# CARDIAC TROPONIN T AND MYOCARDIAL DAMAGE

by

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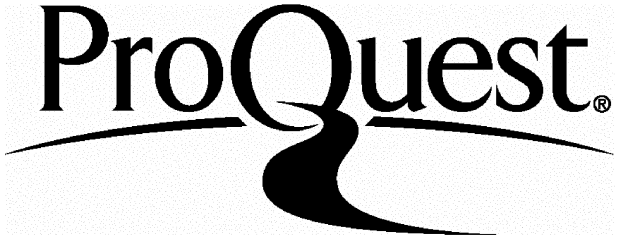

# Abstract

An accurate diagnosis of patients presenting with chest pain is required for appropriate patient management and to define prognosis. Current methods used to diagnose myocardial infarction were reviewed and an assessment of the biochemical markers currently available for identifying myocyte damage revealed a potential role for a more cardiac specific marker. Further review of current strategies to risk stratify patients with unstable angina identified a continuing problem between available investigations and predictive power for future cardiac events. An assay for the cardiac specific protein Troponin T (cTnT) has recently become available and its potential role in acute coronary syndromes was studied.

The cardiac specificity of cTnT was compared with creatine kinase MB isoenzyme (CK-MB) concentration measurement for the differential diagnosis of elevated creatine kinase (CK) produced by arduous physical training in 219 Royal Marine Commandos with no evidence of cardiovascular disease. CK was elevated up to 22.6 times and CK-MB mass up to 6.6 times the upper reference limit. Only 2 individuals had detectable cTnT, neither exceeded 0.2 micrograms/litre. Cardiac Troponin T was considered to be superior and more cardiac specific than the other biochemical markers.

The potential role of cTnT measurement in the differential diagnosis of suspected ischaemic myocardial damage was examined in 93 consecutive patients admitted to a Coronary Care Unit. The study showed that the cut off value should be 0.2 micrograms/litre, and that a single measurement at either 12-24 hours from admission or 12-48 hours from the onset of chest pain will confirm or exclude myocardial infarction. Cardiac Troponin T was also identified in a subset of patients with unstable angina and the prognostic potential and usefulness in risk stratification was examined.

183 patients admitted with unstable angina were followed for a median of 1032 days. The presence of cTnT in the serum when tested at 12-24 hours from admission in this syndrome identified a high risk subgroup that had a higher frequency of cardiac death. Its presence also identified a subgroup that had a higher frequency of coronary

revascularisation, cardiac death or revascularisation and cardiac death or readmission with non fatal myocardial infarction as first events. Further studies were undertaken to try and identify a pathophysiological explanation for the adverse prognosis of this subgroup.

The angiographic culprit lesion morphological findings were compared in 46 unstable angina patients during the repair phase of the syndrome. No major differences in the extent or severity of coronary artery disease, or the location or morphological findings of the culprit lesion between the cTnT positive and cTnT negative unstable angina patients were identified.

Activation of coagulation on admission in unstable angina in relation to cTnT concentrations were studied in 96 patients by examining variations in prothrombin fragment 1+2 and fibrinogen concentrations. No significant differences were observed between the two unstable angina groups.

Variations in Lipoprotein (a) concentrations in patients admitted with unstable angina in relation to cardiac Troponin T levels were examined. Lipoprotein (a) concentrations were significantly higher in the cTnT positive than in the cTnT negative unstable angina patients and these findings represent the first demonstration of a risk factor that may play a role in the pathogenesis of this acute coronary syndrome subgroup.

The cardiac specificity and long diagnostic window of the biochemical marker cardiac Troponin T offers advantages over existing biochemical markers for the confirmation of myocardial infarction. The identification by its presence in the acute syndrome of unstable angina of a high risk subgroup justifies its routine measurement in clinical practice.

# Acknowledgements

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Finally and by no means least I would like to thank the staff at West Middlesex University Hospital, the local General Practioners and most of all the patients without whose help and cooperation this thesis would not have been possible.

# Dedication

This thesis is dedicated to my wife Sarah and to my children Emily, Charles and Alexander for the times that I was not there.

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# Chapter One

# Coronary Artery Disease

## Introduction

In most westernised countries coronary artery disease is the largest single contributor to the morbidity and mortality of the population. It is responsible for between 160,000 and 180,000 deaths annually in the UK and accounts for 27% of all deaths. It has been estimated that coronary artery disease accounts for some 10% of all working days lost due to illness (35 million days/year) (Bourn, 1989).

Coronary artery disease is expressed clinically as stable chronic angina and the acute ischaemic syndromes of unstable angina, acute myocardial infarction, and sudden ischaemic death.

The underlying pathophysiology of these acute syndromes has been debated for over 200 years and only relatively recently has it been more clearly defined (Fuster et al, 1992). In myocardial infarction, atheromatous plaque disruption may be associated with deep arterial damage and ulceration, which results in the formation of a fixed thrombus. These thrombi are occlusive and lead to an abrupt cessation of myocardial perfusion with eventual necrosis of the involved myocardium. The coronary lesion responsible for the infarction is frequently only mildly or moderately stenotic (Ambrose et al,1985), suggesting that plaque disruption with superimposed thrombus, rather than the severity of the underlying lesion, is the primary determinant of the acute occlusion. This knowledge of the primary role of thrombus formation (Falk,1985, Davies,1986, Davies,1988) has led to more rational therapies and following large clinical trials (GISSI, 1986. ISIS-2, 1988. AIMS,1988. ASSET, 1990.), pharmacological lysis with thrombolytic agents is now established therapy for patients presenting with acute myocardial infarction.

In unstable angina, (Fuster et al, 1988. Fuster et al, 1992.) a relatively small fissuring or disruption of an atherosclerotic plaque may lead to a sudden change in plaque morphology and a reduction in coronary flow, resulting in an exacerbation of angina. Recurrent episodes of thrombotic vessel occlusion at the site of plaque disruption or residual thrombus may lead to angina at rest. The thrombus is usually labile, causing short lived vessel occlusion. In addition, the release of a vaso-active substance by the thrombus and vasoconstriction due to endothelial vasodilator dysfunction may further impede coronary flow (Maseri, 1986). Overall, alterations in perfusion and myocardial oxygen supply probably account for two thirds of the episodes of unstable angina. The rest may be caused by transient increases in myocardial oxygen demand or by complete occlusion of an artery with insufficient collateral supply.

Sudden ischaemic death frequently involves a rapidly progressing coronary lesion, in which plaque disruption and resultant thrombosis abruptly lead to ischaemia and fatal ventricular arrythmias. The absence of collateral blood flow to the myocardium distal to the occlusion or platelet microemboli may contribute to the sudden ischaemic death (Falk, 1985).

The most common presentation of the acute coronary syndromes is with chest pain. Patients are usually classified into the different ischaemic groups according to clinical, electrocardiographic and biochemical markers of myocardial damage findings.

## Definition of Myocardial Infarction

The most commonly quoted definition of myocardial infarction is that of the World Health Organisation (WHO,1971) (Table 1.1). This offers three diagnostic possibilities, definite infarction, possible infarction or no myocardial infarction. The definition is based on the components, the clinical history, the electrocardiographic (ECG) findings and the concentrations of biochemical markers of cardiac muscle enzyme release.

Table 1.1 WHO definitions of acute myocardial infarction

| | Criteria | Definitions |
|---|---|---|
| Definite myocardial infarction | The ECG shows unequivocal serial changes | Unequivocal ECG changes are the development of a pathological Q wave and/or the evolution of an injury current that lasts more than one day: at least two ECG's are necessary for the changes to be regarded as unequivocal. |
| | The history is typical or atypical with equivocal ECG and elevated cardiac enzymes | An equivocal ECG is the evolution of an injury current that disappears within 24 hours or when only one ECG is available: a stationary injury current; symmetrical inversion of the T wave; bundle branch block with additional Q waves; or pathological Q waves in a single ECG. |
| | The history is typical, the cardiac enzymes are elevated but the ECG is negative or unavailable | |
| | Fatal cases, whether sudden or not, with naked eye detection of fresh myocardial infarction and/or recent coronary occlusion | |
| Possible myocardial infarction | Living patients with typical pain whose ECG and enzyme results do not put them in the first category and in whom there is no good evidence for another diagnosis | Typical pain is diffuse through the chest, may remain localised, or may radiate to the shoulder, arm, jaw or abdomen on one side or both; is resistant to nitroglycerine if this is taken during the attack; is of more than 20 minutes duration; is usually severe and at times of agonizing intensity |
| | | Atypical pain is "characterised by a sense of suffocation, indigestion, syncope, general malaise, or acute cardiac failure" |
| | Fatal cases, whether sudden or not, where there is no good evidence for another cause of death clinically or at autopsy when the patient has a history of pain, typical or atypical; there is no history of chest pain but there is autopsy evidence of coronary disease; or there is clinical evidence of ischaemic heart disease | |
| No myocardial infarction | Living patients with an equivocal ECG without a typical history or elevated cardiac enzymes | |
| | Where the chest pain has been explained by another diagnosis | |
| | Fatal cases where another diagnosis has been made clinically or at autopsy | |
| | "Fatal cases with insufficient data" where the cause of death is completely unknown | |

## Chest pain.

The chest pain of acute myocardial infarction is classically described as 'crushing' in nature, lasts at least 30 minutes and occurs in the centre of the chest with radiation down the left arm and/or to the jaw. Patients may present however with atypical or no pain. Accurate diagnosis on the basis of history alone remains difficult. A well documented series of 604 patients presenting with chest pain to a casualty department (Emerson et al,1989) not due to trauma, found that 11.8% were sent home inappropriately. In a prospective study of 2320 patients in 6 hospitals (Pozen et al,1984) the number of admissions to the coronary care unit (CCU) with non-specific chest pain was 54%. The NORDKEM (1981) study of suspected acute myocardial infarction found that of 481 patients admitted to hospital with chest pain, 85% were admitted to the CCU and of these 47% had myocardial infarction (Gerhardt et al, 1982). Whilst the findings of these studies are probably dependent in some part on availability of CCU beds and include patients with other ischaemic syndromes, they do show that clinical history alone is not a sufficient discriminant to be used as a 'stand alone' marker for the diagnosis of myocardial infarction.

## Electrocardiography.

The electrocardiographic findings remain the mainstay for the diagnosis of acute coronary syndromes. A study of the admission ECG in 469 consecutive hospital admissions with chest pain found that 167 had a negative and 302 a positive initial electrocardiogram (Brush et al, 1985). Using a concensus final diagnosis based on serial electrocardiograms and cardiac enzymes, the admission ECG had a sensitivity for the diagnosis of MI of 56.6% with a specificity of 85%. Similar findings have been reported by other workers. In 475 patients admitted with suspected uncomplicated acute myocardial infarction (Yusuf et al, 1984), the admission ECG had an overall sensitivity of 75.3% with a specificity of 98.6% using ST elevation as the diagnostic criterion. The sensitivity of diagnosis based on

the ECG increased to 94.3% when ST depression or T wave inversion was included but specificity fell to 49.3%, even when the incidence of acute myocardial infarction was 85.1%. In another study (Rude et al,1983) the overall accuracy of the admission electrocardiogram in 3697 patients with prolonged chest pain and no history of previous infarction was only 75%. In at least 20% of all myocardial infarctions the ECG is indeterminate, mainly because of the presence of left bundle branch block or non specific ST-T wave abnormalities (Turi et al,1985).

ST segment elevation can occur very early in the course of myocardial infarction and this subgroup are important as they form the group that on current concensus are most likely to benefit from therapeutic interventions with thrombolytic agents. In a more recent series however (Norris et al, 1994) they formed only 51% of patients admitted to a district general hospital with a final diagnosis of myocardial infarction.

## Biochemical markers for the diagnosis of myocardial infarction

In the last several decades serum levels of cardiac enzymes and isoenzymes have become the final arbiters by which myocardial damage is diagnosed or excluded (Mair et al,1992).

In a review of the diagnostic accuracy of cardiac enzyme assays in the diagnosis of myocardial infarction (Lee and Goldman,1986), it was considered that for CCU admissions, serial measurement of CK-MB was the best test and should be considered the 'Gold standard' for diagnosis. Although the sensitivity of total creatine kinase was good at 98%, the false positive rates may be as high as 15%.

In the NORDKEM study an elevated CK-MB measured by an immunoinhibition method showed a sensitivity of 99.5% with a specificity of 93.1 % for the diagnosis of myocardial infarction (Gerhardt et al,1982). The CK-MB isoenzyme is not however a cardiospecific molecule, and its variable concentrations and transient increase during the course of myocardial infarction, further limit its diagnostic value (White et al,1985). The ratio of CK-MB/CK offers better specificity than measurements of MB alone but with an unacceptable loss of sensitivity (Thompson et al,1988).

Isoforms or sub-bands of creatine kinase were initially demonstrated in serum after MI when high resolution electrophoresis of CK on polyacrylamide gel revealed multiple bands (Smith, 1972). Subsequent studies (Wevers and Soons, 1986) showed that they could be demonstrated by prolonging the usual time for standard electrophoresis on agarose gels.

CK isoforms are produced by post translational modification of the M subunit of the CK dimer. Removal of the C terminal lysine by a plasma carboxypeptidase results in increased anodal mobility of the M subunit. Hence, there are 5 possible isoforms. These are named according to their increasing anodal mobility, for the 3 isoforms of CK-MM : no modified M subunits - MM3; 1 modified M subunit - MM2; 2 modified M subunits - MM1 and for the 2 isoforms of CK-MB:no modified M subunit - MB2 and 1 modified M subunit - MB1.

It has been proposed that changes in absolute amounts of these isoforms, and in particular the ratio of MM3 to MM1 can be used for diagnosis of acute myocardial infarction (Wevers et al, 1977. Wevers et al, 1978). This was studied in 28 patients with acute myocardial infarction confirmed by serial CK and CK-MB estimations, 26 normal controls and 7 patients with angiographically proven ischaemic heart disease with serial CK-MB within the reference range (Jaffe et al, 1986). Isoforms were determined by imunoblotting. An MM3:MM1 isoform ratio which exceeded their upper reference limit of 2.5 could be demonstrated in 24/28 patients (85.7%) in the first blood sample taken on admission, a mean of 3.9 +/- 0.4 h from onset of chest pain. A more rigorous analysis of the diagnostic performance of MM3:MM1 ratio in patients admitted to the CCU for evaluation of acute chest pain has been performed (Wu et al, 1987). A final consensus diagnosis based on clinical features, serial ECG and serum enzymes (CK, CK-MB, total LactateDehydrogenase (LD) and isoenzyme LD-1) was used to assign patients to MI and non-MI catagories. A total of 69 patients were studied. MI was confirmed in 35 and excluded in 34. Isoforms were quantified by high voltage electrophoresis and liquid chromatography. The MM3:MM1 ratio was compared with CK-MB by ROC analysis and was superior to CK-MB 3-9 hours from onset of chest pain but thereafter was less efficient.

It has also been suggested that the isoenzymes of lactate dehydrogenase, rather than CK-MB, can be used for early diagnosis of acute myocardial infarction in addition to their accepted role as a late marker. Changes in the LD-1/LD-2 ratio during the first 48 hours from onset of chest pain were studied in 128 patients admitted to the CCU (Jablonsky et al, 1985). Diagnosis of acute myocardial infarction was established by clinical features, serial ECG and cardiac imaging. In patients where cardiac enzymes were normal on admission, the LD-1/LD-2 ratio exceeded the upper reference limit of 0.74 at the same time as did CK-MB in 59.1% (39/66). In 16.7% (11/66) of the patients studied, the LD1/LD2 ratio exceeded the upper reference limit before CK-MB. When one or more enzymes were initially abnormal on admission, these figures increased to 71.1% (27/38) and 26.3% (10/38) respectively.

Myoglobin is a low molecular weight protein present in striated and cardiac muscle. In a study comparing myoglobin measured by radioimmunoassay with other enzyme markers in 76 patients admitted within six hours of onset of chest pain, it had the best performance at 5 and 8 hours from onset of chest pain but thereafter CK and its isoenzymes performed better (Van Steirteghem et al, 1982).

When measured by a latex agglutination method in 38 patients admitted with chest pain (Chapelle et al, 1985), the authors concluded that a failure to demonstrate myoglobin by this method categorically ruled out myocardial infarction, but that a positive result must be confirmed by other investigations.

A series of studies (Loughlin et al,1988. Leung et al, 1989. Pellar et al, 1989) of patients admitted to the CCU in whom a diagnosis of acute myocardial infarction was established by clinical features, serial ECG and cardiac imaging, systematically evaluated the different enzyme markers in respect of their time series by rigorous methods, including Receiver Operator Characteristic (ROC) curves and likelihood ratios (explained in detail in chapter two). They included Aspartate Transaminase, which is one of the routine biochemical markers used in the UK, as well as isoenzyme ratios of the biochemical marker Lactate Dehydrogenase. Overall, CK-MB was the best performer for the diagnosis of Myocardial Infarction(Table 1.2).

Table 1.2 Comparative analysis of biochemical markers for the diagnosis of acute myocardial infarction (AMI). The optimal time window is defined as time from onset of chest pain in hours showing the maximum area under the ROC curve. Likelihood ratios at 95% sensitivity are shown for each optimal time window. Data is taken from the papers listed below. Total CK measured at 37$^{o}$C, CK-MB by electrophoresis and scanning densitometry, AST at 37$^{o}$C, LD at 37$^{o}$C, LD isoenzymes by electrophoresis and scanning densitometry.

| Analyte with cut-off value in parentheses. | AMI | No AMI | Optimal time window | Likelihood ratio at 95% sensitivity | Area under ROC curve |
|---|---|---|---|---|---|
| CK-MB[a] (16 U/l) | 151 | 159 | 19-24 | 28 | 0.9905 |
| CK-MB%[a] (5%) | 151 | 159 | 13-18 | 13.5 | 0.9778 |
| CK[a] (200 U/l) | 151 | 159 | 19-24 | 6.1 | 0.9652 |
| AST[b] (39 U/l) | 136 | 114 | 19-24 | 7.9 | 0.9647 |
| LD-1/LD-2[c] (0.92) | 69 | 216 | 55-60 | 1.2 | |
| LD-1/LD-3[c] (1.4) | 69 | 216 | 31-36 | 0.62 | |
| LD-1/LD-4[c] (3.25) | 69 | 216 | 31-36 | 1.25 | |
| LD-1/LD-5[c] (2.5) | 69 | 216 | 19-24 | 0.16 | |

[a] Clin Chem 1989; 35: 1435-1440
[b] Ann Clin Biochem 1989; 26: 533-537
[c] Clin Chem 1988; 34: 1960-1965

## Definition of Unstable angina

The phrase "unstable angina" was first used to describe acute coronary insufficiency in 1971 (Fowler, 1971) and consists of a number of conditions, all characterised by severe transient ischaemia which lie between chronic stable angina and myocardial infarction. It encompasses previously used terms such as 'impending coronary occlusion' (Sampson et al, 1937), 'acute coronary insufficiency' (Master et al,1956), 'the intermediate coronary syndrome' (Graybiel, 1955), 'coronary failure' (Freedberg et al, 1948), 'status anginosus'

(Papp et al,1960), 'pre-infarction angina' (Resnick, 1962) and 'accelerated angina' (Scanlon et al , 1973). This large number of designations, as well as the lack of a clear, agreed upon definition, reflects the ambiguity that has continued to be associated with this 'catch-all' syndrome (Braunwald, 1989). Currently, a broad spectrum of patients with ischaemic episodes varying widely in cause, severity, prognosis, and responsiveness to therapy are lumped together under the broad umbrella of unstable angina, which impedes the ability to accurately define it. This makes it very difficult to obtain a clear understanding of the natural history of the syndrome, to identify which patients would benefit from different therapeutic strategies and to assess the level of risk for a future adverse prognosis. Various classifications for risk stratification have been proposed that not only use information obtained from the clinical history, but also from routine electrocardiography, exercise electrocardiography, coronary arteriography, perfusion scinigraphy, activation of coagulation markers and changes in levels of biochemical markers.

## Clinically based classifications of unstable angina.

The currently most quoted classification in clinical trials is that of Braunwald (Table 1.3). This classification primarily draws on the various components of the presentation history as well as the prior cardiac history in an attempt to quantify level of risk; this includes the nature of the chest pain, the pattern of the chest pain and whether this occurs in the presence or absence of known heart disease. It also allows further group subsets according to whether the ECG has evidence of ST or T wave changes. The classification is based on two premises: that the patient's symptoms are actually caused by myocardial ischaemia and that in patients with prolonged ischaemia, the diagnosis of acute myocardial infarction is excluded by ECG or serum enzyme determinations. It has been noted however, that its usefulness needs to be validated prospectively not only in research studies but also in the clinical setting (Betriu et al, 1992). It has also been acknowleged that to fulfill the classification (Braunwald, 1989) the diagnosis will inevitably be retrospective and therefore be unsuitable for early risk factor stratification.

Table 1.3. Braunwald classification of unstable angina

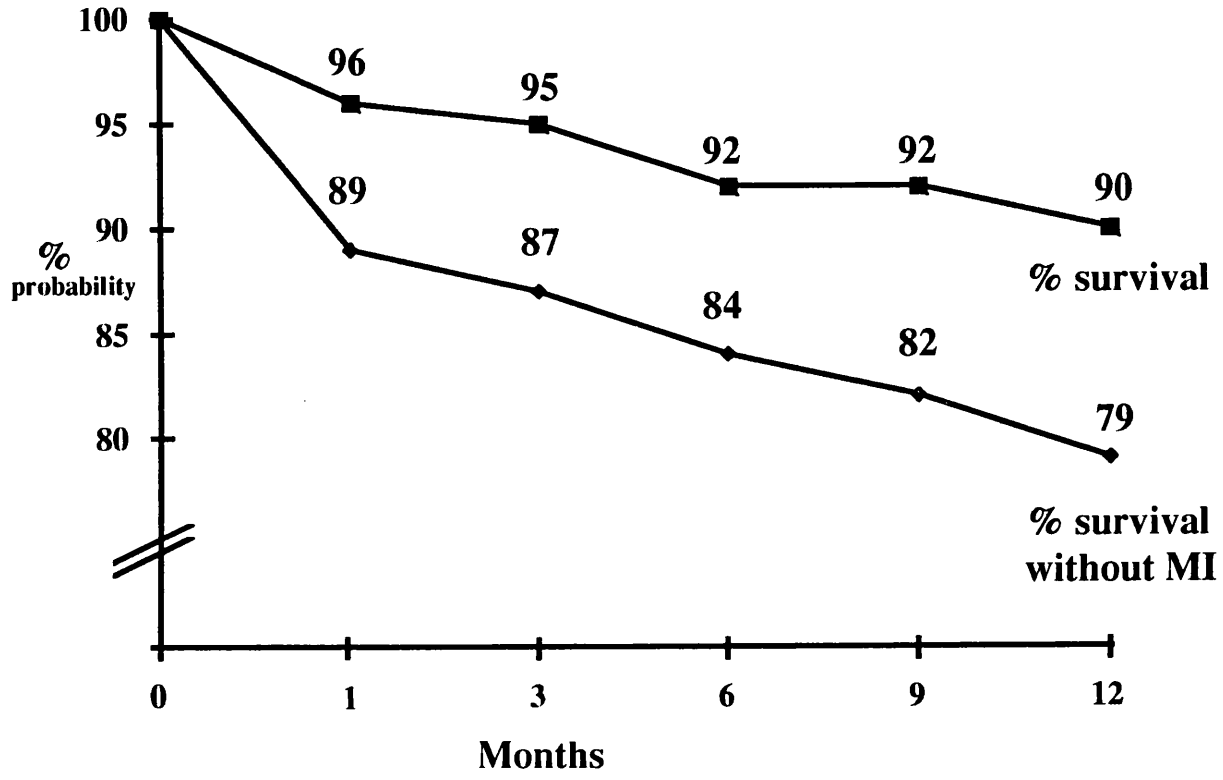
| Severity | Clinical circumstances: A. Develops in presence of extracardiac condition that intensifies myocardial ischaemia (secondary UA) | B. Develops in absence of extracardiac condition (primary UA) | C. Develops within 2 wk after AMI (postinfarction UA) |
|---|---|---|---|
| I. New onset of severe anginaor accelerated angina; no rest pain | IA | IB | IC |
| II. Angina at rest within past month but not within preceding 48 hr (Angina at rest, subacute) | IIA | IIB | IIC |
| III. Angina at rest within 48 hr (Angina at rest, acute ) | IIIA | IIIB | IIIC |

Patients with UA may also be divided into three groups depending on whether UA occurs 1) in the absence of treatment for chronic stable angina, 2) during treatment for chronic stable angina, or 3) despite maximal anti- ischaemic therapy. These three groups may be designated by subscripts 1, 2, or 3, respectively.
Patients with UA may be further divided into those with and without transient ST-T wave changes during pain. UA, unstable angina; AMI, acute myocardial infarction. (from Braunwald E, Unstable Angina, a classification Circulation 1989;80:410-414).

