief and the feasibility of this intervention have driven an expansion of its applications. The guidelines for its applications are continuously changing to include many patients who may have been managed medically or who were candidates for CABG. Currently PCI is mainly performed in hospitals that have cardiothoracic surgical backup in case urgent CABG is required.

The development of coronary stents had been a revolutionary advance in terms of the success and safety of PCI. The introduction of new and powerful regimes of antiplatelets therapy has also reduced the complications associated with PCI. With further improvement in experience, medical management (e.g. antiplatelets, antithrombotic), and technological advancement (e.g. stents, catheters, and guide wires), the complication rates associated with PCI could be further diminished. When this is achieved, it may become feasible to perform PCI in hospitals that do not have cardiothoracic surgery backup in site e.g. district general hospital.

This study has shown that elevated cardiac markers (CK-MB mass, H-FABP, myoglobin, cTnI, and cTnT) concentrations were present in patients who had undergone visually successful and uncomplicated PCI. These elevations in cardiac markers concentrations may have prognostic implications, and were associated with

significantly reduced event-free survival, and with the development of more complications on long-term follow-up (Chapter 5). These complications included a higher incidence of uncontrolled angina, and the need for revascularisation. Some of the findings in this study are in agreement with previously published data. However, there are new findings that can be specifically drawn from this study.

1. This study has found cTnI to be the most sensitive marker for the detection of myocardial damage during and after PCI. It has shown the most frequent increase after PCI compared to cTnT, CK-MB mass, H-FABP, and myoglobin, reflecting superior sensitivity of cTnI assay used by Stratus CS. Cardiac-TnI testing should be the standard cardiac marker used for evaluation of myocardial injury following PCI.

2. Total cTnI elevation was associated with reported complications in 62% of patients. Its specificity and positive predictive value for complications were 67% and 62% respectively. Therefore, elevated cardiac markers post-PCI may or may not be associated with angiographically demonstrable complications during the procedure. Measurement of cTnI is more sensitive for the detection of myocardial injury than contrast angiography and should form part of the routine assessment of all patients following PCI to exclude myocardial damage not detect or noticed during PCI.

3. Occurrence of complications (major dissections, side branch occlusion, transient vessel closure, and bail out stent) during PCI was the variable that correlated most with cardiac markers elevations. The significance of these complications was related to the number of elevated cardiac markers, and the magnitude of elevation. The

measurement of cTnI is a useful adjunct to PCI, and can help provide a quantitative assessment of myocardial damage as a result of complications during PCI.

4. Measurements of cardiac markers post-PCI may be useful for the early detection of evolving MI within the first 2 hours. In patients with chest pain post-PCI, measurement of H-FABP before the procedure (stored serum) and after the onset of chest pain may offer reliable detection of early evolving myocardial infarctions. The combination testing of H-FABP and cTnI increased the specificity for the diagnosis of AMI. This testing may allow the selection of high-risk patients for early re-catheterisation to ascertain the diagnosis and re-establish early reperfusion of the occluded artery.

5. Elevated cTnI $\geq$ 0.18 μg/l [cTnT $\geq$ 0.1 μg/l] post-PCI may guide further management. This elevation was associated with increased risk of complications. Thirty-two per cent of patients with elevated cTnI post-PCI were not treated with antiplatelets other than aspirin. These patients had more episodes of angina and more revascularisation procedures on long-term follow-up (66.7%).

It is not clear at present whether further treatment of this group e.g. clopidogrel is required or might have any impact on their outcome. Many studies have convincingly demonstrated adverse prognostic outcome in patients with ACS and elevated troponins.[1] Even small increases of cTnI $\geq$ 0.08 μg/l were associated with appreciable risks (as suggested by this study as well).[2] Further randomised trials are needed to examine the benefits of long-term antiplatelet treatment in patients with

elevated troponins post-PCI, in the absence of any demonstrable clinical and angiographic complications.

6. The reporting of angiographic successes may be overestimated by reliance on visual assessment alone. Even in the absence of complications at angiography, significant numbers of patients had cTnI elevations. Contrast angiography is less sensitive than coronary angioscopy for the detection of complex plaques and thrombi.[3] This may explain the low positive predictive value of cTnI. The correlation and the predictive accuracy of cTnI for the detection of complications post-PCI should be compared with the more sensitive coronary angioscopy.