## Nature of chest pain in unstable angina.

Accelerated angina and angina at rest account for the majority of patients with the diagnosis of unstable angina (Betriu et al,1992). To assess the overall prognosis of these clinical symptoms they pooled 10 representative series with a total of almost 2000 patients (Figure 1.1). The in hospital mortality rate ranged between 2% and 8% with a mean survival rate of 96%. At one year the mean survival rate was 90%. The probability of survival without infarction was 89% at one month and 79% at one year. This pooled analysis however, included trials that required additional inclusion criteria such as admission ECG changes (53% of patients); 39% of the patients had also sustained a previous myocardial infarction and the degree of left ventricular impairment has a prognostic significance in its own right (Yusuf et al, 1988).

Figure 1.1. Pooled analysis of the prognosis of 2000 patients admitted with unstable angina with a history of accelerated angina or angina at rest (Betriu et al,1992).




A number of trials have attempted to identify high risk unstable angina patients by subgrouping patients according to the various components of the pre-admission and post-admission chest pain. Patients presenting with angina at rest and ECG changes have been reported to have a poorer prognosis ( Farhi et al, 1986. Cohen et al, 1990).

This patient group however, included a number of patients with non-Q wave myocardial infarction, which is a continuing problem in trials which diagnose and risk stratify patients on admission, the 'early entry trials' (Lubsen, 1990).

Gazes and coworkers (1973) in a prospective study of 140 patients admitted with unstable angina, found that the probability of survival at one year was significantly decreased (from 96% to 57%) in patients who had persistent angina within 48 hours after hospital admission. Similarly, Mulcahy et al (1981) studied 101 patients and reported a decreased event free probability ( 87% to 65%) in 22 patients with repeated episodes of chest pain

after admission. Patients who develop angina post infarction are also considered to be a high risk group. Bosch et al (1987) noted a prevalence of post myocardial infarction angina of 18% and was more common in patients with non-Q wave infarction, multivessel disease or previous angina. This group had a significantly lower survival rate (92% versus 83%) during a mean follow up of 14 months. Benhorin et al (1988) using a different criterion of only counting episodes of angina that occured between discharge from the CCU and hospital discharge, found a prevalence of post infarction angina of 33%. They found no difference in survival rates in those with or without post infarction angina on 1-4 year follow up, except a sub group who experienced more than one episode of angina a day.

A number of problems exist however with quoted trials in unstable angina with regard to criteria used to define the population, the population actually studied, the variations in medical managements and the thresholds for intervention. Many studies are based on the experience of a secondary or tertiary cardiac centre. They are largely concerned with patients who have failed to respond to conventional medical treatment. Confusion has been caused by extrapolating these reported experiences to patients presenting to a district general hospital ( Patterson, 1988).

Risk stratification classifications that place great import on the occurence of rest pain in this syndrome are not of great value in routine district general hospital practice, because almost all who are admitted to the CCU will have had rest pain ( Murphy et al, 1992).

## The prognostic role of exercise treadmill testing in unstable angina

The role of exercise testing in identifying high risk patients admitted with unstable angina has been studied by a number of investigators, the results of which are summarised in Table 1.4.

Table 1.4 The prognostic role of exercise testing in patients admitted with unstable angina.

| Study | No. | Protocol | End point | Independent variable | Sens. | Spec. | +PV | -PV |
|---|---|---|---|---|---|---|---|---|
| Nixon | 55 | Upright bicycle exercise stopped for ECG changes, symptoms,or target heart rate. | Severe angina unstable angina | ECG changes | 71% | 70% | 71% | 70% |
| Butman | 125 | Modified Naughton treadmill;exercise stopped for ECG changes,symptoms, or target heart rate. | Severe angina CABG,MI death | ECG changes or chest pain | 76% | 85% | 88% | 71% |
| Swahn | 275 | Upright bicycle; exercise stopped for symptoms (or target heart rate for Non-Q MI, n=74) | CABG,MI death | ECG changes, limiting chest pain,or RPP <13,500 | 92% | 70% | 40% | 98% |
| Wilcox | 107 | Modified Naughton treadmill; exercise stopped for ECG changes or symptoms | Unstable angina,MI death | ECG changes and RPP <18,000 | 38% | 85% | 48% | 79% |
| | | | | ECG changes and RPP<18,000 or ECG changes alone or RPP <18,000 alone | 86% | 32% | 32% | 86% |
| Brown | 52 | Treadmill, Bruce symptom limited | Unstable angina, MI death | ECG changes | 30% | 80% | 55% | 59% |
| | | | | Reversible thallium defect | 70% | 76% | 70% | 76% |

Sens. = sensitivity; Spec. = specificity; +PV = positive predictive value; -PV = negative predictive value. RPP = rate pressure product.

Nixon and coworkers (1980) reported that men with new onset or progressive effort angina and a positive exercise ECG were more likely to have recurrent unstable angina during short term follow up (mean 18 weeks). Their study excluded however, any patients presenting with angina at rest. None of their patients died or had a myocardial infarction during the period of follow up.

Butman et al (1984) performed sub maximal exercise tests on 125 patients admitted with unstable angina. They showed that an unfavourable outcome was associated with a shorter exercise duration, an achievement of a lower maximal rate-pressure product and was more likely to occur if the exercise test evoked angina or ST segment depression. Patients with congestive heart failure or previous myocardial infarcton within three months were excluded, as were women. Swahn et al (1987) included both men and women in their study and showed that the exercise test was prognostically important only in men with unstable angina and that ST depression during exercise and a low rate-pressure product were the only independent predictors of subsequent cardiac death or myocardial infarction; however, none of the clinical or ECG descriptors were independent predictors for future adverse events. Wilcox et al (1991) studied 107 unstable angina patients and concluded that two exercise test variables - a positive exercise ECG and a low rate-pressure product- were independent predictors of an adverse outcome. They excluded however 64% of their initial unstable angina cohort (296 patients ). Severi et al (1988) and Murphy et al (1992) found that a positive exercise test had little or no relationship with subsequent adverse events.

## The prognostic role of myocardial scintigraphy in unstable angina

It has been noted that 25-42% of patients with unstable angina have positive myocardial scintigrams (Willerson et al, 1975. Donsky et al, 1976. Abdulla et al,1976.) Olson et al (1981) studied 150 patients, 1-4 days after admission to the Coronary Care Unit using technicium-99m stannous pyrophosphate scintigraphy; 33% of patients had a positive scintigram. They concluded that patients with continuing angina, an ischaemic ECG and a

positive scintigram constituted a high-risk unstable angina subgroup with a survival rate of 42% on 36 month follow up, whilst those with a negative scintigram had an 88% survival rate. In a multiple stepwise regression model however, the myocardial scintigram was of neglible value compared to clinical history and ECG changes.

**The prognostic role of coronary angiographic findings in unstable angina.**

A number of investigators have examined the prognostic significance of the extent of coronary disease in unstable angina patients. Most of the studies have compared medical versus surgical strategies. Conti et al (1973) examined the morbidity and mortality in 57 unstable angina patients examined angiographically and followed for ten months; 32 patients had three vessel disease and 5 at least a 50% left main stem lesion. Although the authors do not clearly state if those patients with a left main stem lesion were randomised, in the medically randomised group there were no common coronary arterial patterns associated with subsequent increased morbidity or mortality in those with two or three vessel disease.

In the National Cooperative Study Group Trial to compare surgical and medical therapy in unstable angina (Russell et al, 1978) 147 patients were randomised to medical therapy and followed for an average of 30 months. Patients with a left main stem lesion or significant left ventricular dysfunction were excluded. In the first year, patients with two or three vessel disease had more severe angina than patients with single vessel disease and a more significant cross over to revascularisation in patients with more severe disease. There were however, no significant differences between the number of vessels diseased and the survival curves. In a substudy from the same group (Russell et al, 1981) the prognosis of the location of the disease in the left anterior descending artery was examined. There was no significant difference in the in hospital or follow up cardiac death rate, subsequent non fatal infarction rate, need for revascularisation or angina class between those with a proximal lesion versus those with a distal lesion.

## Coronary artery morphological findings in unstable angina

Several studies have examined the coronary artery morphology of the 'culprit' lesion considered to be the stenosis causing the ischaemia. Ambrose et al (1985) reported that in patients with unstable angina a characteristic coronary lesion is found. An eccentric stenosis in the form of a convex intraluminal obstruction with a narrow neck due to one or more overhanging edges or irregular or scalloped borders, or both, is present in approximately 70% of patients with unstable angina. He designated this type of lesion as an Eccentric lesion type II. The underlying pathophysiology was postulated to be a significant plaque disruption, or a partially occlusive thrombus, or both. In a recent editorial (Chesebro et al, 1992) it was considered that a partially occlusive thrombus was more likely to occur in patients with unstable angina and pain at rest. Braunwald recently tested his unstable angina classification (Table 1.3) and compared this with early visual and morphological angiographic findings (Ahmed et al, 1993). There was no difference between the minimal lesion diameter and percent stenosis among the three classes and types of angina, nor was there any difference in the site of the ischaemia related artery in the unstable group compared to the stable angina group. There was also no difference between the incidences of intracoronary thrombus in the three groups (17% in Class I, 13% in Class II, 17% in Class III ). Interestingly, in the same study, there was no significant association between the presence of ST segment change on the ECG in the unstable angina group and the finding of intracoronary thrombus.

## Thrombosis related markers in unstable angina

A number of different markers of coagulation activation have been studied. Alexopoulos et al (1991) studied Fibrin D-dimer and plasminogen activator inhibitor levels both peripherally and from the coronary sinus in patients with unstable angina and rest pain in the 24 hours prior to sampling. They were unable to demonstrate any differences between levels of these coagulation markers in the unstable angina group compared to controls.

They concluded that either the amount of thrombus in the coronary artery was too small or that ongoing acute thrombus formation with a resultant decrease in coronary perfusion may be unimportant as a mechanism of rest pain. Merlini et al (1994) examined plasma levels of Fibrinopeptide A and the prothrombin fragment 1+2 in patients with acute coronary syndromes (unstable angina, n=80) compared with a control group of patients with chronic stable angina (n=37). They were able to demonstrate increased levels of both markers in the acute phase of unstable angina as compared to the control group and interestingly this 'hypercoagulable state' was still evident when tested at six months following admission..

## Biochemical markers of myocardial injury in unstable angina

The potential role of minor rises in biochemical markers of myocardial damage in risk stratification of unstable angina patients, has been examined by a number of investigators. Armstrong and coworkers (1982) prospectively studied 199 patients with unstable angina to assess whether frequent serial sampling of serum creatine kinase (CK) was useful in predicting prognosis. 19% of the patients had transient elevations of CK within the first 24 hours that were missed by the routine daily enzyme estimations and 16% of this group had died on one year follow up compared to 5% of the remaining patients; 14% of this subgroup suffered a non fatal myocardial infarction during the same period of follow up compared to 2% of the no CK elevation group. Another study (Wilcox ,1991) however, failed to show any association between an adverse prognosis and minor rises in creatine kinase. Minor rises in the CK-MB isoenzyme have also been studied (Botker et al, 1991. Ravkilde et al ,1992.)

Botker et al evaluated a newly developed highly sensitive immunoassay for CK-MB in 68 patients admitted with unstable angina. They showed that serial changes in concentration of this marker correlated with signs of repetitive ischaemic episodes deduced from continuous ST segment monitoring in a subgroup and suggested that this may carry an adverse prognosis. This cohort was followed for a period of 30 months by Ravkilde et al

(1992). The subset of patients with changing CK-MB concentrations had a cumulative probability not to suffer cardiac death of 66 +/-10% versus 95 +/-3% in the group with stable in patient CK-MB levels. Rotenberg et al (1986) studied the role of lactate dehydrogenase isoenzymes in 50 patients admitted with angina. The mean LD-1 value and the mean LD 1:2 ratio was significantly higher in those patients whose angina was considered unstable versus those whose angina was stable.

The prognostic role of a minor rise in the enzymes aspartate transaminase and hydroxybutyrate dehydrogenase on daily testing was evaluated by Murphy et al (1992). They followed 149 patients admitted to a district general hospital CCU for a median of three years; 8 of the 13 early events occured in 35 patients with minor enzyme rises (sensitivity 69%, specificity 71%). In combination with either ST or T wave changes or any further pain although the sensitivity improved (77% and 92% respectively) this was at the price of a very low specificity (25% and 18% respectively).

## Biochemical markers of myocardial damage: the need for a sensitive and cardiac specific marker.

In the WHO classification (Table 1.1) and in routine clinical practice serum enzymes and isoenzymes levels form the basis on which most myocardial infarctions are finally classified. For the most part this is a retrospective confirmatory role. On available evidence CK-MB measurements are considered to be the most accurate single biochemical marker to confirm or exclude myocardial damage. Problems exist however with the sensitivity and specificity of this marker for myocardial damage, which limits its ability to be a 'catch-all' diagnostic tool. Increase in CK-MB concentrations not associated with myocardial damage are shown in Table 1.5. Conventionally used enzymes also fail to diagnose myocardial damage efficiently in patients with myocardial injury and skeletal muscle damage - e.g., after cardiac surgery, polytrauma, or multiorgan damage. There may be a role therefore for newer more cardiac specific markers of myocyte damage.

Table 1.5
Causes of CK-MB increases not associated with myocardial damage

| |
|---|
| Release of nonmyocardial CK-MB |
| Trauma to muscle |
| Crush injury |
| Burns |
| Electrical injuries |
| Noncardiac surgery |
| Extreme physical exercise |
| Grand mal seizures |
| Various inflammatory and noninflammatory myopathies |
| Chronic renal failure |
| Hypothroidism |
| Chronic alcoholism |
| Myositis |
| Rhabdomyolysis |
| Hyperthermia and hypothermia |
| Ectopic CK-MB production in tumour patients |
| Decreased clearance of serum CK-MB |
| Hypo- and hyperthyroidism |

In unstable angina, the heterogeneity of the syndrome continues to make risk stratification difficult. Reliable tests that may be of help to physicians in routine clinical practice in deciding who should be referred for more intensive investigation, have to be non invasive, possible to carry out in a District General Hospital setting, and be measurable at a routinely convenient timepoint. Biochemical markers would appear to meet these criteria, but existing markers have produced mixed results as detailed above, perhaps due to problems with specificity. There may be a role therefore, for newer more cardiac specific markers in this acute coronary syndrome as well.

## Cardiac Troponin T

Cardiac Troponin T (cTnT) is a 37-kDa polypeptide sub-unit of the myofibrillar regulatory troponin complex (Greaser et al, 1973). It is found on the thin filament of striated muscle fibres along with actin, tropomyosin and the other protein sub-units of the troponin complex, Troponin C and Troponin I. The function of Troponin T is to bind the troponin complex to tropomyosin. It is mainly intracellular in its structurally bound form. In human

heart muscle approximately 6% of the total myocardial cTnT is found as a soluble, cytoplasmic pool, which probably serves as a precursor pool for the synthesis of the troponin complex (Mair et al, 1992). It is not present in the serum of the normal population. Because the amino acid sequence is unique to cardiac muscle, one can immunologically differentiate skeletal muscle and cardiac protein isoforms (Pearlstone et al, 1986) In the setting of acute myocardial ischaemia, where there is loss of integrity of myocardial cell membranes, cTnT is released into the circulation along with other proteins of the cardiac contractile apparatus (Katus et al, 1989).

Cardiac Troponin T assay

An enzyme-linked immunosorbent assay (ELISA) specific for cardiac TnT has recently been developed (Katus et al, 1989). The immunoassay is based on a single-step sandwich principle, with streptavidin-coated tubes as the solid phase and two monoclonal anti-human cardiac troponin T antibodies. Troponin T is bound on different epitopes by the capture and signal antibodies. The capture antibody is biotinylated, binds completely and reproducibly to the streptavidin-coated tube, and is 99% specific for cardiac troponin (Katus et al, 1992). The second antibody is labelled with horseradish peroxidase and has about 20% crossreactivity with skeletal muscle troponin T. Because of the capture antibody's high specificity, the assay is immunologically specific for cardiac troponin T. However, a cross-reactivity of 1-2% with purified skeletal muscle TnT at high concentrations was found due to non-specific absorption of skeletal muscle TnT to the assay tubes ( Katus et al 1991). The clinical experience with the cTnT assay by contrast (Mair et al, 1991), suggests that this theoretical cross-reactivity with purified skeletal muscle TnT may not be a relevant problem in clinical practice. The assay takes about 90 minutes and requires 200 microlitres of serum.

Cardiac troponin T may have several advantages over traditional serological markers of myocardial injury. For example, in acute myocardial infarction, cTnT appears in the circulation within 3.5 hours of the onset of chest pain, slightly earlier than the rise in CK activity, and concentrations remain increased for at least six days (Katus et al,1991). The

half life of cTnT is 120 minutes, so the sustained increase in serum concentration probably reflects continuing release of protein from disintegrating myofilaments (Lancet Editorial, 1991). Consequently, there is a long diagnostic window of 10.5-140 hours after the onset of pain (Katus et al, 1991), six times longer than the corresponding time interval for CK and nearly twice that for lactate dehydrogenase. The biggest advantage of cTnT may be its cardiospecificity. As previously mentioned, in situations where there is concommitant skeletal muscle damage, identification of myocardial cell necrosis can pose a problem for the laboratory, particularly if the ECG is unhelpful.

Katus et al have also noticed that minor rises of cTnT can also be found in unstable angina patients (Katus et al, 1991), and that some association existed between cTnT presence and severe coronary artery narrowing at subsequent angiography.

It is postulated that there may be a place for this marker in patients with acute ischaemic syndromes, and the examination of its potential role in routine clinical practice forms the purpose of this thesis.

**Intended Investigation.**

The specific aims of this thesis are:

1. To examine the cardiac specificity of cardiac Troponin T compared to other biochemical markers in the presence of skeletal muscle injury.
2. To examine the diagnostic performance of cardiac Troponin T in the differential diagnosis of ischaemic cardiac damage in patients admitted with chest pain
3. To examine the long term prognostic value of cardiac Troponin T concentrations in patients admitted with unstable angina.
4. To evaluate the potential role of cardiac Troponin T in risk stratification in patients admitted with unstable angina to a Coronary Care Unit compared with clinical and electrocardiographic parameters.
5. To qualitatively and quantitatively examine the coronary anatomy and culprit lesion morphology in patients with unstable angina in relation to cardiac Troponin T concentrations.
6. To examine the relationship between markers of activation of coagulation and cardiac Troponin T concentrations in patients admitted with unstable angina.
7. To examine the relationship between Lipoprotein (a) concentrations and cardiac Troponin T concentrations in patients admitted with unstable angina.

# Chapter Two

## Laboratory analytical methods

In this chapter, the methodologies used for the measurement of biochemical markers of myocyte damage and for the measurement of lipid and coagulation variables examined in this thesis are described.

### Biochemical markers of myocyte damage.

Total creatine kinase (CK)

Activity was measured using the reaction sequence:-

$$\text{creatine phosphate + ADP} \xrightarrow{\text{creatine kinase}} \text{creatine + ATP}$$

$$\text{ATP + glucose} \xrightarrow{\text{hexokinase}} \text{glucose 6 phosphate + ADP}$$

$$\text{glucose 6 phosphate + NADP} \xrightarrow{\text{glucose 6 phosphate dehydrogenase}} \text{6 phosphogluconate + NADPH}$$

Activity was measured at 37$^{O}$C, pH 6.6 using optimised reagents supplied by Boehringer (CK-NAC opt., BCL, Lewes, Sussex, cat no.475734) on a centrifugal analyzer (Encore, Baker Instruments) and a discrete analyzer (RA1000, Bayer Technicon Instruments) at different stages of the study.

Creatine kinase MB

Creatine kinase MB activity was measured using the Hybritech Tandem-E CKMB assay system. This is a solid phase two site immunoenzymetric "sandwich" assay. The solid phase consists of a monoclonal anti M antibody coupled to a plastic bead. This binds the M subunit of the CK-MB dimer. A second monoclonal anti-B antibody coupled to alkaline phosphatase binds to the B subunit of the MB dimer. Samples are incubated in the presence of both antibodies. The formation of a solid phase/anti-M/CK-MB/anti-B /alkaline phosphatase "sandwich" then occurs.

The bead is then washed to remove unbound anti-B. The assay system is specific for CK-MB. The anti B antibody does not bind to CK-MM. Hence although CK-MM may be bound to the solid phase there is no alkaline phosphatase activity linked to it. CK-BB is not bound by the anti-M linked to the solid phase. Hence any anti-B bound to CK-BB is washed away. The enzyme activity of the sandwich is measured using a two point assay by addition of para-nitrophenol phosphate as substrate for the alkaline phosphatase and measurement of the liberated para-nitrophenol. The change in absorbance is directly proportional to CK-MB concentration.

For the assay one bead was placed into each tube after blotting the residual droplet of storage buffer. The beads were not allowed to dry out. 100 microlitres of antibody conjugate was pipetted into each tube using a semiautomatic pipette followed by 100 microlitres of standard, zero, control or test as appropriate. The tubes were shaken to allow adequate mixing, covered with parafilm then incubated at room temperature for one hour on a horizontal rotator at 170 +/- 10 r.p.m.

Beads were then washed three times in 2 ml of a wash solution with total wash time not exceeding 15 minutes. 200 microlitres of freshly prepared enzyme substrate (para-nitrophenolphosphate in buffer) were then added, the tubes mixed, covered, and incubated for 30 minutes at room temperature. The reaction was then stopped by addition of 1.5ml of quench reagent, the tubes mixed and the absorbance read at 405 nm. Results were calculated as shown below.

CK-MB concentration =(Abs Sample-Abs zero blank) /(Abs Standard-Abs zero blank)

x F x S

Where F = dilution factor and S = standard concentration

Assays were performed in duplicate with each run containing 2 zero blanks, 2 standards, 2 low and 2 high controls. Samples were diluted with zero diluent where appropriate so that the maximum total CK activity in any sample did not exceed 500 U/l at $30^{o}C$.

Data is given by the manufacturer for within run and between run imprecision. Within run coefficient of variation (CV) is 9.2% at 16.3 micrograms/l CK-MB and 5.1% at 68.7 micrograms/l CK-MB. Between run CV is 10.3% at 16.5 micrograms/l CK-MB and 6.7% at 65.8 micrograms/l CK-MB.

Hydroxybutyrate dehydrogenase

Total hydroxybutyrate dehydrogenase activity was measured using the reaction:-

$$\text{alpha-oxobutyrate + NADH} \xrightarrow{\text{hydroxybutyrate dehydrogenase}} \text{alpha-hydroxybutyrate + NAD}$$

At 25$^{o}$C, pH 7.5, using optimised reagents supplied by Boehringer (HBD opt., BCL, Lewes, Sussex ) on a centrifugal analyzer ( ENCORE, Baker instruments), at 30$^{o}$C, pH 7.5 by an optimised method with commercially supplied reagents (Merckotest HBDH, BDH Diagnostics, Poole, Dorset); also using a centrifugal analyzer (Encore, Baker Instruments, Windsor, Berkshire) and at 37$^{o}$C, pH 7.5 by an optimised method with commercially supplied reagents (Merckotest HBDH, BDH Diagnostics,Poole, Dorset) on a PERSPECTIVE analyzer (American Monitor, Burgess Hill, West Sussex, UK) at different stages in the study.

Aspartate Transaminase

Aspartate transaminase was measured using the reaction sequence:-

$$\text{aspartate + alpha-ketoglutarate} \xrightarrow{\text{aspartate transaminase}} \text{oxaloacetate + glutamate}$$

$$\text{oxaloacetate + NADH} \xrightarrow{\text{malate dehydrogenase}} \text{malate + NAD}$$

At 37$^{O}$C on a SMAC I ( Technicon Instruments, Basingstoke) by the manufacturers recommended method, at 30$^{O}$C by an optimised method with commercially supplied reagents (Merckotest GOT(ASAT)) using a centrifugal analyzer (Encore, Baker Instruments, Windsor, Berkshire); also at 37$^{O}$C on a PERSPECTIVE analyzer (American Monitor, Burgess Hill, West Sussex, UK) by the manufacturer's recommended method with the manufacturer's reagents at different stages of the study.