7. Heart-FABP has been shown in this model of myocardial injury to be a truly an early marker of myocardial damage, with concentration peaks occurring two hours after PCI, and rapidly returns to normal concentration. This early release characteristic of H-FABP is superior to myoglobin, CK-MB mass, and troponins. Heart-FABP was also the most useful test for the very early detection of evolving AMI, but is less sensitive than cTnI for the detection and quantification of other complications post-PCI.

8. A significant numbers of patients (6 out of 7) had cTnI elevation following investigations by intravascular ultrasound or pressure wire studies. It is not known whether such investigations contribute to myocardial injury during PCI and if so by what mechanism(s). This finding suggests that cTnI check should be performed in all patients following these investigations to exclude myocardial damage.

9. Percutaneous coronary intervention technology e.g. stents, balloons, and guide wires is advancing rapidly. Recent years have also witnessed the introduction of many investigative and therapeutic compounds for use with PCI. The exceptional sensitivity of cTnI for the detection of subtle damages to the myocardium will provide a reliable quantitative assessment of the direct impact and safety of all these methods.

Cardiac markers testing post-PCI (using cTnI) should be part of the routine patients' care and will help provide retrospective accurate validation of the success of the procedure, verify suspicious complications during PCI, detect evolving myocardial infarction early (using H-FABP), provide long-term prognostic risk and may guide further antiplatelet treatment.

8.2 CARDIAC MARKERS AND NON-Q WAVE MYOCARDIAL INFARCTION AND UNSTABLE ANGINA

In this study, H-FABP was shown to be superior or similar to myoglobin (that depends on the threshold) for the detection of myocardial injury in patients with non-Q wave MI within 7 hours after symptoms onset. Heart-FABP was increased in 79% of patients at presentation; all the other markers including myoglobin were increased in < 71%. Myocardial infarction can be confirmed using H-FABP, myoglobin, cTnI, and CK-MB mass in 93%, 93%, 86%, and 79% respectively within the first 2 hours of presentation. This suggests that H-FABP is the most sensitive marker for the very early detection of non-Q wave MI. The sensitivity of cTnI was superior to that of cTnT and CK-MB mass. These features of cTnI, combined with the slightly reported

higher specificity over cTnT in patients with renal failure and skeletal muscle disease in the literature, will support the preferential use of cTnI.[4]

Cardiac-TnI and cTnT were more sensitive (55% and 40% respectively) and specific than CK-MB mass, myoglobin, and H-FABP for the diagnosis of UA. Heart-FABP was increased in a fairly high numbers of patients with UA (40%), and showed an earlier concentration peak (within two hours), and might be useful for the earlier detection of these patients. However, H-FABP lacked specificity. There was some overlap between the concentration of H-FABP in some patients with UA and those with NSTEMI and STEMI. This overlap could limit the usefulness of H-FABP as the sole marker for the diagnosis of patients with UA. This problem could be overcome by; using concentration of H-FABP more than five times the upper limit of normal that is specific for the diagnosis of non-Q wave MI; or the combination of H-FABP with cardiac troponins may improve the specificity for the early diagnosis of UA.

Implementation of serial cardiac markers testing in patients presenting to A&E department with acute chest pain is warranted. Point of care testing of two cardiac markers, one that is totally specific e.g. cTnI, and one that is early and sensitive e.g. H-FABP at two intervals within the first 8 hours after presentation is simple and may be cost effective. This policy will reduce the pressure on the main hospital laboratory, and speed up the decision process regarding many aspects of patients' management such as diagnoses confirmation, triage policy (from A&E department and within hospital), risk stratifications (high and low-risk groups), and treatment (antiplatelets, antithrombotic, thrombolytic therapy, and PCI).

Serial cardiac markers testing can clarify atypical chest pain, non-diagnostic ECG or both. Patients may present with subtle changes of ischaemia on the admission ECG such as; minimal ST elevation < 1 mm; transient ST elevation; significant ST elevation in one lead only; suspected but not evident changes of posterior MI; marked and deep T wave inversion; poor R wave progression; conduction abnormality (paced rhythm and the presence of old left bundle branch block); 'Q wave equivalent' changes; and ischaemic left ventricular failure. Concomitant serial cardiac markers testing at admission or shortly afterwards, will help to clarify the diagnoses of AMI in the majority of these patients very early. Patients with non-Q wave MI have a higher rate of complex lesions and total coronary artery occlusion.[5] Some patients may have missed posterior MI, missed Q wave equivalent or confounding ECG changes that preclude the ECG diagnosis of AMI, and may benefit from early PCI.