Cardiac Troponin T

Cardiac Troponin T was determined by an enzyme linked immunosorbent assay (ELISA) using the ES-300 Immunoassay analyzer (Boehringer Mannheim, Lewes, Sussex). 200 microlitres of serum sample was pipetted into streptavidin coated tubes and incubated at 37$^{O}$C for 60 minutes with antibody reagent.

Cardiac Troponin T in the sample binds to 2 antibodies, one labelled with biotin the other with horse-radish peroxidase. The streptavidin binds the complex to the wall of the assay tube. Washing of the coated tube removes unbound enzyme conjugate.

Quantitation was achieved by the addition of a sodium perborate substrate and monitoring the increase in absorbance of a di-ammonium 2,2 azino-bis (3-ethylbenzothiazoline -6-sulphate) chromogen at 422nm after 25 minutes at 37$^{O}$C.

With an initial incubation of 60 minutes to allow antibody/antigen complex formation, the total assay time was 2 hours including sample handling. Inter-assay imprecision (coefficient of variation) was 12.3%, 7.7% and 4.2% at mean troponin T levels of 0.13 micrograms/L, 1.6 micrograms/L and 7.1 micrograms/L respectively. Intra-assay imprecision was 2.0% and 1.9% at mean troponin T values of 1.7 micrograms/L and 7.0 micrograms/L respectively.

## Measurement of Lipid variables

### Triglyceride

Triglyceride was measured using the reaction sequence:-

| | | |
|---|---|---|
| Triglycerides + $3H_2O$ | Lipoprotein lipase → | Glycerol + 3 fatty acids |
| Glycerol + ATP | Glycerol kinase → | Glycerol-3-phosphate + ADP |
| Glycerol-3-phosphate + ATP | Glycerol phosphate oxidase → | $2H_2O_2$ + ADP |
| Cholorophenol + 4-Aminoanti-pyrine + $H_2O_2$ | Peroxidase → | Red violet quinonemine dye + $4H_2O$ + HCL |

The principle of the procedure is that triglycerides are hydrolysed to glycerol and free fatty acids by lipoprotein lipase. The glycerol is then converted to glycerol-3-phosphate by glycerol kinase in the presence of ATP. The glycerol-3-phosphate is reacted with oxygen in the presence of glycerol-3-phosphate-oxidase, generating hydrogen peroxide.

This in turn, forms a coloured complex with aminoantipyrine and cholorophenol in the presence of the peroxidase. The intensity of the coloured complex is directly proportional to the triglycerides concentration of the sample and is measured photometrically at 525 nm.

All triglyceride measurement was by this enzymatic method on an automated analyser (Bayer Technicon, Basingstoke Hampshire) by the manufacturers recommended method (within run CV 3.0%, between run CV 5%). The reference range was 0.9 - 2.0 mmol/l.

Total Cholesterol

Cholesterol was measured using the reaction sequence :-

| | | |
|---|---|---|
| Cholesterol esters | Cholesterol esterase → | Cholesterol and fatty acids |
| Cholesterol + $0_2$ | Cholesterol oxidase → | Cholest-4-en-one + $H_2O_2$ |
| $2H_2O_2$ + 4-Aminoantipyrine + p-Hydroxybenzoate | Peroxidase → | $4H_2O$ + Quinoneimine dye + $CO_2$ |

The principle of the procedure is that cholesterol esterase completely hydrolyses cholesterol esters in serum to free cholesterol. Free cholesterol in the presence of oxygen and cholesterol oxidase generates hydrogen peroxide, which in turn is combined with 4-aminoantipyrine and a phenol derivative to form a quinoneimine dye. A red colour is produced which is directly proportional to the cholesterol concentration in the sample and is quantitated by an endpoint measurement at 525 nm.

All total cholesterol measurement was by this enzymatic method on an automated analyser (Bayer Technicon, Basingstoke Hampshire) by the manufacturers recommended method (within run CV 2.2%, between run CV 5%).The reference range was 3.5 - 6.5 mmol/l.

Apolipoproteins A-1 and B

Apoprotein A-1 (APA) and reagent, used in conjunction with a specific analyser and apolipoprotein calibrator (Beckman array, Beckman Instruments, High Wickham, Bucks.) was used for the quantitative determination of human APA by rate nephelometry. The method employed measures the rate of increase in light scattered from particles suspended

in solution as a result of complexes formed during an antigen-antibody reaction. In the chemical sequence human APA antibody is brought into contact with human APA in a sample. The increase in light scatter resulting from the antigen-antibody reaction is converted to a peak rate signal which is a function of the sample APA concentration. Following calibration, the peak rate signal is automatically converted to concentration by the analyser. The same method was used for apolipoprotein B estimation using human apolipoprotein B antibody.

The within run CV for apolipoprotein A was 2.5%, for apolipoprotein B 1.8%. The between run CV for apolipoprotein A was 5.7%, for apolipoprotein B 3.8%.

The reference ranges are for Apolipoprotein A -males 0.94-1.78, females 1.01-1.98g/l, and for Apolipoprotein B -males 0.63-1.33, females 0.60-1.26g/l.

Lipoprotein (a)

Lipoprotein (a) (Lp(a)) was determined by an enzyme-linked immunosorbent assay (ELISA) using sheep polyclonal monospecific antibodies against purified human Lp(a) (Biopool AB, Umea, Sweden). The flat bottomed polystyrene microtitre plate used, contained 96 wells of 12 columns and 8 rows. Human Lp(a) standards were pre-filled and lyophilized in column 1 of the plate. Sample blanks, controls and test samples were diluted with sample buffer and aliquots (20 microlitres) added to columns 2-12. The microtitre plate was then incubated for 2 hours at 25°C. The plate was then washed using a micro plate washer (Denley, Billinghurst, UK). This was followed by the addition of 200 microlitres of substrate mixture into all the wells. The plate was then incubated for 15 minutes at room temperature.

The enzymatic reaction was terminated by adding 50 microlitres of 3 mmol/l of sulphuric acid into all wells. The amount of Lp(a) in the serum samples were calculated by comparing the absorbance of the samples and standards at 492nm. The standard calibration curve was linear in the range 0-60 mg/dl. Samples of higher concentration were diluted with sample buffer and reanalysed.

All samples were measured in batches in duplicate by a single operator blinded to the final diagnostic classification of the patients The within run CV was 3.2%, and the between run CV was 6%. The reference median Lp(a) concentration for the laboratory was 7.9 mg/dl.

## Measurement of markers of coagulation

### Prothrombin fragment 1+2

Cleavage of prothrombin by factor Xa liberates the activation fragment 1+2 ($F_{1+2}$) which is a direct molecular marker of in vivo factor Xa activity and total thrombin activity. $F_{1+2}$ concentrations were measured by a new ELISA method (Boisclair et al, 1993). A microtitre plate for $F_{1+2}$ was developed based on the sandwich principle. The wells of a microtitre plate were first incubated for 16 hours at 4°C with 100 microlitres of an affinity purified neoantigen-specific antibody to $F_{1+2}$ in 100 mM sodium bicarbonate, pH 9.6 and were subsequently blocked with peptide-BSA conjugate (Pharmacia; Milton Keynes, UK). The wells were then incubated for 60 minutes at 37°C with 100 microlitres of a peroxidase conjugated IgG fraction of rabbit antiserum to prothrombin (Dakopatts; High Wycombe, UK).

Bound enzyme-antibody conjugate was visualised by incubation for 30 minutes at room temperature with 100 microlitres of a solution of 0.667 mg/ml 1,2-pheny-lenediamine in 100 mM citric acid-phosphate buffer, pH 5.0 containing 0.0125% hydrogen peroxide. After stoppinig the reaction absorbances were read on a plate reader at 490 nm ( MR700 from Dynatech; Billinghurst, UK).

Venepuncture was performed by trained senior nursing staff on patients admitted to the coronary care unit (CCU) prior to the initiation of antithrombotic therapy. Samples were collected into vacutainer tubes for biochemical and haemotological estimations; 4.5 mls of blood for measurement of activation of coagulation was taken into bottles containing 0.5 ml of 0.105M buffered sodium citrate (equivalent to 3.2%). Plasma samples were immediately mixed, centrifuged, the supernatants aliquoted and flash frozen in liquid

nitrogen on the CCU before transfer to a -80°C freezer. Samples were batch assayed in duplicate by a single operator blinded to the final diagnostic classification of the patients. The reference $F_{1+2}$ concentration obtained from healthy individuals was 19.2 ± 11.8 ng/ml (mean ± SD).

Fibrinogen

Fibrinogen was measured using a very high sensitivity calcium thromboplastin (PT-Fibrinogen HS PLUS) by a nephelometric method. The PT-Fibrinogen HS PLUS is a lyophilized extract from rabbit brain with the addition of an optimal concentraton of calcium ions. The reconstituted vial is mixed with citrated plasma aliquots and analysed on an automated coagulation laboratory analyser (Instrumentation Laboratory, Milan, Italy). This analyser is a nephelometric centrifugal analyser which measures the intensity of the light scattered by plasma before, during and after clot formation.
The delta light scatter reached at equilibrium is proportional to fibrin (and therefore to total clottable fibrinogen) concentration. All samples were collected into citrate and stored as above. Samples were batch assayed by a single operator blinded to the final diagnostic classification of the patients. The within run CV was 4.6%, and the between run CV was 6.2%. The reference range for the assay is <4.0 g/l.

**Methods for evaluation of diagnostic techniques.**

The diagnostic power of any test can be expressed as its sensitivity, the number of patients with disease correctly identified out of the total population studied. The total population with disease will therefore comprise patients correctly identified - those with a true positive diagnosis (TP) plus those in whom there has been a false negatives diagnosis (FN)). Sensitivity is usually expressed as

TP/TP + FN

Any test must not only be sensitive but also specific. It must recognise those without disease - the true negatives (TN). The total population without disease will comprise all those with a true negatives plus those giving a false positive (FP) diagnosis.

Hence specificity is calculated as

$$TN/TN + FP$$

The relationship of sensitivity and specificity can be expressed as test efficiency, calculated as

$$TP + TN/ TP + FN + TN + FP$$

Tests such as electrocardiography make a True/False (Boolean) classification - the patient has had a myocardial infarction or has not. Biochemical test results comprise numeric data with the intervals between data points affected by analytical imprecision. This permits a more sophisticated form of analysis.

A minimum threshold value can be defined optimising the sensitivity of a test - to "rule out" the diagnosis with maximum sensitivity. Similarly a maximum threshold value can be defined to optimise the specificity of the test - to "rule in". These two values are often not the same. The relationship between sensitivity and specificity that occurs at different values can be examined be determining sensitivity and specificity at differing levels of cut off value and plotting the resulting curve - receiver operator characteristic (ROC) curves. The optimum values for sensitivity, specificity and efficiency can be determined from the curve. An ideal test would give a rectangular curve.

In practice a curve approximating to this is seen with the point of inflection corresponding to maximum efficiency. ROC curve analysis has been applied to the assessment of biochemical testing (Robertson, 1981) and provides a convenient method of analysis for testing the cardiac specificity of cardiac Troponin T with other biochemical markers of myocyte damage.

# Chapter Three

## The cardiac specificity of cardiac Troponin T compared to other biochemical markers in the presence of skeletal muscle injury.

### Introduction

The differential diagnosis in patients presenting with chest pain where there may be skeletal muscle trauma creates a problem for the laboratory. A range of approaches have been suggested including creatine kinase (CK) isoenzyme separation, creatine kinase MB (CK-MB) isoenzyme/total CK activity ratio (Schwartz et al, 1988), CK MB concentration measurement and CK-MB mass/total CK activity ratio (Alaf et al, 1988). I compared the diagnostic performance of proposed strategies for the differential diagnosis of cardiac muscle damage with measurement of cardiac troponin T (cTnT) in the situation of elevation of CK due to arduous physical training .

### Methods

Royal Marine recruits were studied during Commando training. All had been pre-screened by full physical examination and electrocardiography both at entry, following basic training and during the training program. None had any cardiac symptoms at any time.

219 males were examined, age range 18 to 26 years, median 20.8 years. Serial blood samples were obtained from individuals during training at 1, 12, 19, 24 and 29 weeks. Sera were separated and stored at -70$^{0}$C prior to estimation for aspartate transaminase activity (AST), CK activity, CK-MB concentration and cTnT.

AST and CK were measured on all samples. Samples for further study were selected from those with maximal elevation of CK and AST. The majority of these were taken following a period of maximal physical exertion after one of the final qualification assignments. This comprised a 30 mile route march, in full webbing, carrying a forty pound pack, and a weapon. Substantial elevation of muscle enzymes were seen.

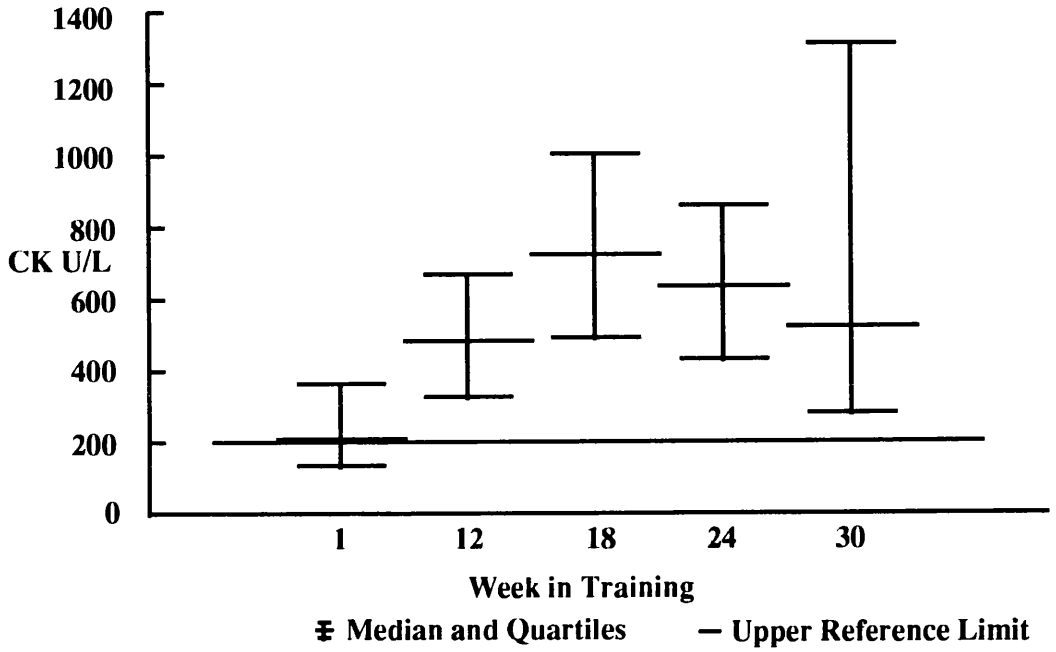
AST was measured at 30$^{O}$C on a SMAC I (Bayer Diagnostics, Basingstoke, Hampshire) by the manufacturers recommended method. Total CK was measured at 37$^{O}$C using commercially supplied reagents on a Technicon AXON by the manufacturers recommended method (Bayer Diagnostics, Basingstoke, Hampshire). CK-MB concentration was measured by a microparticle assay on an Abbot IMx (Abbot diagnostics, Maidenhead, Berkshire) by the manufacturers recommended method. Determination of cTnT was by an enzyme linked immunosorbent assay (ELISA) using the ES-300 Immunoassay analyzer (Boehringer Mannheim, Lewes, Sussex) . The upper reference limits for each analyte were as follows: AST 20 U/l, CK, 200 U/l; CK-MB concentration, 5 micrograms/l; cTnT 0.2 micrograms/l. Detailed methodologies of the assays were as described in the laboratory analytical methods chapter.

Specificity analysis was performed for the extreme case assuming a disease prevalence of zero. Specificity and confidence interval was calculated for each test by stepwise increments of the decision threshold. Each step interval corresponded to half of the quoted upper reference limit. Specificity curves were then generated using multiples of the upper reference limit to normalise the data and permit direct comparison of analytes. For CK-MB concentration/activity ratio a value of 0.04 micrograms/U was used, corresponding to the value described (Alaf et al, 1988) corrected for assay temperature.

## Results

The profile of muscle enzymes (CK and AST) values throughout training is shown in figure 3.1. A total of 235 samples were further studied, with CK-MB available on 189. Medians, ranges and quartiles were as follows: CK 229 (18-4518, lower quartile 133.5, upper quartile 363.7); CK-MB 4 (0-33, lower quartile 2.7, upper quartile 5.5); cTnT 0.0 (0.0-.17, lower quartile 0, upper quartile 0); CK-MB/CK ratio 0.02 (0.0-0.1988, lower quartile 0.0132, upper quartile 0.0305).

Figure 3.1 Median and quartiles for CK (upper graph) and AST (lower graph) values seen during Commando training of Royal Marine recruits.



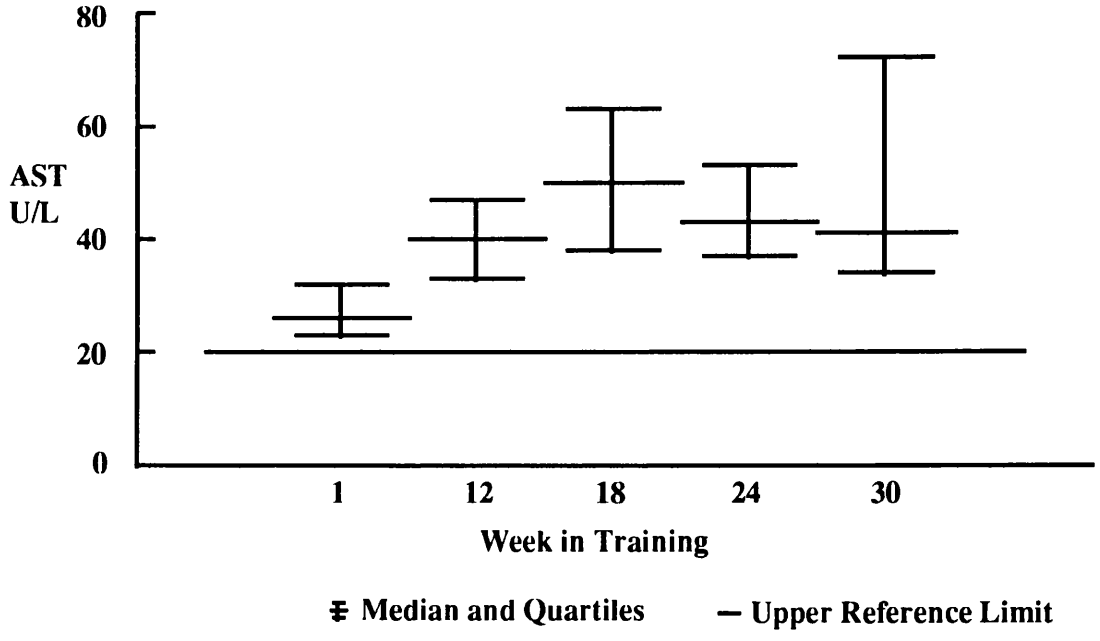
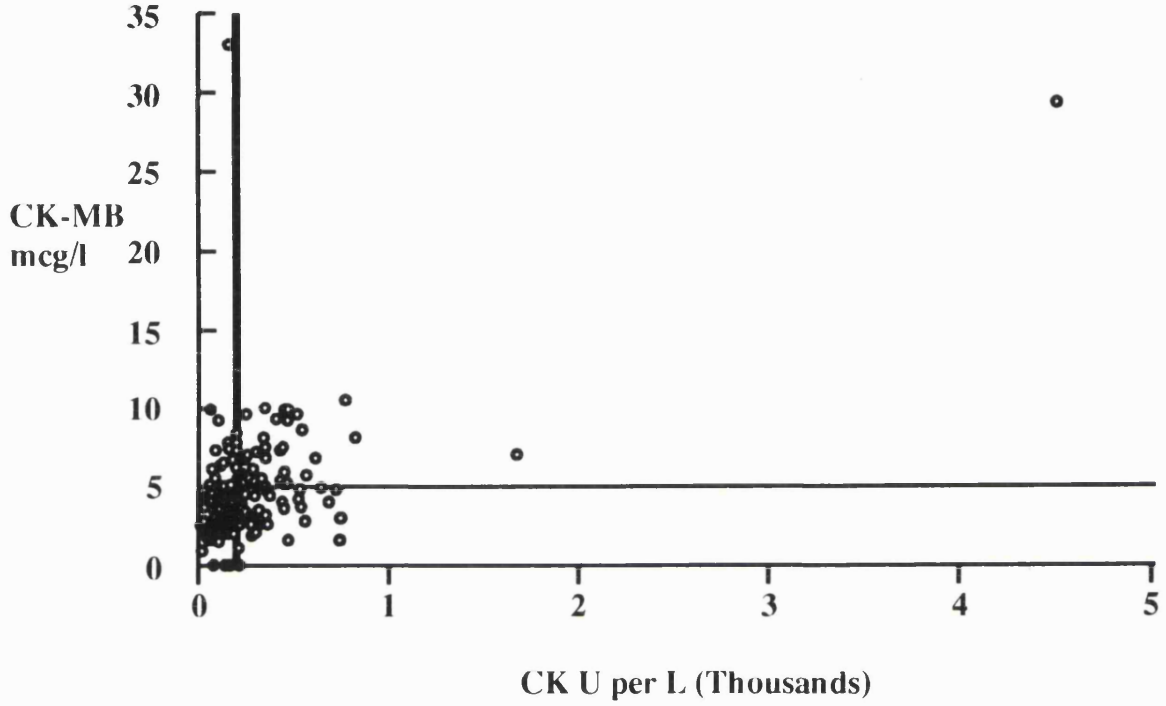



Figure 3.2 CK-MB concentration (upper graph) and cardiac Troponin T (lower graph) plotted against CK value for individual samples. Upper reference limits are shown for CK-MB ( horizontal line, 5 micrograms/L), cardiac Troponin T (horizontal line, 0.2 micrograms/L) and CK (200 U/l, vertical line solid line, both graphs).



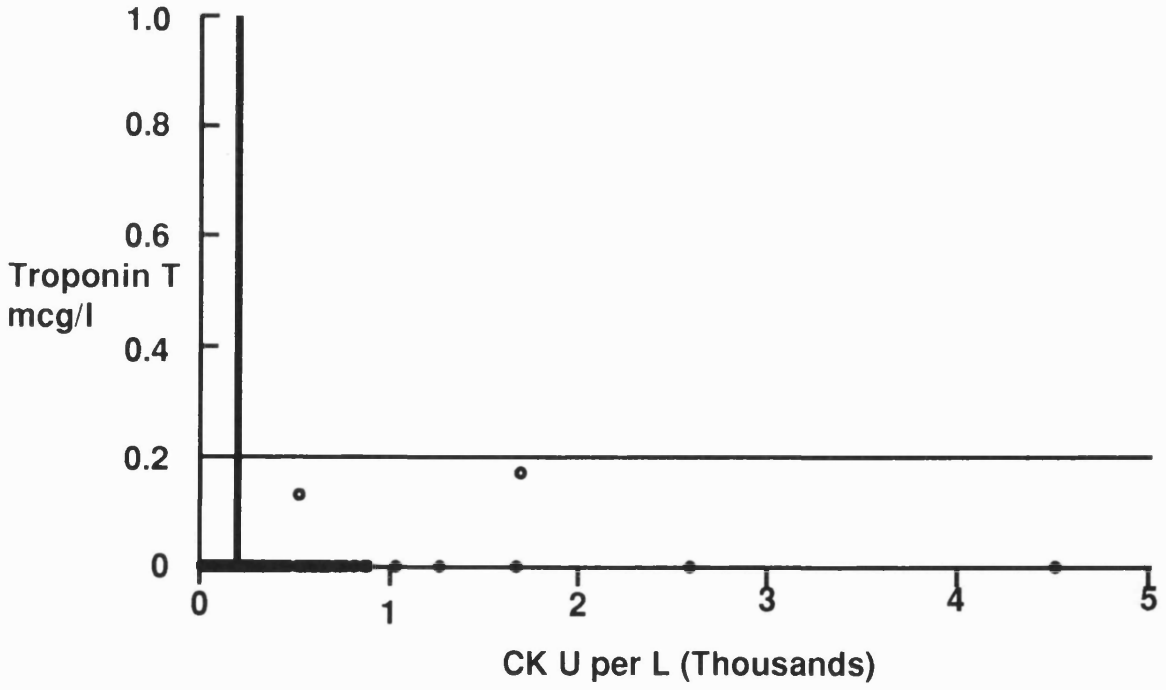
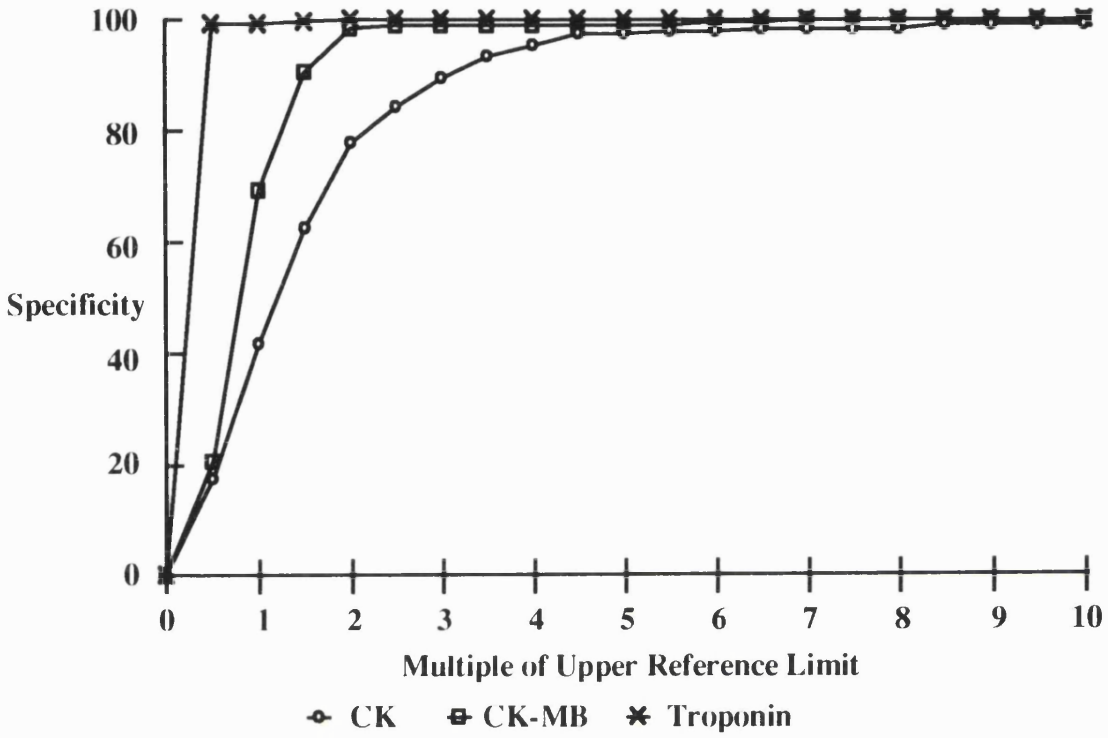



Figure 3.3 Specificity curves for CK, CK-MB concentration and cTnT normalised to multiples of the upper reference limit.




CK was elevated up to 22.6 times and CK-MB mass up to 6.6 times the upper reference limit. Only 2 individuals had detectable cTnT, neither exceeded 0.2 micrograms/L. Plots of CK-MB mass against CK and cTnT against CK are shown in figure 3.2.

Regression of CK-MB mass against CK by Deming's method (Deming, 1943) produced a best estimate of CK-MB mass/CK activity ratio of 0.0052 micrograms/U. Specificity curves for all three analytes are shown in figure 3.3, with data for CK-MB concentration/CK activity ratio in table 3.1. At the current decision thresholds for CK of 200 U/l (Collinson et al, 1989), CK-MB mass of 10 micrograms/l (Collinson et al, 1992), CK-MB concentration/CK activity ratio 0.04 and cTnT of 0.2 micrograms/l (Gerhardt et al, 1991) specificity with 95% confidence intervals were: CK 41.7% (35.4-48.0), CK-MB 98.4 (95.4-99.7), CK-MB concentration/CK activity ratio 86.24 (80.5-90.8), cTnT 100 (98.4-100.0).

Table 3.1 Specificity with confidence intervals for CK-MB concentration/activity ratio at increasing ratio values.

| CK-MB concentration/CK activity ratio micrograms/U | Specificity (Confidence interval) | |
|---|---|---|
| 0.03 | 73.54 | (66.7-79.7) |
| 0.04 | 86.24 | (80.5-90.8) |
| 0.05 | 90.48 | (85.4-94.3) |
| 0.06 | 92.59 | (87.9-95.9) |
| 0.07 | 93.65 | (89.2-96.7) |
| 0.08 | 94.71 | (90.5-97.4) |
| 0.09 | 96.83 | (93.2-98.8) |
| 0.1 | 96.83 | (93.2-98.8) |
| 0.11 | 97.88 | (94.7-99.4) |
| 0.12 | 97.88 | (94.7-99.4) |
| 0.13 | 97.88 | (94.7-99.4) |
| 0.14 | 98.94 | (96.2-99.9) |
| 0.15 | 99.47 | (97.1-100) |
| 0.16 | 99.47 | (97.1-100) |
| 0.17 | 99.47 | (97.1-100) |
| 0.18 | 99.47 | (97.1-100) |
| 0.19 | 99.47 | (97.1-100) |
| 0.2 | 100 | (98.1-100) |

## Discussion

In this study population, only cTnT was able to exclude myocardial damage with 100% specificity at the optimised decision threshold. None of the study group had cardiac symptoms or other evidence of cardiac disease despite large elevations of CK and elevations of CK-MB. There was no consistent relationship between CK value and CK-MB concentration, as shown by the poor performance of the CK-MB concentration/ activity ratio. Other studies on polytraumatised patients (Mair et al, 1991) and in CK and CK-MB increases following exercise (Mair et al, 1992) are in agreement with these findings. There has been one report in the rat that skeletal Troponin T may revert to a cardiac isoform in regenerating skeletal muscle (Sagin et al, 1990) producing false positive elevation of cTnT. This was not observed in this study group. The muscle enzyme profile

suggests that throughout the study period there was progressive muscle microtrauma, with presumed regeneration and repair. This indicates that in short term muscle trauma, cTnT determinations are cardiac specific. This does not mimic the situation seen in polmyositis. To date there is only one report of elevated cTnT in patients with polymyositis (Kobayashi et al,1992). Further studies need to be performed to clarify the situation in such patients, although providing categorical diagnosis may require skeletal and cardiac muscle biopsy. In conclusion, this study shows that in individuals undergoing arduous physical training, cTnT estimation allows accurate exclusion of a cardiac cause of CK elevation. Cardiac troponin T measurement is the investigation of choice for the differential diagnosis of patients with an elevated CK of skeletal muscle origin to exclude myocardial damage.
The high cardiospecificity of this marker suggested that its measurement may have a place in the differential diagnosis of patients presenting to hospitals with chest pain.

# Chapter Four

## Cardiac Troponin T measurement in the differential diagnosis of suspected ischaemic myocardial damage

### Introduction

The aims of this study were to examine the diagnostic performance of cardiac Troponin T (cTnT) for the differential diagnosis of suspected myocardial infarction; to examine the possible confounding effect of sample timing and thrombolytic therapy on diagnostic accuracy and to directly compare the diagnostic performance of cTnT determinations with that of an accurately timed CK-MB concentration measurement.

### Methods

Consecutive patients admitted to the coronary care unit with cardiac symptoms were studied. All were assessed by clinical examination, serial electrocardiography, measurement of aspartate transaminase (AST) and hydroxybutyrate dehydrogenase (HBD) for three consecutive days after admission. Time from onset of symptoms to time of first sample was recorded in all cases. Full clinical details were obtained on all patients including follow up data and clinical outcome.

On patients in whom myocardial infarction had been excluded further investigation was carried out by stress electrocardiography or radionuclide (dipyridamole enhanced thallium) scintigraphy. Coronary angiography was performed on patients with positive stress electrocardiograms or radionuclide scans, or when equivocal investigations were accompanied by a strong clinical suspicion of ischaemic heart disease. When ischaemic heart disease was excluded an alternative diagnosis was sought.

Infarction was judged to have occurred in patients with a history of chest pain accompanied by the development of Q waves, or ischaemic ECG changes and sudden death within 72 hours of admission. S-T and T wave changes were taken to indicate

infarction when serial AST and HBD values exceeded twice the upper reference limit. Alternatively, infarction was judged to have occured if there was evidence from cardiac imaging in patients without previous myocardial infarction (occluded vessels on subsequent angiography, an akinetic or hypokinetic segment on echocardiography or angiography or a persistent filling defect on radionuclide scintigraphy).

A diagnosis of angina was made in patients with clinical features suggestive of ischaemic heart disease prior to admission, no further symptoms following admission and no change in serial AST and HBD values and either transient S-T changes or a positive stress ECG.

Unstable angina was diagnosed in patients with chest pain lasting for 30 or more minutes when one (or more) of the following occurred after admission:

i) Persistent S-T segment shift or T wave changes without changes in serial AST or HBD values.

ii) Further cardiac symptoms (chest pain, chest discomfort or dyspnoea) with development of S-T segment shift or T wave changes.

iii) An initially normal ECG with serial AST and HBD below the upper reference limit during the first 48 hours following admission but persistent chest pain and development of either ECG changes typical of myocardial infarction or serial AST and HBD values exceeding the upper reference limit or both 48 hours after admission.

Non-ischaemic chest pain (NICP) was diagnosed when angina could be excluded by a definite alternative source of the chest pain or an atypical history with a negative stress ECG.

93 patients were studied, 66 males and 27 females, age range 36 to 82 years, median 62 years. Using the criteria defined above, 53 patients were considered to have sustained acute myocardial infarction, 4 to have had unstable angina, 23 to have had ischaemic heart disease without acute infarction and 13 to have had non-ischaemic chest pain. In the acute

myocardial infarction group, 22 received thrombolytic therapy with acyl plasminogen streptokinase activated complex (APSAC, Eminase), 8 with streptokinase.

Blood samples were obtained from patients on admission and 2,4,8,12,24 and 48 hours from admission. Sera were separated on the CCU, stored at 4 $^{O}$C and analyzed for CK within 24 hours, stored frozen at -20 $^{O}$C until analyzed for CK-MB concentration or stored frozen at -70 $^{O}$C until analyzed for cardiac Troponin T concentration. CK-MB determinations were performed only on the 0-8 hour samples, since previous studies had shown maximal diagnostic accuracy 8 hours from admission (Collinson et al, 1989), CK and cardiac Troponin T were determined on all samples. Assay procedures were as previously described.

Receiver operator characteristic (ROC) curves were generated from all the cTnT data using sample time from admission and sample time corrected for time of onset of chest symptoms with values grouped into 4 hour intervals. The optimised cut-off for cTnT was selected from the point of inflection of the ROC curves showing the closest approximation to the idealised ROC function.

The diagnostic performance of cTnT was then examined by sensitivity and specificity analysis using the selected cut-off for cTnT. Optimised cutoffs for CK and CK-MB concentration, taken from previous studies (Collinson et al, 1992), were CK, 120 U/L and CK-MB 10 micrograms/L.

Sensitivity and 100 - specificity was plotted for time from admission for all cTnT data without correction for time of onset of chest pain, including patients with symptoms of more than 12 hours duration on admission, to determine the time criticality of measurements.

Direct comparison with an optimised diagnostic strategy was then performed by exclusion of patients with symptoms of more than 12 hours duration on admission. Sensitivity and 100 - specificity plots were generated for cTnT, CK and CK-MB using time from admission and time from onset of chest pain. All plots were constructed including and excluding patients receiving thrombolytic therapy.

## Results

The optimised diagnostic cutoff for cardiac Troponin T was 0.2 micrograms/L for all the sample times studied. The effect of altering the diagnostic cut-off on sensitivity, specificity and efficiency at 12 hours is illustrated in Figure 4.1 and summarised in table 4.1. Efficiency is maximal at 96.92% for values between 0.2 to 0.3 micrograms/L but sensitivity falls with a value above 0.2 micrograms/L.

Sensitivity and 100 - specificity curves following admission for all patients, including those with symptoms of more than 12 hours duration on admission are shown in figures 4.1, 4.2 and 4.3. Cardiac Troponin T reaches optimum diagnostic performance 12 hours from admission and remains 100% sensitive up to 24 hours. Comparison of the curves shows that exclusion of patients receiving thrombolytic therapy has no effect on the diagnostic performance of cTnT estimation. Sensitivity and specificity at 12 hours are respectively 100% and 93.9% excluding thrombolysed and 100% and 93.9% including thrombolysed patients.

Correcting for time from onset of chest pain, or excluding those with symptoms of more than 12 hours duration on admission improves the diagnostic performance . Sensitivity for cTnT increases to 100% at 48 hours from admission or onset of symptoms; hence the diagnostic window for cTnT is increased to 48 hours from admission or onset of symptoms.

2 patients classified non-infarct showed cTnT values exceeding 0.2 micrograms per litre. One had sustained a myocardial infarction 10 days previously. The second had unstable angina with elevation of CK-MB concentrations which just failed to exceed the diagnostic cut off. It is likely that this patient had a non Q wave infarction. Data for both of these patients is presented in table 4.2. Exclusion of both these cases increases the specificity of cTnT to 100%.

Figure 4.1. Sensitivity, specificity and efficiency curves for cardiac Troponin T twelve hours from admission at increasing cut off levels.

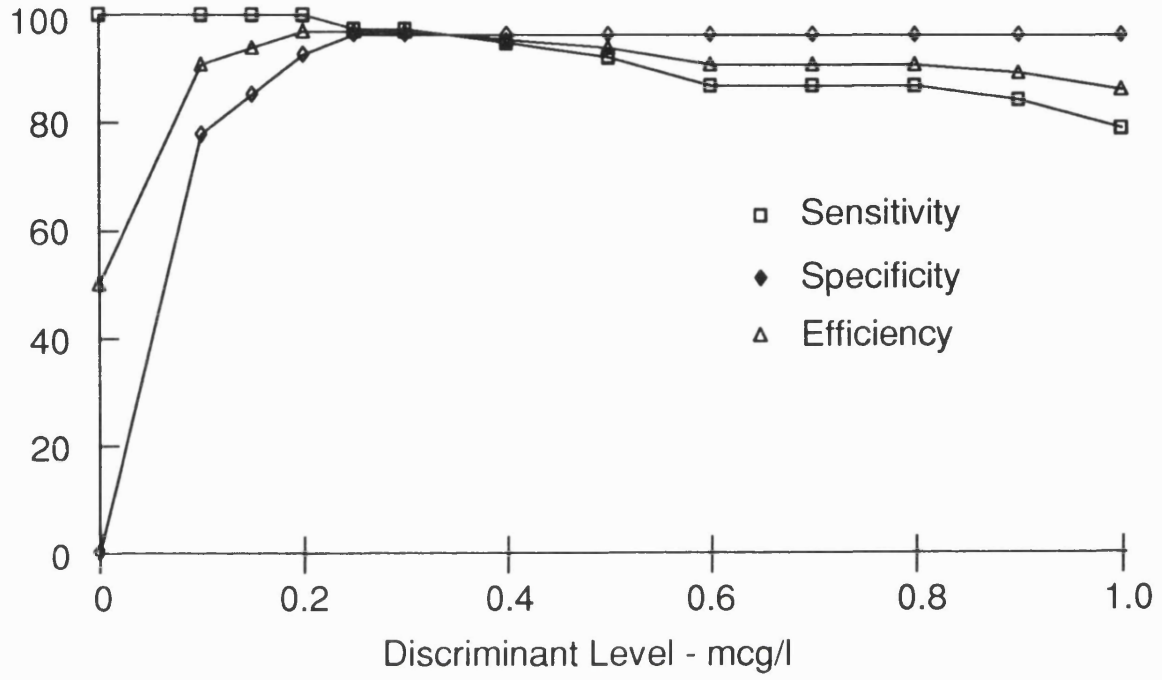
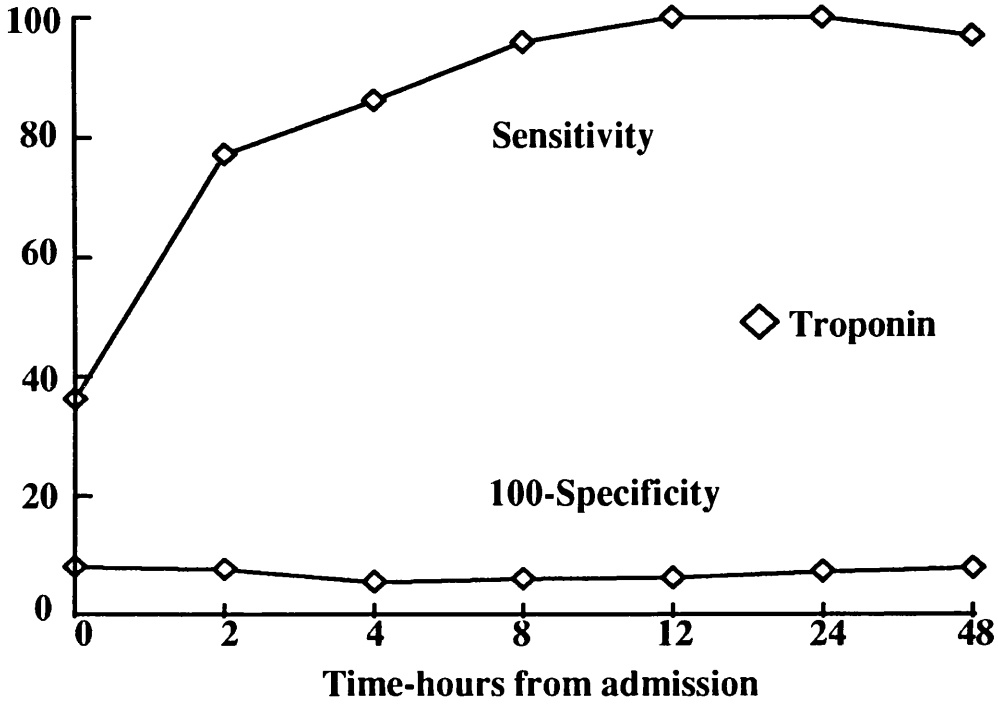



Table 4.1 Sensitivity, specificity and efficiency values for cardiac Troponin T twelve hours from admission at increasing cut off levels.

| Cutoff - micrograms/L | Sensitivity | Specificity | Efficiency |
|---|---|---|---|
| 0.1 | 100 | 77.78 | 90.77 |
| 0.15 | 100 | 85.19 | 93.85 |
| 0.2 | 100 | 92.59 | 96.92 |
| 0.25 | 97.37 | 96.3 | 96.92 |
| 0.3 | 97.37 | 96.3 | 96.92 |
| 0.4 | 94.74 | 96.3 | 95.38 |
| 0.5 | 92.11 | 96.3 | 93.85 |
| 0.6 | 86.84 | 96.3 | 90.77 |
| 0.7 | 86.84 | 96.3 | 90.77 |
| 0.8 | 86.84 | 96.3 | 90.77 |
| 0.9 | 84.21 | 96.3 | 89.23 |
| 1.0 | 78.95 | 96.3 | 86.15 |

Figure 4.2 Sensitivity and specificity curves with time for cardiac Troponin T concentration. Graphs are time from admission excluding (top) and including (bottom) thrombolysed patients.



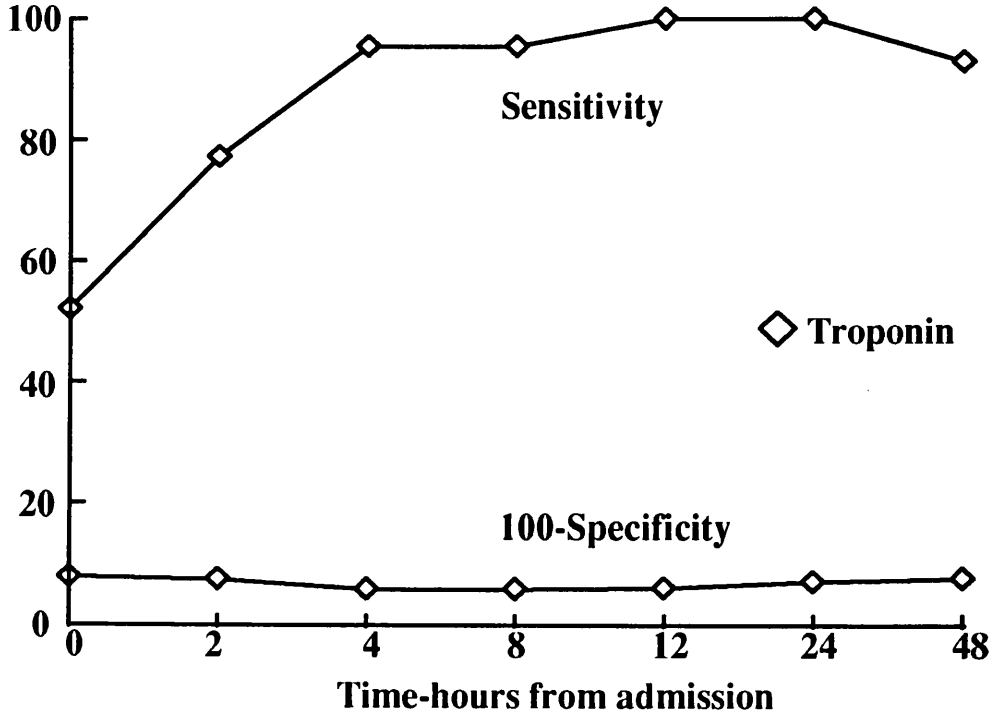
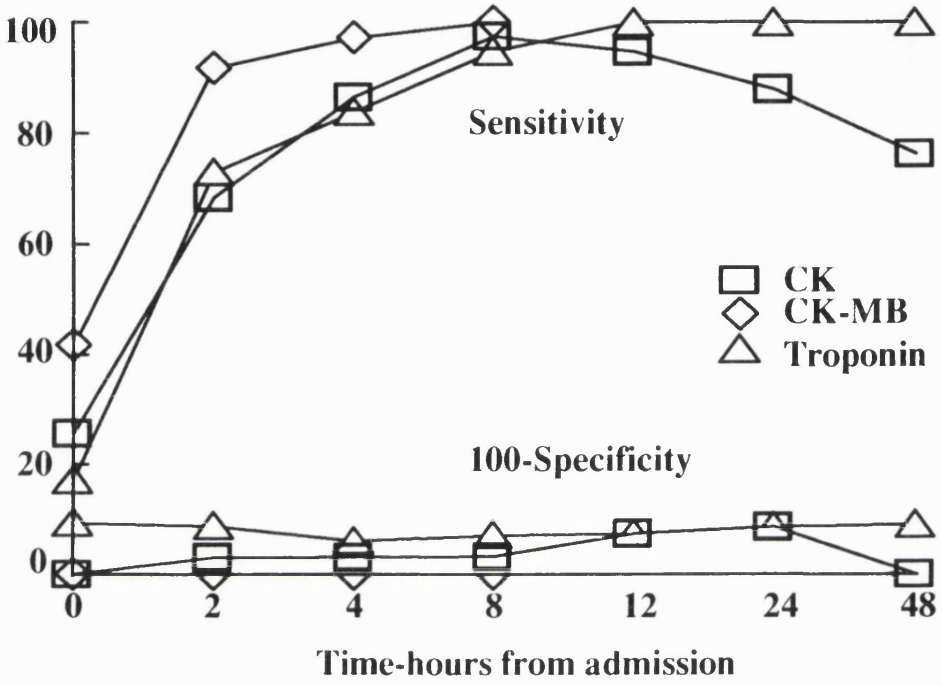



Figure 4.3 Sensitivity and specificity curves with time for CK, CK-MB concentration, and cardiac Troponin T concentration in patients with symptoms of twelve hours or less. Graphs are time from admission including (top) and excluding (bottom) thrombolysed patients.