Serial cardiac markers testing will also help with decisions regarding patients' management such as; (1) Prevention of inadvertent discharge of patients with myocardial damage from A&E departments; (2) Selection of high-risk patients for admission to CCU and other dependent units; (3) Classification of patients into high-risk group may guide early treatment with newer and more potent and expensive antiplatelet regimes; (4) Target the use of invasive procedures such as PCI to the most appropriate high-risk group.

In patients with chest pain but with no ECG changes of ischaemia, serial testing can rule out serious myocardial damage early. This will impact on patients' management

in several ways; (1) Early stratification of such patients into low-risk group. Low-risk patients can be admitted to regular beds and if necessary subjected safely to early exercise tolerance test to rule out ischaemic heart disease and discharged early. Alternatively, low-risk patients can be safely discharged and further investigations planned as outpatients. This will provide better and cost effective management of resources; (2) Avoidance of unnecessary and expensive high-risk invasive investigations in these low-risk patients; (3) There is little evidence to support cost effective use of potent antiplatelets in low-risk patients, hence these treatments should be reserved for high-risk groups.

8.3 CARDIAC MARKERS AND THE EARLY DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION

In patients presenting with AMI within 6 hours after symptom onset, the peak concentrations of H-FABP, myoglobin, cTnI, CK-MB mass, and cTnT were reached at 2 hours, 2 hours, 8 - 10 hours, 8 - 10 hours, and 16 - 24 hours respectively after presentation. Heart-FABP was the most sensitive marker for the very early diagnosis of AMI (75.5%). In comparison, CK-MB mass, cTnT, cTnI and myoglobin had low sensitivity (range 24% - 62%) at presentation. Heart-FABP achieved peak sensitivity (100%) at 2 hours and remained elevated at 100% for the first 2 - 8 hours after presentation. The optimum diagnostic window for myoglobin was between 2 - 8 hours after presentation (sensitivity 91 - 93%). The optimum diagnostic window for CK-MB mass and cTnI was between 2 - 16 hours, but the sensitivity of cTnI (97.7 - 100%) during this interval was superior to CK-MB mass (93 - 100%). Cardiac-TnT

was a late marker, and reliable sensitivity of cTnT was seen at 8 - 10 hours after presentation (Table 5 page 254).

The following observations can be drawn from the results of this study; (1) Heart-FABP was superior to myoglobin for the early diagnosis of AMI within 6 hours after symptom onset (75.5% vs 62%); (2) The diagnostic window of H-FABP was relatively prolonged (up to 14 hours after symptom onset). This diagnostic window will provide ample opportunity for the detection of AMI within the most appropriate time interval for thrombolytic therapy; (3) The sensitivity of cTnI used on Stratus CS was superior to cTnT and CK-MB mass. This will support its use for the early detection of patients with AMI; and (4) In patients who present within 6 hours after symptom onset, serial combinations testing of H-FABP and cTnI at two intervals during the first 8 hours, was sufficiently sensitive for the early diagnosis of all patients with AMI.

This serial combination testing of cTnI and H-FABP may also be useful in the following situations; (1) Timing the onset of AMI event. Heart-FABP concentration has been shown repeatedly to peak between 5 - 10 hours after AMI (8 hours in this study). The time-concentration changes of H-FABP and cTnI may help time the onset of MI event. Rising H-FABP and cTnI concentrations may suggest recent event within the proceeding 12 hours. This information may in turn be a useful help with decision on reperfusion therapy; (2) The early diagnosis of AMI in the presence of ECG features that confound the detection of ischaemia such as left bundle branch block and paced rhythm; (3) In patients with atypical chest pain or equivocal ECG

changes of AMI; (4) Detection of posterior MI; (5) Detection of AMI in the presence of skeletal muscle injury.