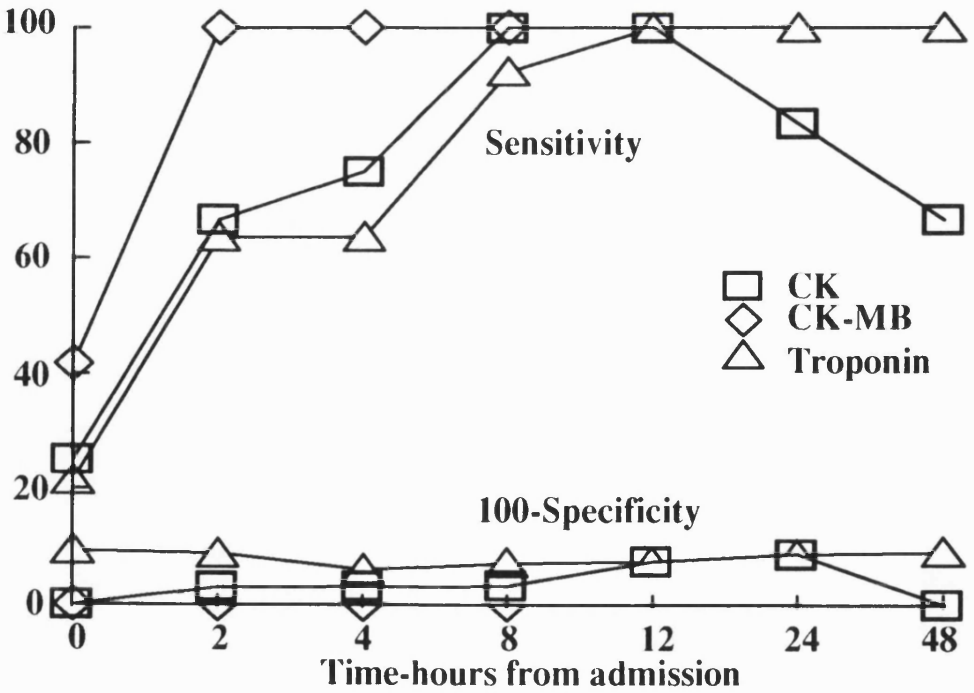
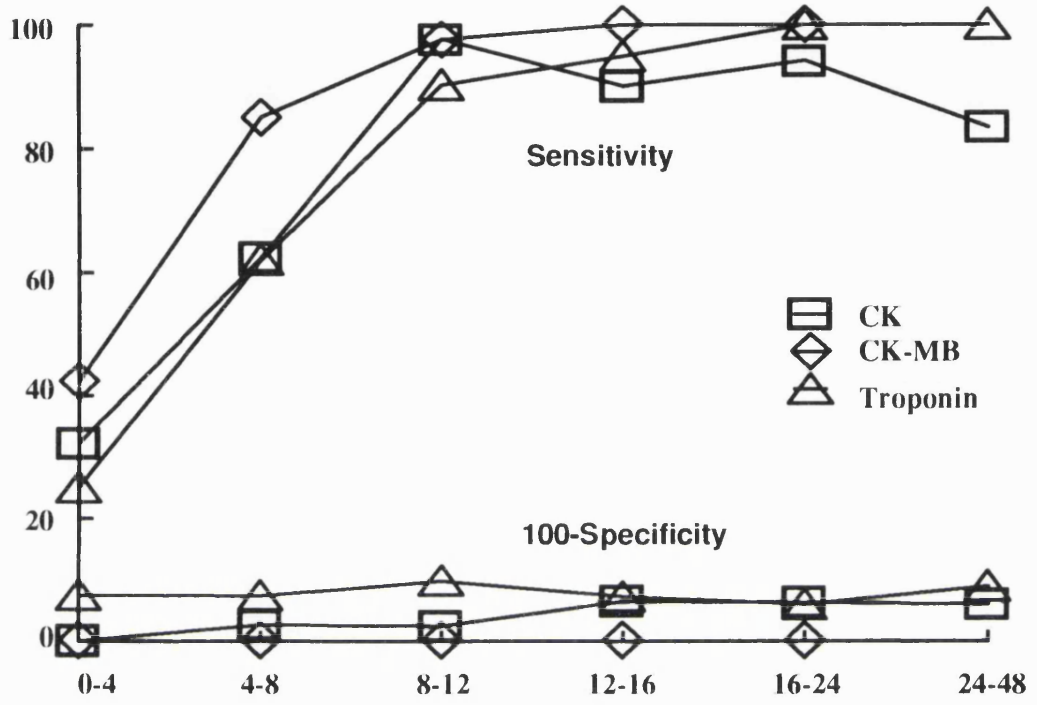



Figure 4.4 Sensitivity and specificity curves with time for CK, CK-MB concentration, and cardiac Troponin T concentration in patients with symptoms of twelve hours or less. Graphs are time from onset of chest pain including (top) and excluding (bottom) thrombolysed patients.



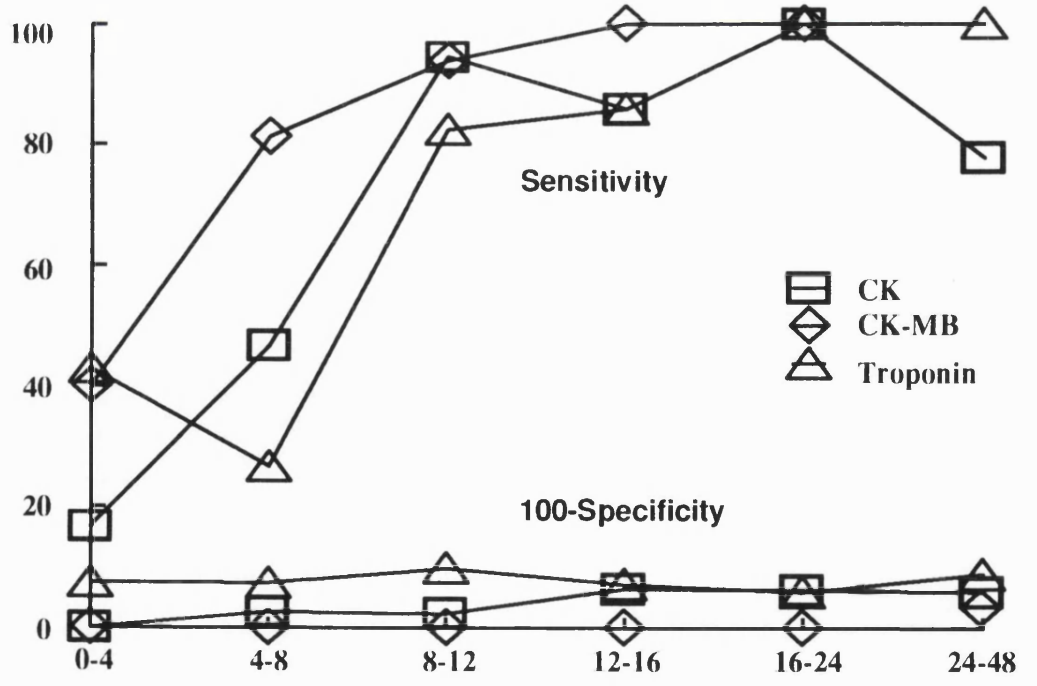



Table 4.2 Summary of results on the two patients classified as unstable angina with cardiac Troponin T exceeding 0.2 micrograms/L.

| Time - hours | CK (u/L) | CK-MB concentration (micrograms/L) | cardiac Troponin T concentration (micrograms/L) |
|---|---|---|---|
| Patient 1 | | | |
| 0 | 97 | 2.7 | 0.28 |
| 2 | 94 | 8.8 | 0.29 |
| 4 | 121 | 2 | 0.22 |
| 8 | 153 | 2 | 0.24 |
| 12 | 139 | | 0.22 |
| 24 | 81 | | 0.22 |
| 48 | 35 | | 0.23 |
| Patient 2 | | | |
| 0 | 32 | 5.6 | 4.36 |
| 2 | 33 | 4.7 | 3.82 |
| 4 | 32 | 6.3 | 3.88 |
| 8 | 31 | 5.1 | 3.7 |
| 12 | 31 | | 3.75 |
| 24 | 30 | | 2.65 |

Four patients with a final diagnosis of angina had detectable (>0.1 mcg/l) cardiac Troponin T values. All four had cardiac events during the subsequent six months. This did not occur in the remaining angina patients. No patients where ischaemic heart disease was excluded had detectable cardiac Troponin T.

## Discussion.

The cut off of 0.2 micrograms per litre exceeds the value quoted by the manafacturers who claim any cardiac Troponin T (>0.1 micrograms/L) indicates myocardial damage. Although initial studies used values of 0.5 micrograms/L and 1.0 micrograms/L (Katus et al, 1991), a subsequent multi-centre study by the same group quotes a cutoff of 0.2 micrograms/L (Gerhardt et al, 1991). A second group derived an upper reference limit of

0.5 micrograms/L (Mair et al,1991). This study demonstrates that a value of 0.2 micrograms/L is appropriate and a value of 0.5 micrograms/L is too high.

Direct comparison of identical patients shows that cTnT offers no advantage over CK-MB, or even CK in the unthrombolysed case when sample timing is accurate and patients with symptoms of more than 12 hours duration on admission are excluded. This represents the ideal situation which may not be achieved in routine clinical practice. Cardiac Troponin T determination is minimally affected by the vagaries of sample timing, reflecting the reported (Katus et al, 1989) long period of elevation (12 hours to 10 days) or thrombolysis. The diagnostic performance of CK-MB is known to reach a peak at 19-24 hours from onset of chest pain and to decline thereafter (Leung et al, 1989). Cardiac Troponin T retains diagnostic accuracy up to 48 hours from onset of chest pain or 24 hours from admission. Hence a single estimation of cTnT twelve hours from admission fulfils the criterion of a single diagnostic test to confirm or exclude myocardial infarction.

Analytical considerations and requirements for clinical management must also be considered. The comment has been made that early confirmation or exclusion of infarction does not identify the problem patients (Eagle,1991), those with unstable angina. Cardiac Troponin T estimations show potential for recognising those patients with angina at high risk of subsequent cardiac events. Elevated cTnT was reported in 16 patients originally classified as unstable angina then reclassified as minimal myocardial damage on the basis of retrospective review of CK-MB measurements (Gerhardt et al, 1991). There were 4 in hospital deaths in this group, but no long term follow up data is presented. This group may comprise small myocardial infarcts missed by conventional markers, a possibility in one of the cases presented in table 4.2 .

In conclusion, the study showed that a single measurement of cardiac Troponin T 12-24 hours from admission or 12-48 hours from onset of chest pain will confirm or exclude myocardial infarction. This study also suggested that cardiac Troponin T may have a role to play in risk factor stratification in patients admitted with unstable angina and so further studies were undertaken.

# Chapter Five

## The study population and methodology for examination of the prognostic role of cardiac Troponin T in patients admitted with chest pain

### Introduction

In the study described in the previous chapter, detectable levels of cardiac Troponin T in patients admitted with angina appeared to be associated with an unfavourable outcome on short term follow up. In order to examine whether this marker had any long term prognostic value and to evaluate any potential role in risk stratification in patients admitted with chest pain, a much larger study was undertaken examining patients admitted to a single Coronary Care Unit in a district general hospital

### Methods

This was an observational study. Guidelines for the initial medical management, and suggestions for subsequent management, of patients admitted to the Coronary Care Unit (CCU) by the admitting physicians were provided by the cardiac team, but clinical freedom was allowed and frequently exercised. Following discharge from the CCU, patients remained under the care of their admitting physician who decided future management both in terms of need for referral for cardiological opinion or investigation. Patients who were referred for invasive investigation underwent coronary angiography at a number of different centres. The need for, and timing of, revascularisation was made by the receiving interventional cardiologists independent of the referring physician. All management decisions were made without knowledge of the patients Troponin T status. Patients admitted to the CCU had frequent blood samples taken throughout the first 48 hours of admission as previously described and approved by the local ethical committee. The samples were separated on the Coronary Care Unit and the serum immediately frozen before storage in a $-80^{0}$C freezer. The routine cardiac enzyme protocol in the hospital of

measurement of aspartate transaminase (AST) and hydroxybutyrate dehydrogenase (HBD) daily for consecutive days formed the biochemical variables used for patient management along with clinical and electrocardiographic (ECG) findings.

Daily creatine kinase (CK) estimations and the daily AST and HBD values were used for the final biochemical diagnostic coding for the admissions.

## Clinical Data

Full clinical details were recorded on all patients by proforma. Particular attention was paid to previous cardiac history, classification of chest pain before entry, incidence of chest pain during admission, admission and subsequent inpatient ECG abnormalities, inpatient and discharge drug management and subsequent investigations and treatment. The various components of the chest pain before entry were recorded and also graded using the commonly used Canadian Cardiovascular Society Angina Classification (CCSC) (Table 5.1).

Table 5.1. Canadian Cardiovascular Society Angina Classification.

| Class | Activity Evoking Angina | Limits to Normal Activity |
|---|---|---|
| 1 | Prolonged exertion | None |
| 11 | Moderate exertion | Slight |
| 111 | Mild exertion | Marked |
| 1V | Minimal or rest | Severe |

Adapted from: Campeau L. Grading of angina pectoris. Circulation 1976;54:522-523.

Follow up for survival, interventions and mortality was by examination of hospital records, postmortem results where available, death certificates, general practitioner questionnaire, patient or next of kin questionnaire, with follow up telephone contact if required. Survival status and cause of death were established for all patients. Cause of death was classified according to American Heart Association criteria (Gillum et al, 1984).

**Diagnostic classifications**

The final diagnostic classification for each patient was made retrospectively, when all of the biochemical, clinical and follow up data had been collected. It was considered that this method would allow a more accurate diagnostic grouping for the patient cohort.

Myocardial infarction was diagnosed using World Health Organisation criteria (WHO, 1971); chest pain, ECG changes, cardiac enzymes at least twice the upper reference limit. Unstable angina was diagnosed if all enzyme measurements (CK, AST, HBD) were below twice the upper reference range throughout the routine sampling period; there was no progression to myocardial infarction within the first 48 hours of admission, and evidence of active ischaemic heart disease was proven by either follow up cardiac event, coronary angiography, exercise treadmill test or by radio-isotope study.

Patients with a final diagnosis of unstable angina were dichotomised for Troponin T status according to their cTnT value at 12-24 hours from admission . A value of $\geq$ 0.2 ng/ml was taken to be positive as this value and timing from the previous studies appeared to be the cut off level at which the marker was at its most efficient.

Non Ischaemic Chest Pain was diagnosed if angina was excluded by an alternative source of pain, or a negative exercise treadmill test, or normal coronary anatomy on subsequent angiography.

## Study endpoints

The primary endpoints of interest were:

1. Cardiac death.
2. Coronary revascularisation (Percutaneous Transluminal Coronary Angioplasty or Coronary Artery Bypass Grafting)
3. Cardiac death or coronary revascularisation as first event.

The secondary endpoints examined were:

1. Cardiac death or readmission with non fatal Myocardial Infarction as first event.
2. Readmission with either myocardial infarction or unstable angina as first event.

A further question it was hoped to answer was the effect of intervention compared to no intervention on survival in the Troponin T positive unstable angina subgroup.

## Statistical Analysis

Baseline demographics were expressed as percentages or means and standard errors. Differences in the baseline demographics between the two unstable angina groups were tested by chi-squared statistics for categorical variables and by Mann Whitney U tests for ordinal variables.

Survival curves for the whole study population for the end point cardiac death and for the Troponin T status groups for all end points were computed using the Kaplan-Meier method.

The differences between the positive and negative Troponin T status unstable angina groups with regard to frequency of the determined endpoint were tested by the chi-

squared statistic for the end points coronary revascularisation and for cardiac death or revascularisation, and by the Mantel-Haenszel statistic for the end points cardiac death and cardiac death or readmission with non fatal myocardial infarction. This latter statistic was used to allow for the potential confounding influence of coronary revascularisation.

All of the clinical, risk factor and management variables were entered into multiple logistic regression analyses for each endpoint. The predetermined variables entered were Troponin T status, thrombolysis and the collapsed variables of further chest pain or ECG changes (ST segment depression or T wave inversion) following admission and further chest pain or a rise in any of the conventional cardiac enzyme measurements to above the upper reference but to less than twice the upper reference limit. These two groups of collapsed variables were an attempt to emulate indices that have been used to risk stratify unstable angina patients (Heng et al 1976, Nordlander et al, 1979, Fahri et al, 1986, Murphy et al, 1992).

# Chapter Six

# The prognostic role of cardiac Troponin T in patients admitted with chest pain

## Study results

Cardiac Troponin T results were available for 513 admissions. Only the first admission was included in the analysis and so 460 patients were finally studied. The patients were followed for a median of 1032 days (lower quartile 858 days, upper quartile 1307 days).

## Baseline population demographics

These are summarised in table 6.1. Using the diagnostic classifications previously described (Page 52) , 230 patients had a final diagnosis of Myocardial Infarction, 183 a final diagnosis of unstable angina and 47 patients a final diagnosis of non ischaemic chest pain. Risk factors and cardiac symptoms prior to entry were not significantly different in the acute coronary syndrome groups with the exception of an increase in the percentage of patients who had suffered a previous myocardial infarction in the unstable angina group. Of particular interest is the uniformity of rest pain in the forty eight hours prior to admission across the groups, and the lack of difference between the unstable angina and non ischaemic chest pain groups with rest pain on admission, which has been noted by others (Murphy et al, 1992) to be of limited value in risk stratification in a similar population. 33.9% (62/183) patients whose admission was classified as unstable angina were positive for cardiac Troponin T, comparable with findings of other groups (Hamm et al, 1992., Katus et al, 1991., Seino et al, 1993., Ravkilde et al,1993).

Table 6.1.Baseline study population demographics.Values are expressed as percentages or means with standard errors.

| Variable | Myocardial Infarction | Unstable Angina | Non Ischaemic Chest Pain | Unstable Angina | | p value |
|---|---|---|---|---|---|---|
| | | | | TnT +ve | TnT -ve | |
| Number | 230 | 183 | 47 | 62 | 121 | - |
| Age (years) | 61.7(.69) | 60.9(.85) | 51.2(1.4) | 62.8(1.3) | 59.9(1.1) | +.0892 |
| Male sex | 76.1% | 71.6% | 59.6% | 67.2% | 75.6% | .2311 |
| FHIHD(<55) | 21.3% | 18.0% | 25.5% | 11.29% | 21.49% | .0850 |
| Hypertension | 26.5% | 26.8% | 25.5% | 27.42% | 26.45% | .8884 |
| Diabetes Mellitus | 15.2% | 15.8% | 2.1% | 20.97% | 13.22% | .1820 |
| Current smoker | 37.4% | 30.6% | 51.1% | 37.09% | 27.28% | .1575 |
| Previous MI | 16.1% | 36.1% | 4.3% | 32.26% | 38.02% | .4438 |
| CCSC 3 or 4 | 10.9% | 15.3% | 10.6% | 14.51% | 15.70% | .8333 |
| Accelerated angina | 40.0% | 41.5% | 17.0% | 43.55% | 40.50% | .6924 |
| Rest pain before admn. | 34.8% | 41.0% | 38.3% | 38.71% | 42.15% | .6552 |
| Rest pain on admn. | 87% | 68% | 66% | 70.1% | 66.1% | .5074 |
| Time to CCU from worst pain (hrs) | 8.14(.66) | 8.01(.65) | 11.71(1.9) | 6.64(.88) | 8.84(.91) | +.3130 |
| Abnormal ECG on admission(ST or T) | 88.3% | 42.6% | 19.1% | 50.00% | 40.87% | .1620 |
| Heparin on CCU | 57% | 46.4% | 51.1% | 40.32% | 49.59% | .2356 |
| Duration of Heparin(hrs) | 64.4(3.2) | 62.3(4.4) | 60.8(14.2) | 72.6(8.2) | 57.9(5.2) | +.0447 |
| Inpatient Aspirin | 78.2% | 72.1% | 67.9% | 71.4% | 74.7% | .5506 |
| Inpatient IV nitrates | 72.6% | 53.8% | 48.2% | 58.9% | 54.0% | .6440 |
| Inpatient B-blocker | 25.6% | 25.4% | 12.5% | 32.1% | 27.03% | .4917 |
| Inpatient Ca-antagonist | 16.5% | 43.1% | 17.9% | 44.6% | 39.6% | .5365 |
| Pain in CCU (≥1 ) | 41.7% | 27.9% | 34.0% | 25.81% | 28.93% | .6320 |
| Pain as inpatient (≥1 ) | 23.5% | 23.0% | 19.1% | 27.42% | 20.66% | .3048 |
| ECG changes with pain | 9.6% | 12.6% | 0% | 16.13% | 10.74% | .2290 |
| Thrombolysed | 77.8% | 21.9% | 8.5% | 32.26% | 16.53% | .0151 |
| Length of stay (days) | 13.9(2.3) | 9.05(1.1) | 4.65(.5) | 9.7(.58) | 7.1(.47) | +.0001 |
| Diag.Troponin(≥0.2mcg/l) | 9.1(.72) | 0.33(.07) | 0.02 | 1.0(.13) | 0.02 | +.00001 |
| Discharge Aspirin | 71.7% | 72.7% | 53.2% | 77.42% | 70.25% | .3041 |
| Discharge Warfarin | 8.7% | 3.8% | 4.3% | 4.84% | 3.31% | .6170 |
| Discharge B-blocker | 25.2% | 26.2% | 17.0% | 29.03% | 24.79% | .4770 |
| Discharge Nitrate | 23.5% | 37.2% | 19.1% | 46.77% | 32.3% | .0450 |
| Discharge Ca-antagonist | 5.2% | 36.6% | 12.8% | 40.32% | 34.71% | .4570 |

Legend : FHIHD= Family history of heart disease less than 55 years of age; CCSC 3 or 4= Canadian Cardiac Society Angina Class 3 or 4 before admission; Accelerated Angina=Increasing chest pain in the 48 hours before admission;ST or T= ST segment changes or T wave inversion on the ECG; Diag.Troponin=The cardiac Troponin T value at 12-24 hours from admission.The statistical significance column represents the differences in baseline demographics between the Troponin T positive and Troponin T negative unstable angina patients as assessed by the Chi-squared statistic for categorical variables and +Mann U Whitney statistic for ordinal variables.

Patients with unstable angina who were cTnT positive were more likely to receive thrombolytic therapy on clinical grounds (p=0.01), to receive heparin for a longer period (p=0.04), to require more discharge anti-anginal medications (mean 1.94 v 1.63, p= 0.035) and to stay in hospital longer (p=0.0001). The cTnT -ve group were slightly but not significantly younger (p=0.08) and more likely to have a family history of premature heart disease (p=0.09).

No other significant differences between the two unstable angina groups were seen with regard to risk factors for heart disease, cardiac symptoms prior to admission, initial medical management on CCU and discharge therapy, ECG abnormalities on admission or whilst an inpatient, or the incidence of further chest pain after admission.

## Statistical analyses.

### All patients

The survival curves for the study diagnostic groups for the end point cardiac death are shown in Figure 6.1. There have been no deaths or interventions in the non-ischaemic chest pain group.

14.2% (26/183) of the unstable angina group and 18.3% (42/230) of the myocardial infarction have died (p= NS) (Table 6.2).

### Unstable angina patients

Survival curves for the unstable angina groups dichotomised according to cTnT status were generated for each endpoint, as were frequency statistical analyses. All of the variables in Table 6.1 were entered into the multiple logistic regression model but only those variables that were either predetermined as defined in the previous chapter or that reached conventional statistical significance (p = <0.05) are shown .

Figure 6.1. Kaplan Meier survival curves of all diagnostic groups for the endpoint cardiac death.

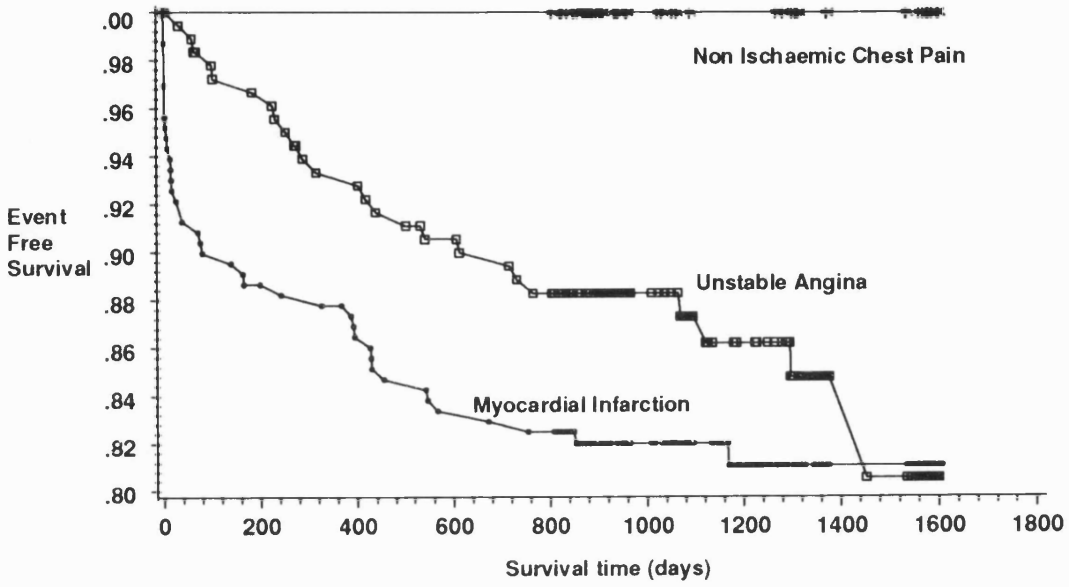



Table 6.2. Frequency of cardiac death for all final diagnostic groups

| Diagnostic classification | Number | mortality | percent |
|---|---|---|---|
| Non Ischaemic Chest Pain | 47 | 0 | 0 |
| Unstable Angina | 183 | 26 | 14.2 |
| Myocardial Infarction | 230 | 42 | 18.3 |

## Cardiac Death

19.3% (12/62) of the cTnT positive unstable angina group versus 11.5% (14/121) of the cTnT negative unstable angina group have died. Examination of the event free survival curve (Figure 6.2) shows that the curves continue to separate until about four years from admission and then begin to converge.

The difference in frequencies of this endpoint between the cTnT +ve and the cTnT -ve unstable angina groups was not significant (p=0.11) when tested by the chi-squared statistic. When allowance was made for coronary revascularisation using the Mantel-Haenszel statistic, the frequencies were significantly different (p=0.035) with a relative risk (RR) of 2.58 (95% confidence intervals (CI) 1.07,6.24) (Table 6.3).

Without any allowance made for revascularisation in the logistic regression model, only previous myocardial infarction reached conventional statistical significance (p=0.04) as a risk factor for cardiac death in unstable angina (lTable 6.4). This group had a lower revascularisation rate (RR=0.3, p=0.008), which may have been indicative of a more conservative strategy being practised by the physicians when previous myocardial damage was present. Troponin T status fared no better or worse as a predictor of cardiac death in unstable angina than the collapsed variables groups, further chest pain or ECG changes (ST segment depression or T wave inversion) and further chest pain or a minimal rise in conventional cardiac enzymes.

Of the sixty two unstable angina patients who were positive for cardiac Troponin T, 22 (35.5%) have undergone revascularisation and only one has died (4.5%) (Figure 6.3), whereas of the 40 patients who did not undergo revascularisation 11 (27.5%) have died (p=0.028), with a relative risk of death without revascularisation of 7.96 (95% CI 0.95,66.54), indicating that revascularisation may be of benefit to this subgroup (Table 6.5).

Figure 6.2. Kaplan Meier survival curves for the endpoint cardiac death for unstable angina patients according toTroponin T status.

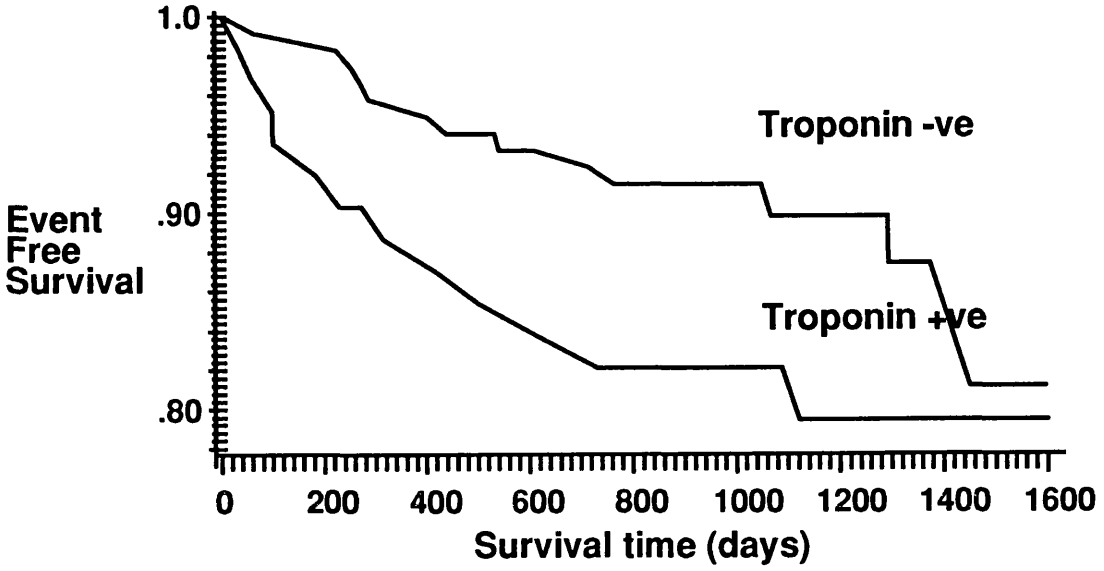



Table 6.3. Mantel-Haenszel statistical analysis for the end point cardiac death in unstable angina patients according to Troponin T status.

| End point | Troponin +ve | Troponin -ve | Significance | RR | 95%CI |
|---|---|---|---|---|---|
| Cardiac Death | 12/62 (19.3%) | 14/121(11.5%) | 0.035 | 2.58 | 1.07-6.24 |

Table 6.4. Multiple logistic regression analysis of unstable angina variables for the end point cardiac death.

| Variable | Significance | Relative Risk | 95% CI |
|---|---|---|---|
| Previous MI | 0.040 | 3.66 | 1.061-12.658 |
| Thrombolysis | 0.150 | 0.31 | 0.064-1.527 |
| Troponin T status | 0.507 | 1.50 | 0.434-5.410 |
| Pain or minimal rise in conventional enzymes | 0.403 | 1.71 | 0.485-6.051 |
| Pain or ECG changes | 0.765 | 1.20 | 0.359-4.025 |

Figure 6.3. Kaplan Meier survival curves for the endpoint cardiac death for cardiac Troponin T positive unstable angina patients who did or did not receive revascularisation on follow up.

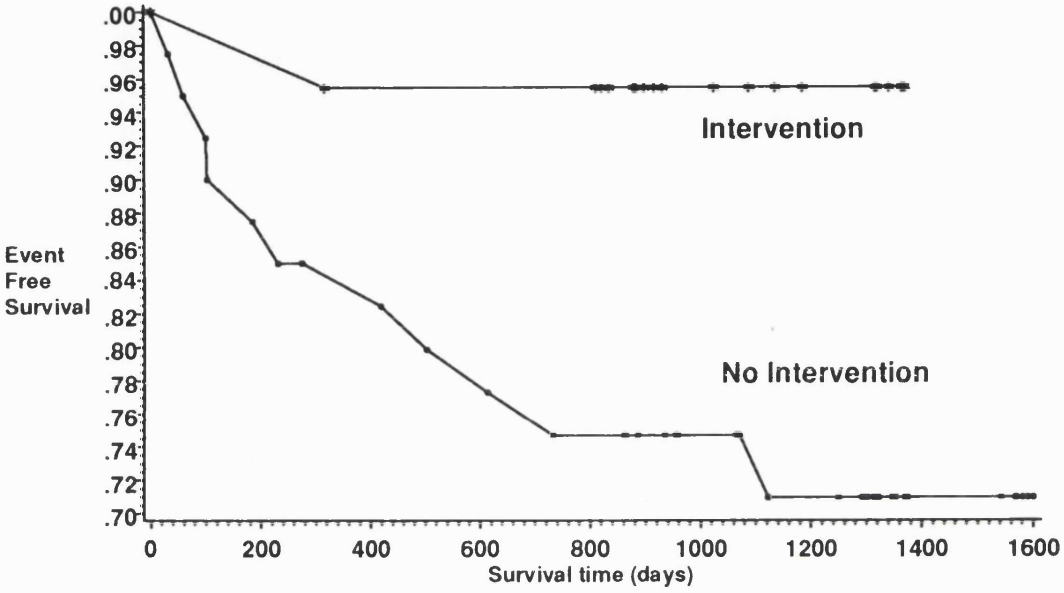



Table 6.5 . Chi-squared statistical analysis for the end point cardiac death for cardiac Troponin T positive unstable angina patients who did or did not receive revascularisation on follow up.

| End point | Intervention n=22 | No Intervention n=40 | Significance | RR | 95%CI |
|---|---|---|---|---|---|
| Cardiac Death | 1/22(4.5%) | 11/40(27.5%) | 0.028 | 7.96 | 0.95-66.54 |

## Coronary revascularisaton

35.5% (22/62) of the cTnT positive unstable angina group versus 21.5% (26/121) of the cTnT negative unstable angina group underwent either Coronary artery bypass grafting (CABG) or Percutaneous Transluminal Coronary Angioplasty (PTCA), (p=0.032, RR =2.09, 95% CI 1.06,4.03) (Table 6.6).

Examination of the event free survival curve (Figure 6.4) shows that most of the revascularisation procedures were carried out within the first two years of admission.

In the regression model (Table 6.7), both accelerated angina in the 48 hours before admission and Troponin T status were highly significant for this endpoint (p=0.001 and p=0.007 respectively). The presence of either variable was associated with a greater than five fold relative risk for revascularisation (p=0.0007).

The collapsed variable of further inpatient chest pain or ECG changes was also significant for this endpoint (p=0.017).

## Cardiac death or revascularisation

53.2% (33/62) of the cTnT positive unstable angina group versus 33.05% (40/121) of the cTnT negative unstable angina group have either died or undergone revascularisation as a first event, (p=0.004, RR=2.45, 95% CI 1.30,4.61) (Table 6.8).

The event free survival curve (Figure 6.5) shows that the curves maximally separate at about two years and then run reasonably parallel.

In the logistic regression model (Table 6.9), Troponin T status was highly significant for this endpoint (p=0.008, RR=2.55, 95%CI 1.28,5.08). Accelerated angina before admission and the collapsed variable further inpatient chest pain or ECG changes were also significant (p=0.038 and p= 0.041 respectively). Again the presence of either variable - accelerated angina or Troponin T status - was highly significant for this endpoint (p= 0.0007).

Figure 6.4. Kaplan Meier survival curves for the endpoint coronary revascularisation for unstable angina patients according to Troponin T status.

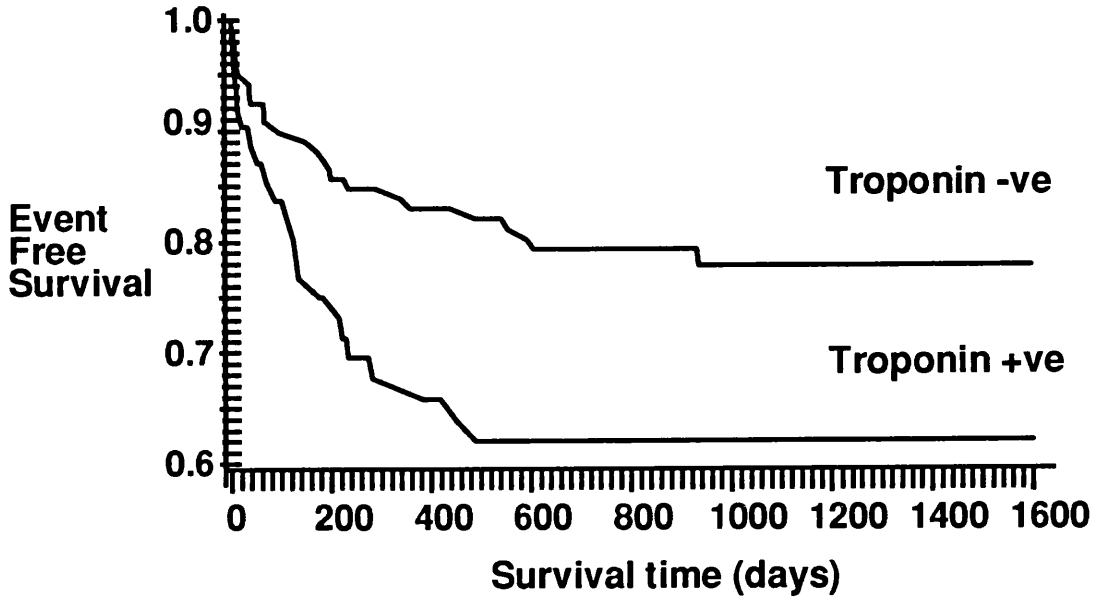



Table 6.6. Chi-squared statistical analysis for the end point coronary revascularisation in unstable angina patients according to Troponin T status.

| End point | Troponin +ve | Troponin -ve | Significance | RR | 95%CI |
|---|---|---|---|---|---|
| PTCA or CABG | 22/62 (35.5%) | 26/121(21.5%) | 0.032 | 2.09 | 1.06-4.13 |

Table 6.7. Multiple logistic regression analysis of unstable angina variables for the end point coronary revascularisation.

| Variable | Significance | Relative Risk | 95% CI |
|---|---|---|---|
| Accelerated Angina | 0.001 | 3.74 | 1.703-8.218 |
| Troponin T status | 0.007 | 3.18 | 1.372-7.368 |
| Previous MI | 0.008 | 0.30 | 0.124-0.730 |
| Troponin T or Accelerated Angina | 0.0007 | 5.39 | 2.039-14.327 |
| Pain or ECG changes | 0.017 | 3.19 | 1.228-8.238 |
| Pain or minimal rise in conventional enzymes | 0.386 | 1.55 | 0.576-4.158 |
| Thrombolysis | 0.316 | 0.56 | 0.179-1.743 |

Figure 6.5. Kaplan Meier survival curves for the endpoint cardiac death or coronary revascularisation as first event for unstable angina patients according to Troponin T status.

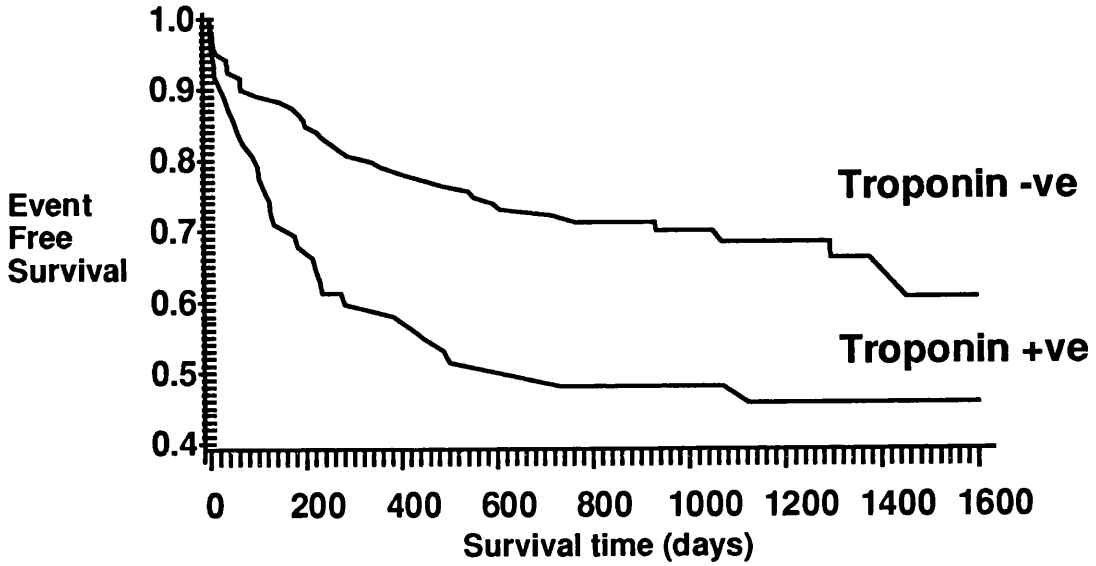



Table 6.8. Chi-squared statistical analysis for the end point cardiac death or coronary revascularisation as first event in unstable angina patients according to Troponin T status.

| End point | Troponin +ve | Troponin -ve | Significance | RR | 95%CI |
|---|---|---|---|---|---|
| Death or Intervention | 33/62 (53.2%) | 40/121(33.05%) | 0.004 | 2.45 | 1.30-4.61 |

Table 6.9. Multiple logistic regression analysis of unstable angina variables for the end point cardiac death or coronary revascularisation as first event.

| Variable | Significance | Relative Risk | 95% CI |
|---|---|---|---|
| Troponin T status | 0.008 | 2.55 | 1.278-5.084 |
| Accelerated Angina | 0.038 | 1.98 | 1.036-3.787 |
| Troponin T or Accelerated Angina | 0.0007 | 3.55 | 1.707-7.396 |
| Pain or ECG changes | 0.041 | 2.16 | 1.029-4.539 |
| Thrombolysis | 0.106 | 0.46 | 0.181-1.178 |
| Pain or minimal rise in conventional enzymes | 0.399 | 1.41 | 0.630-3.182 |

## Cardiac death or readmission with non fatal myocardial infarction

35.5%(22/62) of the TnT positive unstable angina group versus 17.3%(25/121) of the TnT negative unstable angina group have either died or been readmitted with a non fatal myocardial infarction as a first event.

Examination of the event free survival curve (Figure 6.6) shows that the curves maximally separate at about two and a half years from admission and then run reasonably parallel. The difference in frequencies of the endpoint was borderline statistically significant (p=0.07) when tested by the chi-squared statistic.

When allowance was made for coronary revascularisation using the Mantel-Haenszel statistic (Table 6.10), the frequencies were significantly different at p=0.042 with a relative risk of 2.16 (95%CI 1.03,4.53). Without any allowance made for revascularisation in the logistic regression model (Table 6.11), patients who were diabetic, in particular, (p=0.007) but also patients who were older (p=0.009) or had suffered a previous myocardial infarction (p=0.037) had a significantly increased risk for this endpoint.

Troponin T status was a better predictor for this endpoint than the two collapsed variables but did not reach conventional statistical significance (p=0.12).

## Readmission with myocardial infarction or unstable angina

Troponin T status was not significantly associated with an increased risk for this endpoint either with regard to the frequency (35.5% versus 38% cTnT +ve versus cTnT -ve, p=0.789) (Figure 6.7, Table 6.12) or in the logistic regression model.

Only previous myocardial infarction achieved conventional statistical significance (p=0.036) as a predictor (Table 6.13).

Figure 6.6. Kaplan Meier survival curves for the endpoint cardiac death or non fatal myocardial infarction as first event for unstable angina patients according to Troponin T status.

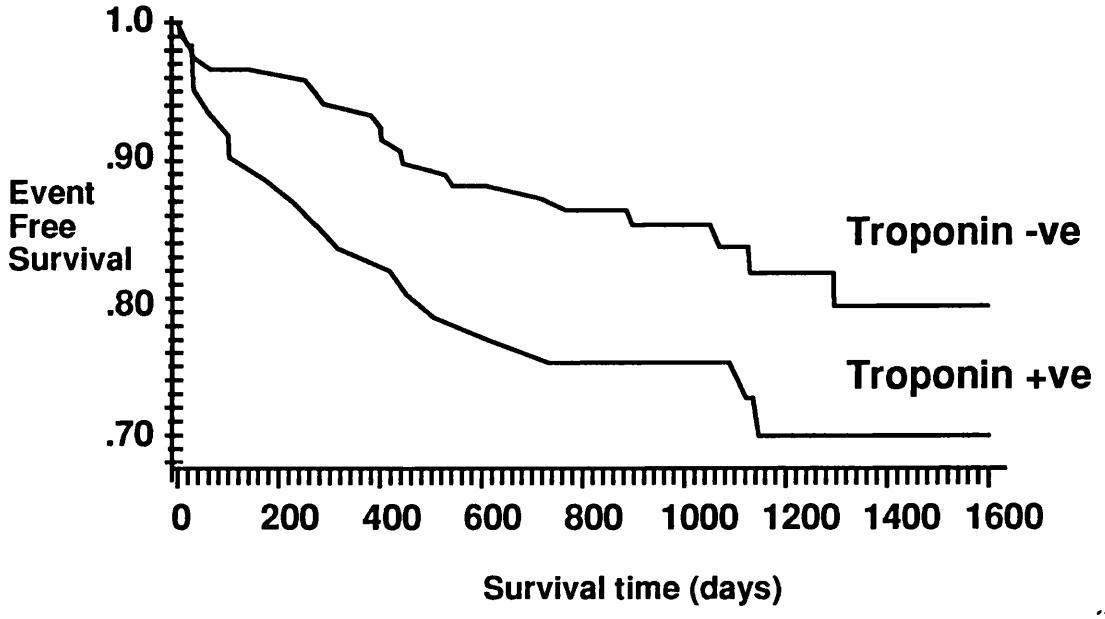



Table 6.10 . Mantel-Haenszel statistical analysis for the end point cardiac death or non fatal myocardial infarction as first event in unstable angina patients according to Troponin T status.

| End point | Troponin +ve | Troponin -ve | Significance | RR | 95%CI |
|---|---|---|---|---|---|
| Death or non Fatal MI | 22/62 (35.5%) | 25/121(17.3%) | 0.042 | 2.16 | 1.03-4.53 |

Table 6.11. Multiple logistic regression analysis of unstable angina variables for the end point cardiac death or non fatal myocardial infarction as first event.

| Variable | Significance | Relative Risk | 95% CI |
|---|---|---|---|
| Diabetes Mellitus | 0.007 | 4.56 | 1.513-13.747 |
| Age | 0.009 | 1.08 | 1.019-1.141 |
| Previous MI | 0.037 | 2.83 | 1.061-7.566 |
| Troponin T status | 0.123 | 1.21 | 0.421-3.472 |
| Pain or minimal rise in conventional enzymes | 0.736 | 1.18 | 0.438-3.216 |
| Pain or ECG changes | 0.739 | 0.84 | 0.312-2.286 |
| Thrombolysis | 0.980 | 1.01 | 0.314-3.281 |

Figure 6.7. Kaplan Meier survival curves for the endpoint non fatal myocardial infarction or readmission with unstable angina as first event for unstableangina patients according to Troponin T status.

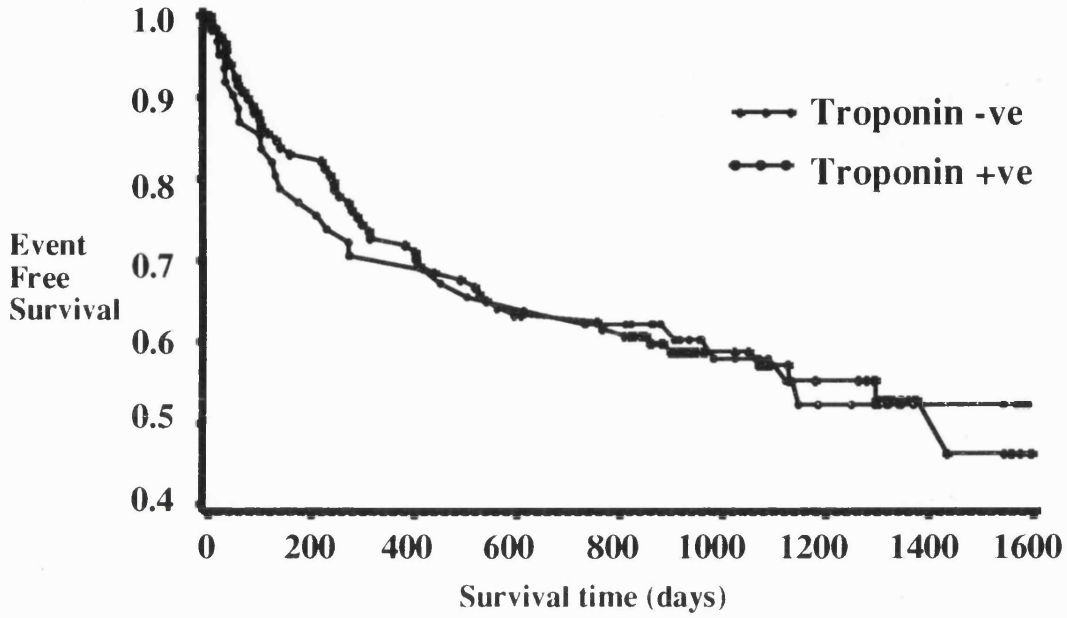



Table 6.12. Chi-squared statistical analysis for the end point non fatal myocardial infarction or readmission with unstable angina as first event in unstable angina patients according to Troponin T status.

| End point | Troponin +ve | Troponin -ve | Significance | RR | 95%CI |
|---|---|---|---|---|---|
| Readmn.MI or UAP | 22/62 (35.5%) | 46/121(38.0%) | 0.789 | 0.92 | 0.48-1.73 |

Table 6.13. Multiple logistic regression analysis of unstable angina variables for the end point non fatal myocardial infarction or readmission with unstable angina as first event.

| Variable | Significance | Relative Risk | 95% CI |
|---|---|---|---|
| Previous MI | 0.036 | 2.25 | 1.054-4.819 |
| Pain or minimal rise in conventional enzymes | 0.151 | 1.76 | 0.813-3.830 |
| Troponin T status | 0.376 | 0.68 | 0.293-1.591 |
| Pain or ECG changes | 0.452 | 1.34 | 0.620-2.917 |
| Thrombolysis | 0.973 | 0.98 | 0.405-2.393 |

## Discussion

The study indicates that the presence of cardiac Troponin T in the serum of patients admitted with unstable angina, when measured at a practical time point in the routine clinical environment, identifies a high risk subgroup that has a higher frequency of cardiac death. Cardiac Troponin T also identifies a subgroup that has a higher frequency of coronary revascularisation, cardiac death or coronary revascularisation and cardiac death or readmission with non-fatal myocardial infarction as first event on long term follow up.