Serial cardiac markers testing may also be useful in the management of patients in the early phase of MI; (1) Early estimation of infarct size within 24 hours can offer important prognostic informations; (2) Detection of re-infarction if it occurs early; (3) Detection of infarct extension; and (4) Detection of reperfusion. The value of CK-MB mass, cTnT, and cTnI in these situations (with the exception of reperfusion) is limited. Heart-FABP and myoglobin were the two markers with possible diagnostic potential in these situations. Myoglobin was more sensitive than H-FABP for detecting re-infarction in the patient who developed this complication. Both markers may be useful for detecting infarct extension.

Heart-FABP and myoglobin were the only two markers that peaked at 8 hours. This early peak suggests that these two markers may be more suitable than CK-MB mass, cTnT and cTnI for detecting successful reperfusion. The sensitivity of myoglobin and H-FABP ratios for detecting successful reperfusion at two hours was moderate (75 - 77%). For better accuracy, this ratio may need to be calculated after one hour following reperfusion. Heart-FABP and myoglobin may also be useful for the early estimation of infarct size. However, this measurement can be obtained by other means that are rapid, less invasive, and fairly reliable such as echocardiography. When accurate early estimation of infarct size is required, serial H-FABP or myoglobin testing can be used.

The troponin proteins are more sensitive and specific than routine standard cardiac markers like CK and CK-MB. The latter markers are cheap and may be used for the confirmation of MI in patients with unequivocal ECG changes of AMI. However, for better management of all other patients with acute chest pain and suspected MI, the serial combinations of H-FABP and cTnI should be used. The results of serial cardiac markers testing along with electrocardiographic finding, and the clinical presentation, will allow satisfactory patients' care in most clinical settings.

8.4 IMPACT OF THE NEW DEFINITION OF ACUTE MYOCARDIAL INFARCTION BY THE EUROPEAN SOCIETY OF CARDIOLOGY AND THE AMERICAN COLLEGE OF CARDIOLOGY

This study examined the clinical impact of the new definition of AMI by the ESC/ACC in the three groups of patients that constituted the study (for definition see page 16). In PCI group, 57.5 per cent [46/80] of patients fulfilled the biochemical criteria for the diagnosis of AMI (i.e. cTnI > 99^{th} percentile of control group = 0.1 μg/l). The diagnosis of AMI in such a high numbers of patients (sometimes in patients with no apparent complications) following this relatively safe procedure will have many adverse repercussions in clinical practice.

This will raise concerns about the safety of the procedure. It will also have important and widespread social, health and legal implications. Ultimately this may impact negatively on the widespread and growing applications of this popular intervention, and will call for drastic measures to reduce these complications. In patients with UA, 75 per cent had elevated cardiac markers concentrations consistent with AMI. This

problem can be reduced significantly by using two different thresholds (based on ROC curve analysis) that define patients with UA from those with myocardial infarction.

The effects of the ESC/ACC definition on the AMI group were an improvement of sensitivity and NPV and a decrease of specificity and PPV. Using the 99th percentile of cTnI, 67% of patients will have evidence of MI at presentation, but on the other hand 50% of patients with UA will be misclassified as MI at presentation. The use of this definition in isolation may lead to erroneous diagnosis of AMI. This definition will be ideal in situations when there are cardiac markers that are 100% sensitive and specific and when used in conjunction with unequivocal ECG changes of AMI. Outside these specific situations, the applications and interpretation of these recommendations may need to be exercised with caution.

8.5 LIMITATIONS OF HEART FATTY ACID BINDING PROTEIN ASSAYS

Heart-FABP is potentially useful for the very early identifications of patients with AMI. In combinations with cardiac specific markers (cTnI), H-FABP can offer very early reliable warning of evolving AMI event. In addition, serial testing of H-FABP can also assist in the triage and management of patients in many different ways as detailed previously. There are however, some limitations that may interfere with the applications of H-FABP in clinical practice:

(A) Reliability of the assay. The normal range quoted by the manufacturer for H-FABP assay was < 5 μg/l. However, the range of H-FABP in our control sample was 1 - 14.5 μg/l, and this may reflect insensitive assay. In addition, the coefficient of variations of the current assay in the intermediate H-FABP concentration range 47.46 ± 9.39 μg/l was 19.8%. Further work is needed to develop and improve the sensitivity and the precision of the assay across all concentration ranges to less than the clinically accepted 10% level.