Cardiac Troponin T may identify minor myocardial damage missed by other biochemical markers (Hamm et al, 1992., Seino et al, 1993., Gerhardt et al, 1991). Minimal rises above the upper reference limit of CK, AST and HBD have been suggested as being potentially useful in risk stratification (Murphy et al, 1992), but in this study they did not achieve significance on any of the end points studied, either alone or as the collapsed variable further chest pain or minimal rise in conventional enzymes.

Troponin T status was also superior to the collapsed variable of either further chest pain or further ECG changes following admission in all of the end points studied. Of all of the clinical variables in Table 6.1, accelerated angina in the 48 hours before admission was the most significant predictor of risk for the endpoints coronary revascularisation and cardiac death or revascularisation as first event (Tables 6.7 and 6.9).

Troponin status was also a good predictor for these endpoints, but the combination of either variable was the most powerful predictor of risk ($p=0.0007$) (Tables 6.7 and 6.9). The differences in frequencies for the combined endpoint cardiac death or readmission with non fatal myocardial infarction as first event ($p=0.042$) is consistent with the Nordic study findings ($p=0.024$) (Ravkilde et al, 1993) with respect to the Mantel-Haenszel statistic.

The role of exercise testing was not formally analysed in this study. This was due to significant variations in uptake, protocol used and reasons for test termination. Whether exercise testing in unstable angina has any prognostic value in a similar population has been questioned (Murphy et al, 1992). One third of the cardiac Troponin T positive group

received thrombolysis. This is explained by the practice of an ISIS 2 strategy (ISIS-2, 1988) by some of the admitting teams. While the role of thrombolysis in unstable angina has been questioned (Williams et al, 1990., Bar et al, 1992), its potential as a holding strategy prior to acceptance for angiography or as a definitive strategy in this high risk subgroup has not been studied. It did not however, appear to be associated with an increased hazard in any of the endpoints studied.

There was no difference between the unstable angina groups with regard to use of those medical therapies which may be of benefit in this syndrome; aspirin, heparin, beta-blockers or calcium antagonists (Wallentin, 1990., Yusuf et al, 1988., Lewis et al, 1983., Theroux et al, 1988., Lubsen, 1990., Cohen, 1994), either whilst an inpatient or on discharge.

The event rate in the cardiac Troponin T negative unstable angina group for the endpoints show that the Troponin status of these patients should not be used however as a sole discriminator of risk.

The cardiac Troponin T positive patients appeared to benefit from revascularisation, but whilst the choice of intervention will be determined by the coronary anatomy, the ideal timing for the intervention remains to be established.

Whether a lower or higher discriminant value (e.g. 0.1 mcg/ml) as quoted by the manafacturers would have significantly affected the prediction of endpoints was further studied for the three primary endpoints on all of the unstable angina patients (Table 6.14).

For the endpoints cardiac death and cardiac death or coronary revascularisation as first event the discriminant value of 0.2 micrograms /litre appears to be the best cardiac Troponin T cutoff. Interestingly however, for the end point need for coronary revascularisation as first event, the higher the cardiac Troponin T level the more significant the risk for this endpoint. What the presence of cTnT in the serum of patients actually represents at a pathophysiological level in unstable angina and why its presence is associated with a poorer prognosis on follow up compared to the cTnT negative unstable angina group has not been studied. It may be related to variations in the degree of plaque rupture and/or the subsequent change in geometry of the culprit lesion during the repair phase.

Table 6.14. The effect of changing the cardiac Troponin T discriminant level on the endpoints:- cardiac death, coronary revascularisation or cardiac death or coronary revascularisation as first event.

a. Cardiac Death

| Chi-square | Significance | RR | 95%CI | TnTmcg/ml |
|---|---|---|---|---|
| 1.24993 | 0.26357 | 1.61640 | 0.69293 -3.77060 | 0.1 |
| 2.50517 | 0.11347 | 1.97538 | 0.84141 -4.63760 | 0.2 |
| 0.16693 | 0.68285 | 1.20856 | 0.48665 -3.00137 | 0.3 |

b. Coronary revascularisation as first event

| Chi-square | Significance | RR | 95%CI | TnTmcg/ml |
|---|---|---|---|---|
| 3.40563 | 0.064974 | 1.87166 | 0.95744 -3.65882 | 0.1 |
| 4.58057 | 0.032336 | 2.09000 | 1.05701 -4.13251 | 0.2 |
| 8.05734 | .0045320 | 2.70968 | 1.34412 -5.46258 | 0.3 |

c. Cardiac death or revascularisation as first event

| Chi-square | Significance | RR | 95%CI | TnTmcg/ml |
|---|---|---|---|---|
| 5.13195 | 0.023489 | 2.00905 | 1.09471 -3.68708 | 0.1 |
| 7.98594 | .0047142 | 2.45554 | 1.30789 -4.61021 | 0.2 |
| 6.73407 | .0094589 | 2.36047 | 1.22452 -4.55020 | 0.3 |

Alternatively, the presence of cTnT may be associated with a more proximal culprit lesion or more extensive disease. Systemic factors which may either impair fibrinolysis such as Lipoprotein (a) or promote a more prothrombotic state such as high levels of circulating fibrinogen could also be playing a role.

The remainder of this thesis has explored some of these possibilities in an attempt to explain why this unstable angina subgroup are at increased risk of future cardiac events.

# Chapter Seven

## Culprit lesion morphology in patients with unstable angina related to cardiac Troponin T concentrations.

### Introduction

At a pathophysiological level, unstable angina represents fissuring of an atheromatous plaque with ulceration and platelet aggregation. This may lead to thrombus formation which is usually non occlusive. These plaque disruptions may cause Type 11 or Type 111 injuries (Ip et al, 1990) and result in varying degrees of smooth muscle proliferation which may result in the progression of the atheromatous plaque (Fuster et al, 1992). This process of organisation and repair of the ruptured plaque may take up to three months (Fuster et al, 1992).

A number of angiographic studies ( Moise et al, 1983., Ambrose et al, 1986. Ambrose et al, 1988) have studied progression of stenoses in patients who have had an episode of unstable angina. Two main conclusions have been drawn from these studies. The first was a common finding that compared to patients with chronic stable angina who served as a control group, patients admitted with unstable angina had evidence of progression of the 'culprit' lesion on sequential angiography. Examination of the morphology of these stenoses also revealed that they are eccentric in shape in the majority of patients with unstable symptoms, but not in patients with chronic stable disease (Ambrose et al, 1985). The findings of Kondo et al (Kondo et al, 1994) of mural thrombus on early qualitative angiography in cTnT positive patients, would suggest that these patients had suffered a more severe plaque injury than the cTnT negative patients. Consequently, in the repair phase one might see differences in the culprit lesion morphology between the two groups.

A number of investigators have also suggested that the angiographic extent and location of coronary disease have some predictive value for subsequent cardiac events (Maseri, 1986. Leeman et al, 1988), although there is disagreement with these findings (Conti et al,1973., Russell et al, 1978). Hamm et al (1992) assessed the early visual angiographic extent and

severity of coronary disease in patients with unstable angina at rest and failed to demonstrate any significant differences between patients who were positive or negative for the presence of cardiac Troponin T.

The purpose of this study was to undertake a detailed angiographic analysis of the coronary anatomy of patients who had been admitted with unstable angina, to test the hypothesis that the presence of cTnT in the serum may be associated with either more extensive or more proximal coronary artery disease, or a more complex morphological appearance of the culprit lesion, and that this may explain their longer term adverse prognosis when compared with the cTnT negative group.

## Methods.

Patients from the study population described in chapter four, who had been admitted with an episode of unstable angina which had stabilised and underwent coronary angiography within 100 days were eligible for the study, providing there was no evidence of progression to myocardial infarction on biochemical or electrocardiographic grounds either during inpatient stay or on follow-up. Patients who had been readmitted with a further episode of unstable angina or who were in Canadian Cardiac Society angina class 111 or 1V were also excluded (Campeau, 1976).

## Angiographic Analyses

Coronary arteriography was performed using the Judkins technique. Low-osmolar contrast medium was used for all the angiograms. A minimum of five views of the left system and two of the right coronary artery were filmed. All coronary angiograms were analysed by two experienced clinicians, who were blinded to the Troponin T status of the patients.

Each of the fifteen angiographic segments was graded according to the European Cooperative Study Group system (Table 7.1). The culprit artery was judged from the electrocardiographic records of the patient's admission and the angiographic appearances. Computerised analysis (Cardiovascular Measurement System, Medis) was performed on the target artery segment. Calibration was from the filled catheter tip and an automated edge detection system was used to minimise user influence. Frames for analysis were selected to show the target lesion in end-diastole in the least foreshortened unobstructed view (Figure 7.1). Values for the minimum lumen diameter of the culprit lesion, reference diameter for the arterial segment , maximum percent stenosis, length of stenosis and an estimate of plaque volume were obtained. Qualitative culprit lesion morphology was also scored visually according to defined criteria for complexity (Ambrose et al, 1988).

Table 7.1 Diameter Stenosis Grading by the European Cooperative Study Group

| Grade of stenosis | |
|---|---|
| 0 | Normal |
| 1 | <50% diameter stenosis |
| 2 | 50% to 90% diameter stenosis |
| 3 | 91% to 99% diameter stenosis, complete filling within three cycles |
| 4 | 91% to 99% diameter stenosis, no complete filling within three cycles |
| 5 | Total occlusion, with or without collateral filling |

## Statistical analysis.

For categorical variables, values were expressed as percentages. Differences between the baseline demographics and angiographic and culprit lesion morphologies were tested using the Chi squared statistic. For ordinal variables, values were expressed as means with standard errors. Differences between the baseline demographics and angiographic and culprit lesion morphologies were tested using the Mann U Whitney statistic. Correlations were examined using the rank Spearman statistic.

## Results.

Baseline demographics.

These are shown in table 7.2. 46 patients who had a final classification of unstable angina as defined in chapter five were studied; 24 who were negative for the presence of cTnT in their serum in the first 48 hours of their study entry admission, and 22 whose level of cTnT exceeded 0.2ng/ml.. No significant differences were seen between the two groups with respect to age, male sex, risk factors for heart disease, cardiac history prior to admission, the frequency of ST or T wave inversion on the admission ECG and medical management whilst on CCU, as inpatients and with regard to discharge medication. There was a higher incidence of previous myocardial infarction in the cTnT negative group (Table 7.2) but this did not reach conventional statistical significance (p=0.09).

## Angiographic and lesion morphology findings.

These are summarised in table 7.3. There was no difference between the groups with respect to time to angiography, the extent (defined as at least one stenosis >50% in a major vessel) or the severity of coronary disease or the location of the culprit lesion. Each of the fifteen coronary segments were analysed and visually scored according to the ECSG system. No significant differences were observed between the groups in the coding score on any of the segments analysed except for the presence of a more severe lesion in Segment 1 in the cTnT positive group (Chi-square 3.97, p=0.046).

Figure 7.1. Example of a coronary lesion assessed by the automated computerised analysis method.

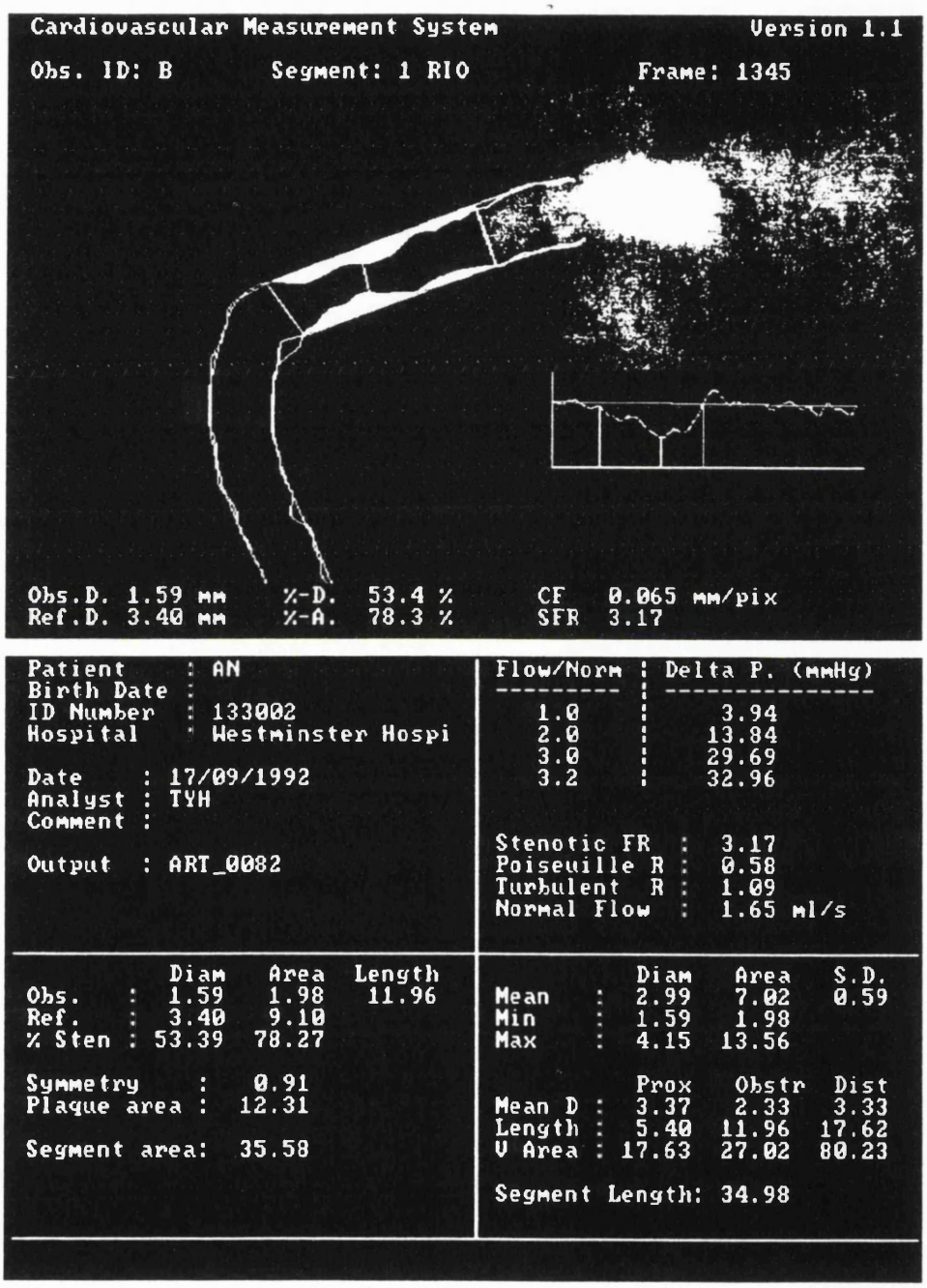


Table 7.2. Baseline demographics.

| Variable | Troponin T -ve | Troponin T +ve | Significance |
|---|---|---|---|
| Number | 24 | 22 | - |
| Age | 59.2 | 57.3 | 0.613 |
| Male sex | 92% | 91% | 0.928 |
| FHIHD (<55) | 29% | 27% | 0.887 |
| Hypertension | 19% | 15% | 0.524 |
| Diabetes Mellitus | 25% | 14% | 0.337 |
| Smoker (current/ex) | 62% | 85% | 0.550 |
| Previous MI | 54% | 27% | 0.067 |
| Accelerated Angina | 69% | 52% | 0.256 |
| Rest pain | 50% | 55% | 0.760 |
| Abnormal ECG on admission (ST or T) | 63% | 60% | 0.891 |
| CCU aspirin | 92% | 82% | 0.327 |
| CCU IV nitrates | 75% | 64% | 0.407 |
| IV Heparin | 75% | 68% | 0.611 |
| Duration of Heparin (hours) | 79.7(14.4) | 65.7(9.6) | 0.456 |
| Troponin T peak (ng/ml) | 0.11(.08) | 0.98(.18) | 0.001 |
| Inpt B blocker | 42% | 46% | 0.797 |
| Inpt $Ca^{2+}$ antagonist | 50% | 55% | 0.760 |
| Discharge Aspirin | 88% | 91% | 0.713 |
| Discharge B blocker | 46% | 32% | 0.335 |
| Discharge $Ca^{2+}$ antagonist | 46% | 41% | 0.739 |

Legend:- FHIHD = Family history of heart disease less than 55 years of age; MI = myocardial infarction; Accelerated angina = increasing chest pain in the 48 hours before admission; CCU= Coronary Care Unit; ECG = Electrocardiogram; ST or T= ST segment changes or T wave inversion; IV = intravenous; B Blocker = beta adrenergic blocking drug; $Ca^{2+}$= Calcium antagonist. The statistical significance column represents the differences between the unstable angina groups as assessed by the Chi-squared statistic for categorical variables and Mann U Whitney statistic for ordinal variables.Values are expressed as percentages or means with standard errors.

Table 7.3. Angiographic and culprit lesion morphology findings.

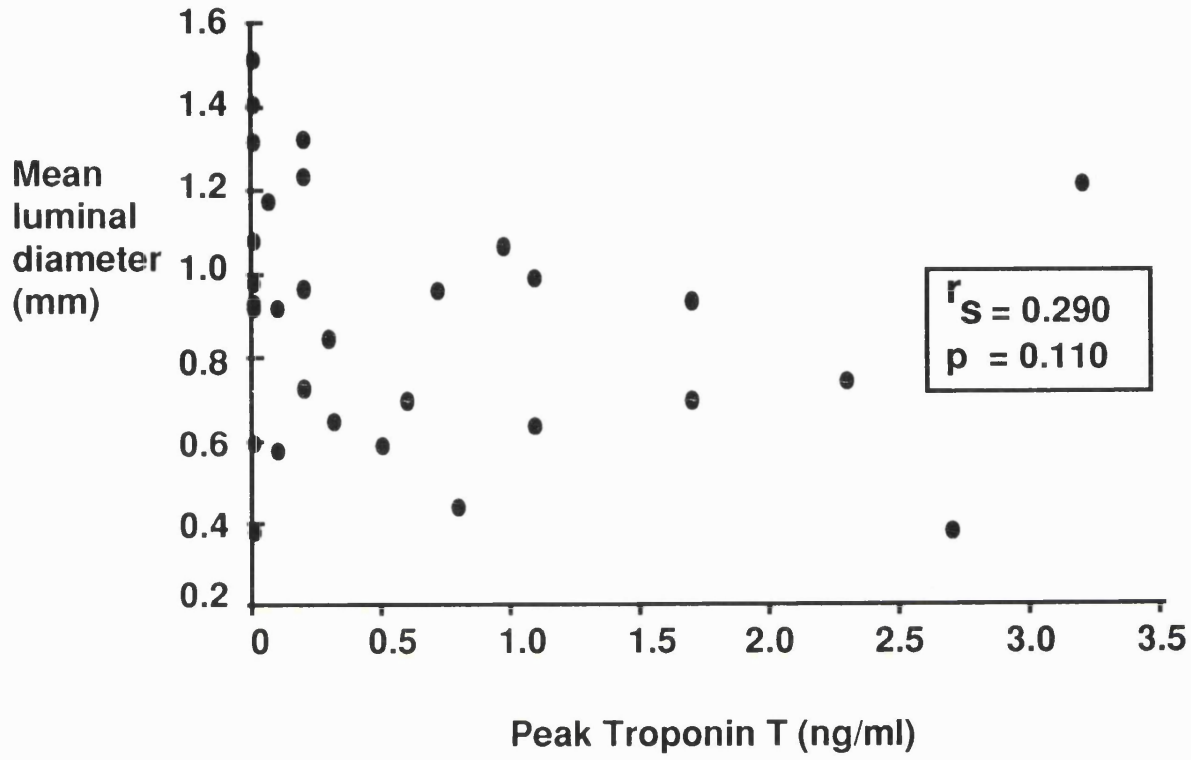
| Variable | Troponin T -ve | Troponin T +ve | Significance |
|---|---|---|---|
| Time to angiography(days) | 34(8.9) | 26.5(6.9) | 0.733 |
| Vessel disease | | | |
| 1 | 48% | 38% | |
| 2 | 22% | 38% | 0.533 |
| 3 | 26% | 24% | |
| Occluded culprit vessel | 22% | 5% | 0.105 |
| Proximal LAD lesion >90%* | 68% | 55% | 0.588 |
| Location of lesion at Bifurcation | 69% | 40% | 0.090 |
| Mean luminal diameter (mm) | 0.9(.09) | 0.84(.06) | 0.237 |
| Diameter stenosis | 63.0%(2.5) | 67.8%(1.4) | 0.960 |
| Plaque length (mm) | 12.19(2.1) | 10.96(0.1) | 0.952 |
| Plaque area ($mm^2$) | 10.45(2.4) | 9.41(1.0) | 0.659 |
| Plaque symmetry | 0.62(.06) | 0.69(.06) | 0.357 |
| Eccentric Lesion | 69% | 70% | 0.936 |
| Irregular contour | 38% | 45% | 0.654 |
| Shoulder present | 6% | 10% | 0.689 |
| Angulation: | | | |
| None - <45° | 31% | 65% | |
| Moderate - 45-90° | 50% | 30% | 0.039 |
| Severe - >90° | 19% | 5% | |

*Proximal LAD lesion:- ECSG stenosis grading >2 in segment 6 or 7.

The statistical significance column represents the differences between the unstable angina groups as assessed by the Chi-squared statistic for categorical variables and Mann U Whitney statistic for ordinal variables.

Values are expressed as percentages or means with standard errors.

Figure 7.2. Scatter plot, Spearman rank correlation and significance between mean luminal diameter and peak cardiac Troponin T value for all patients.



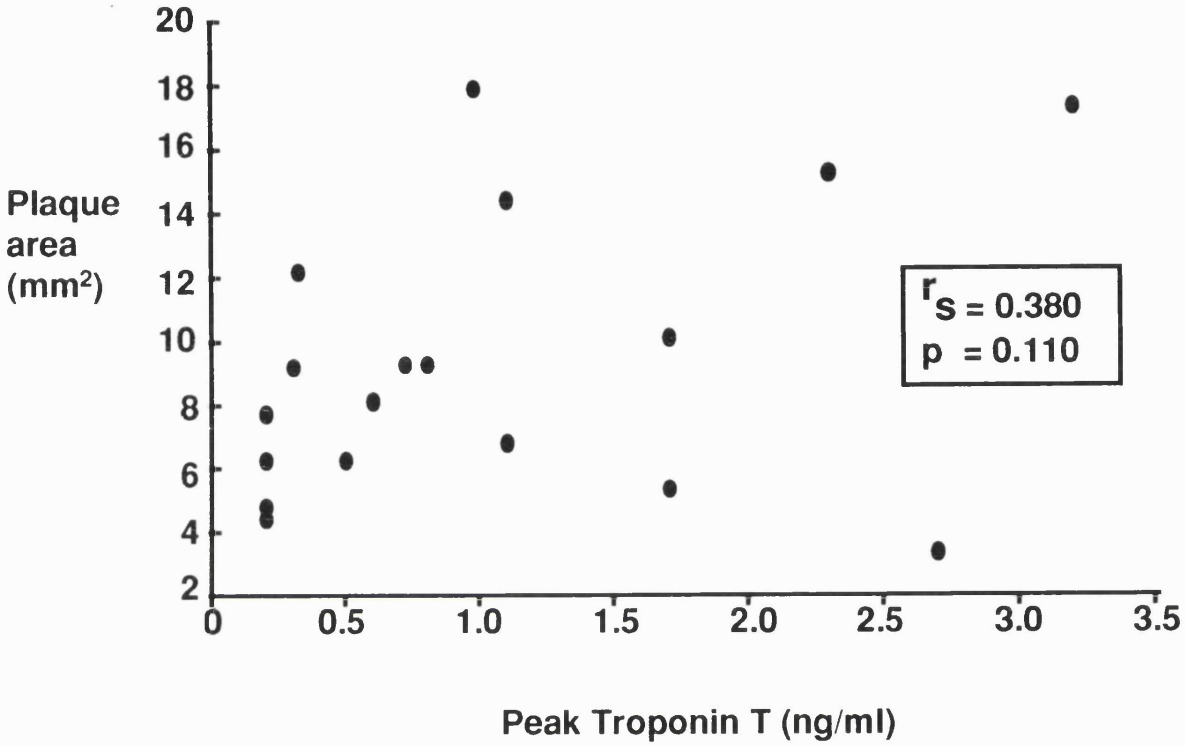

In particular there was no difference between the groups in the frequency or severity of lesions in the proximal left anterior descending artery.