(B) Heart-FABP as a cardiac marker was designed to offer very early results. The current turn around time of the assay is prolonged (60 minutes), and should be reduced further to allow early results to be used immediately in the decision-making process of patients' management. The ultimate goal would be to provide this assay in the A&E department or CCU on a point of care instruments that can offer rapid and reliable early result in less than 20 minutes.

(C) Most of the recent research work on H-FABP was mainly concerned with developing sensitive and specific H-FABP assays, and their use in patients with AMI. There have been few researches in certain key areas that are essential to understanding the full potential of H-FABP for the early diagnosis of ACS. These areas include:

1. The exact route(s) of excretion of H-FABP from the circulation. As suggested by previous studies, the kidney is the major route of excretion of H-FABP from circulation. The effects of disease states and in particular kidney disease on the renal

handling of H-FABP has not yet been fully evaluated. The effects of renal impairment on the level of H-FABP and cTnT concentration were studied in a small group of 16 patients with renal failure [6 females, 10 males, aged between 30 - 70 years old], on haemodialysis or peritoneal dialysis. These patients had a range of conditions that were commonly seen in the renal unit including chronic glomerulonephritis, interstitial nephritis, adult dominant polycystic kidneys disease, and hypertensive nephropathy. The average urea and creatinine concentration in this group of patient was 19 ± 9.6 mmol/l and 531.3 ± 231.2 mmol/l respectively. Heart-FABP was increased in all 16 patients (mean = 81 ± 53.3 μg/l [range 24 - 173 μg/l]). Cardiac-TnT was increased ≥ 0.1 μg/l in 50%, ≥ 0.2 μg/l in 31.3%, and ≥ 0.3 μg/l in 6%. There were no correlations between the concentration of either urea or creatinine and the concentration of H-FABP and cTnT. There was a positive correlation between the concentrations of cTnT and H-FABP ($r = 0.569$, $p < 0.02$).

Gorski et al (1997) reported that H-FABP and myoglobin concentrations were both significantly elevated in patients with renal failure. The concentrations of these markers were not affected by dialysis. In addition, the myoglobin to H-FABP ratio in this group was similar to the ratio found in patients with myocardial infarction.[6] These two studies indicate that the power of H-FABP, myoglobin, and myoglobin to H-FABP ratio for the diagnosis of AMI in the presence of renal failure is limited. The usefulness of H-FABP or myoglobin for the detection of re-infarction, extension of infarction, reperfusion, and estimation of infarct size may also be limited. The presence of renal failure may also interfere greatly with the specificity of cTnT for the detection myocardial injury.[4] This interference should be borne in mind when

interpreting the result of these markers in patients with ACS in the presence of renal impairement.[7]

2- The effects of disease states in particular chronic liver disease on the normal concentration of H-FABP was studied in a group of patients with a mixture of chronic liver disorders [n = 10, mean age ± SD = 58.33 ± 7.19 years (range 45 – 70 years), median = 59 years]. These patients had a range of conditions including infective hepatitis and cirrhosis. The concentration of the following markers were measured in each sample and compared, alanine aminotransferase [ALT], bilirubin, and H-FABP. The normal concentrations of these markers in a normal reference control group were H-FABP = 6.86 ± 2.21 μg/l, ALT = 10 - 50 U/L, and bilirubin = 3 - 16 μmol/l. The concentration of these markers in patients with liver disease were as follows; ALT [mean ± SD = 198.67 ± 122.89 U/L, range (73 – 500 U/L), median = 114 U/L]; bilirubin [mean ± SD = 100.89 ± 87.85 μmol/l, range (17 – 337 μmol/l), median = 66 μmol/l]; and H-FABP [mean ± SD = 6.44 ± 3.06 μg/l, range (2 – 11 μg/l), median = 7 μg/l].

These data illustrate clearly that there is no significant interference with the normal concentration of H-FABP in the presence of liver disease, despite the significant elevation of liver enzymes and proteins. These data are consistent with the reduced cross reactivity (< 0.005) between H-FABP and other FABP including liver- FABP [see page 69]. These data may support a useful role of H-FABP for the diagnosis of myocardial injury in patients with liver disease.

3. The metabolism of H-FABP and the effects of disease states and drugs on the normal concentrations of H-FABP. In particular, the effect of inflammatory conditions such as severe infections or autoimmune disease states. The base line H-FABP was significantly elevated [52 μg/l] in a patient with active rheumatoid arthritis, but without clinically proven myocardial damage. Cardiac-TnI, cTnT, myoglobin and CK-MB mass were within the normal range. The total white cell count was significantly elevated (15.5). This patient was also on immunosuppressive and anti-inflammatory drugs (methotrexate and prednisolone). The exact cause(s) of elevated H-FABP in this patient is not clear. Elevated H-FABP in this patient may be drug related or related to rheumatoid arthritis or to some other unknown factors.

4. Exploring the possibility of existence of isoforms of H-FABP specific to the heart. The abundance of this protein in myocardial cells, together with its proposed multiple functions, raises the possibility of existence of H-FABP specific to the myocardium. This possibility should be explored by further research work. Clarification of all the physiological functions of H-FABP in the heart will help to answer this question.

5. The true distribution of H-FABP outside the heart using recent and more advanced techniques like sensitive and specific H-FABP antibodies, cDNA and mRNA.

6. Direct comparison between H-FABP, myoglobin and CK-MB subforms. Zimmerman et al (1999) showed in a large study (n = 955) that CK-MB subforms [sensitivity = 91%, specificity = 89%], and myoglobin [sensitivity = 78%, specificity

= 89%] were the two most sensitive and specific markers for the early diagnosis of AMI within 6 hours after symptom onset. These were followed by CK-MB mass, cTnI, and cTnT, which were more suitable for the late confirmation of AMI. The authors concluded that combinations of CK-MB subforms and troponins reliably triage patients with chest pain.[8] So far there have been no studies comparing the usefulness of these three early markers (myoglobin, CK-MB subforms, and H-FABP) in patients with AMI. Such study is important especially to compare the release kinetics and sensitivity of H-FABP and CK-MB subforms following myocardial injury.

7. Urinary H-FABP testing. The possibility of detecting AMI from urinary H-FABP testing is an interesting concept. The availability of test methods that can offer reliable warning of ACS from analysis of urine samples might become useful for the management of patients in the community. The early confirmation of AMI (based on qualitative tests using urine samples) may result in a rapid triage of patients to hospital and this will shorten the time to thrombolytic therapy. Several urinary H-FABP assays are available.[9-12] However, the role of urinary H-FABP will depend on the resolution of the above concerns and the development of sensitive assays that are easy to use.

8.6 LIMITATIONS OF STUDIES

The aim of the current study was to investigate specifically the early release feature of H-FABP in patients with ACS and compare it with the current cardiac markers of myocardial injury. Therefore, it was necessary to control some outside factors and

exclude some patients with conditions that could interfere with the normal concentration of H-FABP. The relatively small numbers of patients and the selection criteria in some of the groups have been limiting factors in the current study. Future studies should be designed to eliminate these disadvantages by including a large number of randomly selected groups of patients and employ rigorous validation methods. Heart-FABP may be useful in ACS in different aspects of patients' management [e.g. triage, diagnosis, and treatment]. Future studies should therefore focus on specific areas only. Some ideas or proposal for future studies are discussed below.

8.7 FUTURE STUDIES WITH HEART FATTY ACID BINDING PROTEIN IN PATIENTS WITH ACUTE CORONARY SYNDROMES

1. The usefulness of heart fatty acid binding protein to diagnose or rule out acute myocardial infarction

An important study would be to evaluate the usefulness of H-FABP to diagnose or rule out AMI in a large sample of patients enrolled consecutively, who present with chest pain suggestive of myocardial ischaemia (e.g. chest pain at rest $\geq$ 30 minutes), within 6 – 12 hours after the onset of symptoms to the A&E department. Two serial blood samples for H-FABP analysis are obtained at presentation and 2 hours later. Blood samples are also taken and analysed for CK-MB in the hospital main laboratory using routine methods to verify or rule out the diagnosis of AMI.

The physician in the emergency department should be blind to the study. The triage of patients from the A&E department [i.e. to coronary care unit, or to other medical units, or discharged home], should be left to the discretion of the physician. The diagnostic evaluation and care of all patients should be conducted by physicians who are unaware of the results of H-FABP, and H-FABP should be analysed by a person who is unaware of the clinical outcome of the patients and other clinical data, including the results of routine assays of CK-MB. The final clinical diagnosis should be determined by consultant cardiologist or CCU team. For each patient, the results of the assay for H-FABP and conventional assays [CK-MB] should be analysed separately and independently by at least two of the investigators without knowledge of the clinical findings. The diagnosis of myocardial infarction is confirmed or excluded on the bases of abnormal or normal CK-MB respectively, according to a valid cut-off criterion. The best cut-off concentration for H-FABP for the diagnosis of AMI should be based on ROC curve analysis between patients with confirmed AMI and without AMI.

The following ideas or proposals describe briefly other important areas of future research for heart fatty acid binding. These studies should be designs with rigorous validation methods similar to (1) above [with modifications where necessary].

2. Detection of successful coronary reperfusion following thrombolysis

In patients with unequivocal ECG diagnosis of AMI treated with thrombolytic therapy, H-FABP can be used in these patients to detect successful reperfusion. Heart-FABP concentration is measured immediately before thrombolysis and

repeated again at 60 minutes following thrombolysis. The success or failure of reperfusion should be confirmed immediately and independently with coronary angiography. The best cut-off concentration ratio [i.e. post-thrombolytic concentration divided by pre-thrombolysis concentration] selected to determine successful reperfusion should be based on ROC curve analysis between patients with successful and failed reperfusion. The sensitivity of H-FABP ratio for the detection of successful reperfusion can be compared with other cardiac markers ratios or with clinical markers of reperfusion e.g. decrease ST segment elevation.

3. Detection of early re-infarction or infarct extension following acute myocardial infarction

In this study, patients with AMI should have extended observation. Patients who develop chest pain suggestive of further ischaemia in the early phase of AMI, should have further blood samples taken for H-FABP measurement. This should be compared with the previous H-FABP concentration [measured from stored routine samples if no previous H-FABP result exist]. An increase in concentration compared with the previous sample [i.e. double peak rise] indicates re-infarction.

The diagnosis of re-infarction or infarct extension should be confirmed independently with coronary angiography [± echocardiography]. The best cut-off concentration [or ratio] used to diagnose re-infarction or infarct extension should be based on ROC curve analysis between patients with and without these complications. The sensitivity of H-FABP for the detection of these complications should be

compared with other cardiac markers ± ECG criteria ± echocardiographic features measured at the same time.

4. Timing the approximate onset of acute myocardial infarction event

In patients with unequivocal diagnosis of AMI e.g. significant ST segment elevation but with uncertain time of onset of symptoms. Heart-FABP in combination with cTnI may potentially be useful for estimating the approximate time of onset of AMI within the relevant time window for implementation of thrombolytic therapy [i.e. within 12 hours after symptoms onset]. Heart-FABP [and cTnI] concentration changes between presentation and 30 - 60 minutes later can be used to give an approximate time of onset of AMI [see page 259 for details]. If concentration changes at 30 - 60 minutes suggested a recent AMI event, these patients may be considered for thrombolytic therapy [if they still have ongoing chest pain and no contraindication to thrombolytic therapy] or to early intervention with PCI. The validity of this method, safety and prognostic value of such interventions are then assessed in these patients.

5. Early detection of evolving myocardial infarction following percutaneous coronary intervention

The usefulness of H-FABP for the early detection of high-risk patients with evolving AMI following PCI should be investigated further using large numbers of patients with recurrence of chest pain post-PCI. The confirmation or exclusion of AMI should be validated independently with repeat coronary angiography and with further blood samples taken for CK-MB measurement in the main laboratory. Heart-FABP should be measured in blood samples taken before PCI and after onset of chest pain and the

increase in concentration examined. The best cut-off concentration in these patients should again be based on ROC curve analysis between patients with and without AMI. The value of H-FABP for the detection of AMI post-PCI should be compared with other methods such as ECG criteria or other cardiac markers.

6. The significance of cardiac troponin I elevation following percutaneous coronary intervention

The correlation between the frequency and magnitude of cTnI elevation post-PCI and the types, frequency and severity of complications occurring during PCI should be investigated with more sensitive methods other than contrast coronary angiography e.g. intravascular ultrasound or coronary angioscopy. The results should be reported by at least two independent observers. In addition, the measurement of cTnI on Stratus CS should also be verified independently with extra measurements of cTnI on dimension as well [both systems use cTnI assays with equal sensitivity].

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