Examination of the culprit stenosis morphology revealed that angulation was less likely to be present, or if present was likely to be less severe in the cTnT positive group (p=0.039). The culprit lesion was less likely to be present at a bifurcation in the cTnT positive group but this did not reach conventional significance (p=0.09).

A negative correlation was obtained between the mean luminal diameter and peak cTnT values for the whole patient group ($r_S$ = -0.29, p=0.11) (figure 7.2), and within the cTnT positive group a correlation was obtained between plaque area and peak cTnT value ($r_S$ =0.38, p=0.11) (figure 7.3).

These correlations did not achieve conventional statistical significance however. No other relevant correlations were seen.

Figure 7.3. Scatter plot, Spearman rank correlation and significance between plaque area and peak cardiac Troponin T value in the Troponin T positive unstable angina group.




Discussion.

The findings of this study suggest that the adverse prognosis of the cTnT positive unstable angina group compared to the cTnT negative unstable angina group is not explained by any major differences in the extent or severity of coronary artery disease, or by the location or morphological findings of the culprit lesion during the repair phase of plaque disruption.

Examination of the baseline variables in table 7.1, shows that the groups were well matched for risk factors and cardiac histories prior to admission, and in particular were matched for medical managements that may be of benefit in this acute coronary syndrome and may have an affect on plaque repair (Fuster et al, 1992).

The higher incidence of a totally occluded culprit vessel in the cardiac Troponin T negative group supports the concept this is more likely to occur without plaque disruption

or evidence of any myocardial damage in a vessel with a long standing severe stenosis, perhaps because of well developed collateralls. Unlike small plaques, severe stenotic plaques tend to be very fibrotic and stable, therefore it is conceivable that thrombotic occlusion of these plaques is mainly the result of decreases in flow, rather than plaque disruption (Fuster et al, 1992) which one would expect in the cardiac Troponin T positive group. Correlations were found between the cTnT peak and some aspects of plaque morphology, indicating that this group may be more prone to some progression of the plaque in the repair phase, but this effect is small and appears not to be significant. By restricting this study to patients whose angina was stable and by examining the angiographic findings in the repair phase of the syndrome, I cannot exclude some significant differences between the two unstable angina groups with regard to plaque morphological findings if patients were examined during the acute admission phase.

# Chapter Eight

## Activation of coagulation in unstable angina in relation to cardiac Troponin T concentrations.

### Introduction

Recently, workers have shown that activation of coagulation can be identified in patients admitted with unstable angina by measurement of the prothrombin fragment 1+2 ($F_{1+2}$) (Merlini et al, 1994). A new ELISA method for $F_{1+2}$ measurement that allows reliable assessment of coagulation system activity under in vivo conditions has been developed (Boisclair et al, 1993).

Prothrombin fragment 1+2 is a 31-kd polypeptide released from the amino terminal end of prothrombin during its conversion to thrombin and quantifies factor Xa activity. It was found to be significantly raised on admission in patients with unstable angina when compared to patients with chronic stable angina or healthy controls (Merlini et al, 1994).

It has also recently been reported (Kondo et al, 1994) that intracoronary mural thrombus on early coronary angiography could be identified in over 50% of patients admitted with unstable angina who were cardiac Troponin T (cTnT) positive. No intracoronary thrombus was seen in the cTnT negative group.

The purpose of the present study was to measure the admission $F_{1+2}$ and the subsequent cTnT concentrations in patients admitted with unstable angina, to examine whether the cardiac Troponin T positive sub group had evidence of increased activation of coagulation, on peripheral venous sampling, on admission to the coronary care unit as compared to the cardiac Troponin T negative group.

I also wished to examine whether the more routinely available marker, Fibrinogen, which has also been shown to be raised in acute ischaemic syndromes (Kruskal et al, 1987), might also have a role to play in the early identification of this important unstable angina sub group. Using the final diagnostic classifications described in Chapter four, a subset of the patient cohort were studied.

## Methods

The sampling protocol and measurement of prothrombin fragment 1+2, fibrinogen and cardiac Troponin T were as described in the laboratory analytical methods chapter. Samples were batch assayed by investigators blinded to the final diagnostic classifications.

## Statistical analysis.

Correlations between admission $F_{1+2}$ and Fibrinogen, and between these markers and admission, diagnostic and peak cardiac Troponin T values were tested using the rank Spearman statistical test. Differences between the admission coagulation values and cardiac Troponin T values at various discriminant levels and time points were tested by the Chi-squared statistic.

## Results

96 patients who had a final diagnostic classification of unstable angina were studied. The patient baseline demographics are listed in table 8.1.

There was a significant correlation between the admission $F_{1+2}$ (mean 39.8 ng/ml, range 9.8-141) and Fibrinogen (mean 1.99 g/l, range 1.1-4.1) values, $r_s = 0.253$ $p = 0.025$ (Figure 8.1) and the mean admission $F_{1+2}$ values were significantly higher than the mean value for our healthy controls (39.8 ng/ml v 22.0 ng/ml, $p = <0.001$)

The cardiac Troponin T results for each patient were divided into admission to the CCU values, the diagnostic values- the value at 12-24 hours from admission- and the peak values- the highest value recorded in the first 48 hours of admission.

The cardiac Troponin T results were further classified into the number positive at these three time points according to a discriminant value of 0.1ng/ml or 0.2ng/ml.

Figure 8.1. Scatter plot, spearman rank correlation and significance between admission Fibrinogen and Prothrombin 1+2 concentrations for all unstable angina patients.

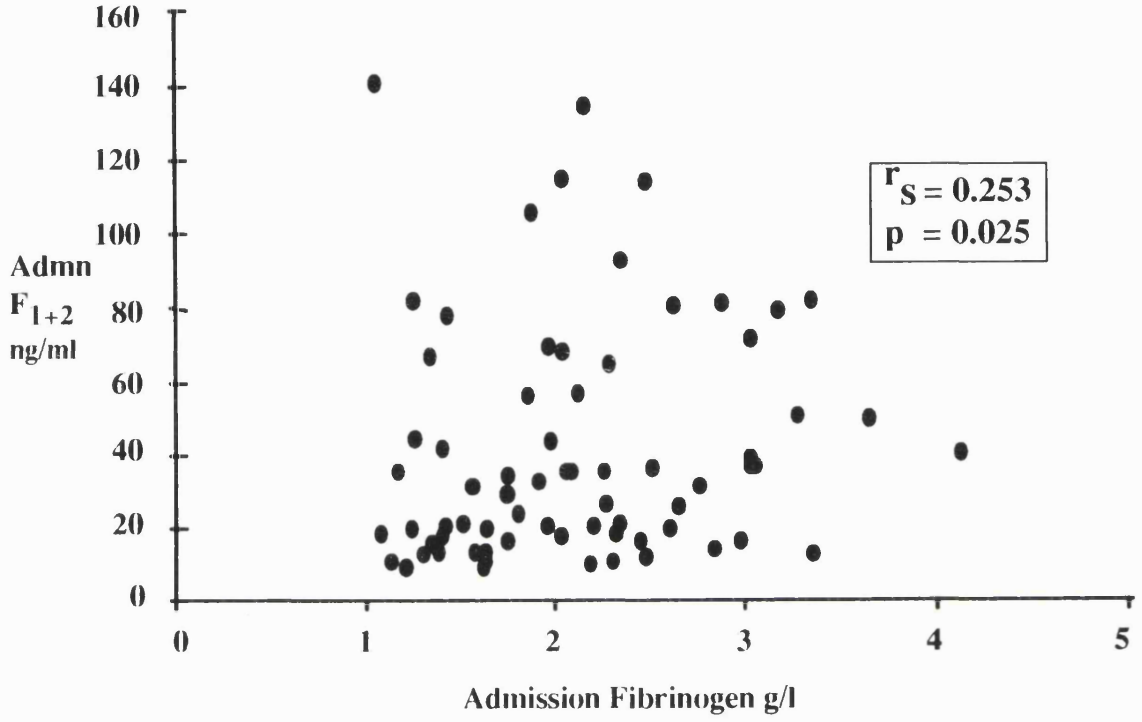


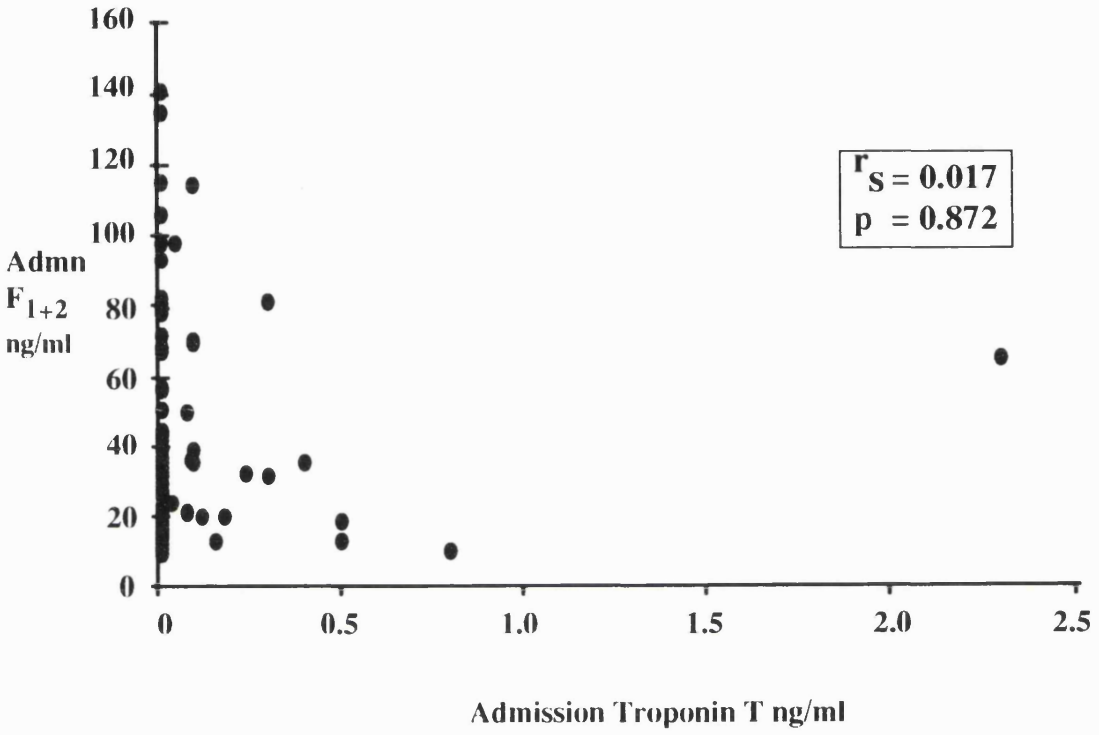
Table 8.1. Baseline demographics.

| Variable | Value |
|---|---|
| Number | 96 |
| Median age(years) | 61.4 |
| Male sex | 78.3% |
| Previous MI | 44.8% |
| Hypertension | 36.7% |
| Diabetes Mellitus | 19.8% |
| Current smoker | 33.3% |
| FHIHD (<55years) | 45.8% |
| Rest pain | 36.7% |
| CCSC 3 or 4 | 16.6% |
| Accelerated angina | 45.6% |
| Abnormal ECG on admission (ST depression or T inversion) | 42.2% |
| Median time to first sample from onset of worst pain (hours) | 4.83 |
| Mean admission Troponin T conc.(<0.2ng/ml) | 0.084 |
| Mean diagnostic Troponin T conc.(<0.2ng/ml) | 0.233 |
| Mean peak Troponin T conc.(<0.2ng/ml) | 0.339 |
| Mean admission $F_{1+2}$ conc.(<50ng/ml) | 39.8 |
| Mean admission Fibrinogen conc.(<4.0g/l) | 1.99 |
| CCU Aspirin | 72.9% |
| CCU IV Heparin | 80.2% |
| Mean duration Heparin(hours) | 61.0 |
| CCU IV Nitrates | 70.8% |

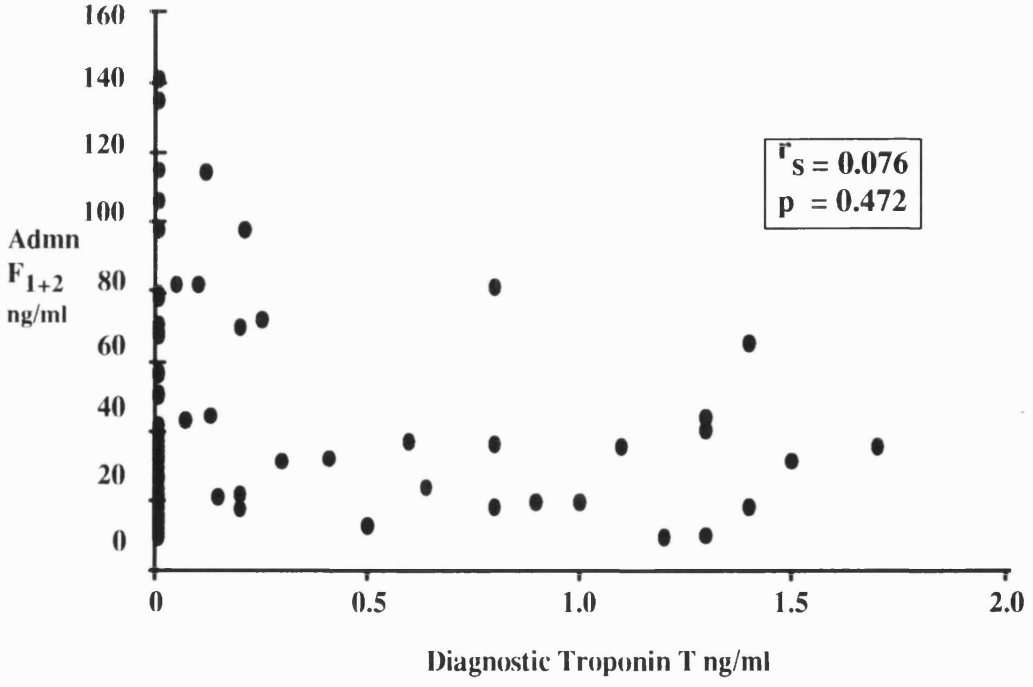
Legend. FHIHD= premature family history of heart disease. CCSC= Canadian Cardiac society angina classification before admission. Accelerated angina=increasing angina in the 48 hours before admission. CCU=Coronary Care Unit. IV=Intravenous.

Figure 8.2. Scatter plots, spearman rank correlations and significances between admission F1+2 concentrations and cardiac Troponin T values at various time points.

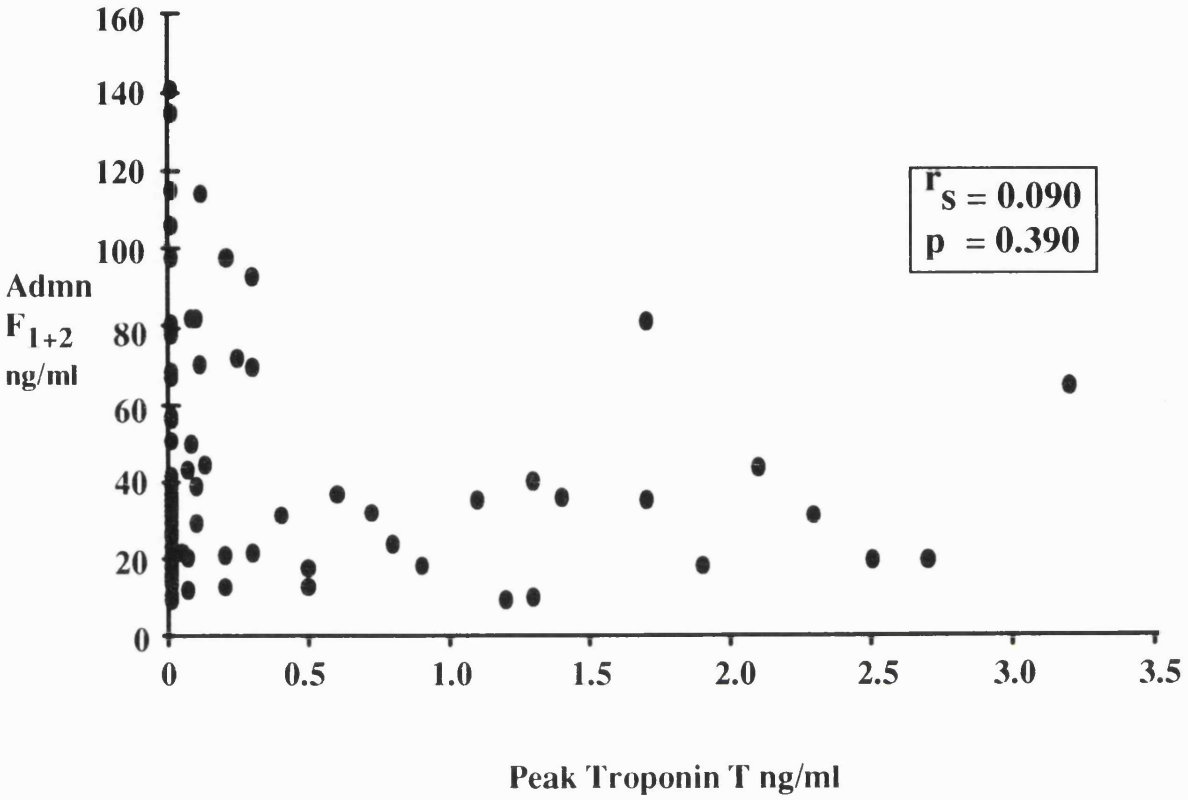
(a). Admission $F_{1+2}$ and admission cardiac Troponin T concentrations.




(b) Admission $F_{1+2}$ and diagnostic cardiac Troponin T concentrations.



(c)Admission $F_{1+2}$ and peak cardiac Troponin T concentrations.

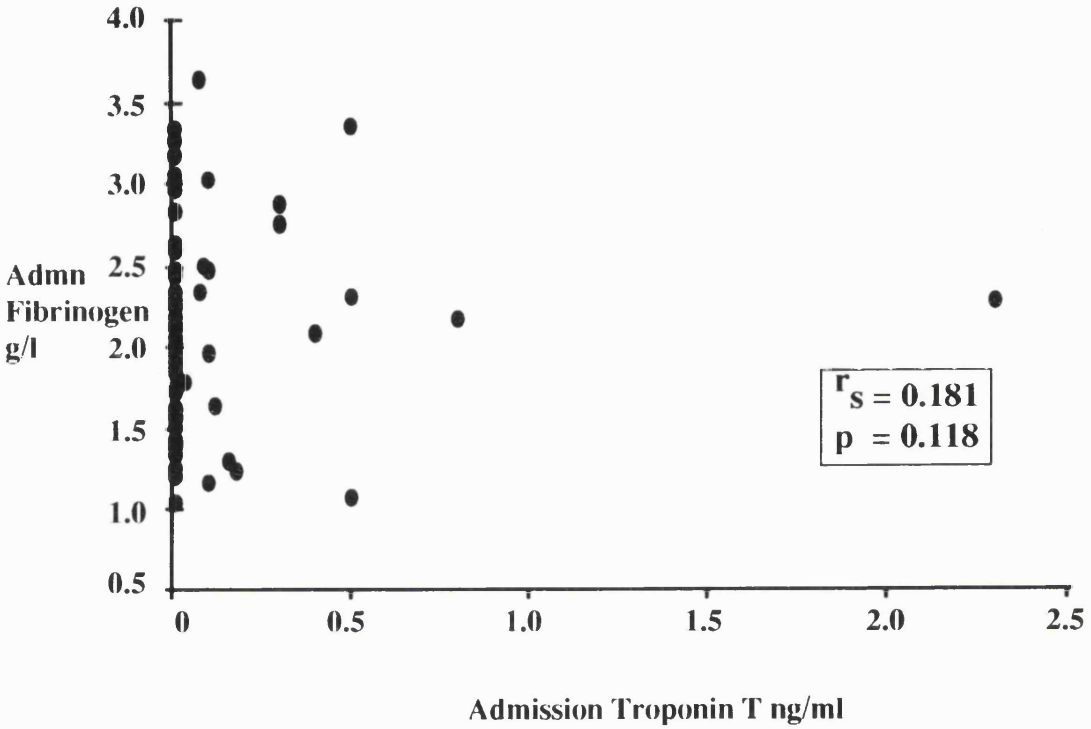



No significant correlations were obtained between $F_{1+2}$ concentrations on admission and admission cTnT values, $r_S$= 0.017 p = 0.872 (Figure 8.2a), diagnostic cTnT values, $r_S$ = 0.076 p = 0.472 (Figure 8.2b), or peak cTnT values, $r_S$ = 0.090 p = 0.390 (Figure 8.2c)

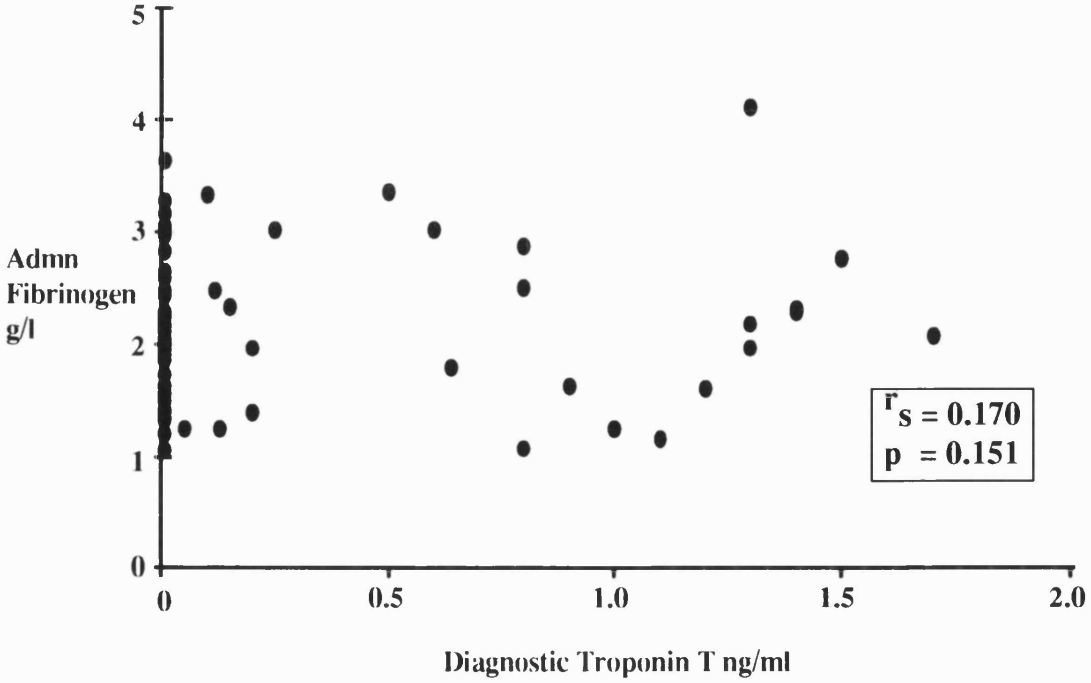
No significant correlations were obtained between admission to the CCU Fibrinogen concentrations and the admission cTnT concentrations, $r_S$ = 0.181 p= 0.118 (Figure 8.3a), the diagnostic cTnT concentrations, $r_S$=0.170 p= 0.151 (Figure 8.3b), or the peak cTnT concentrations, $r_S$=0.206 p= 0.072 (Figure 8.3c).

Figure 8.3. Scatter plots, spearman rank correlations and significances between admission Fibrinogen concentrations and cardiac Troponin T values at various time points.

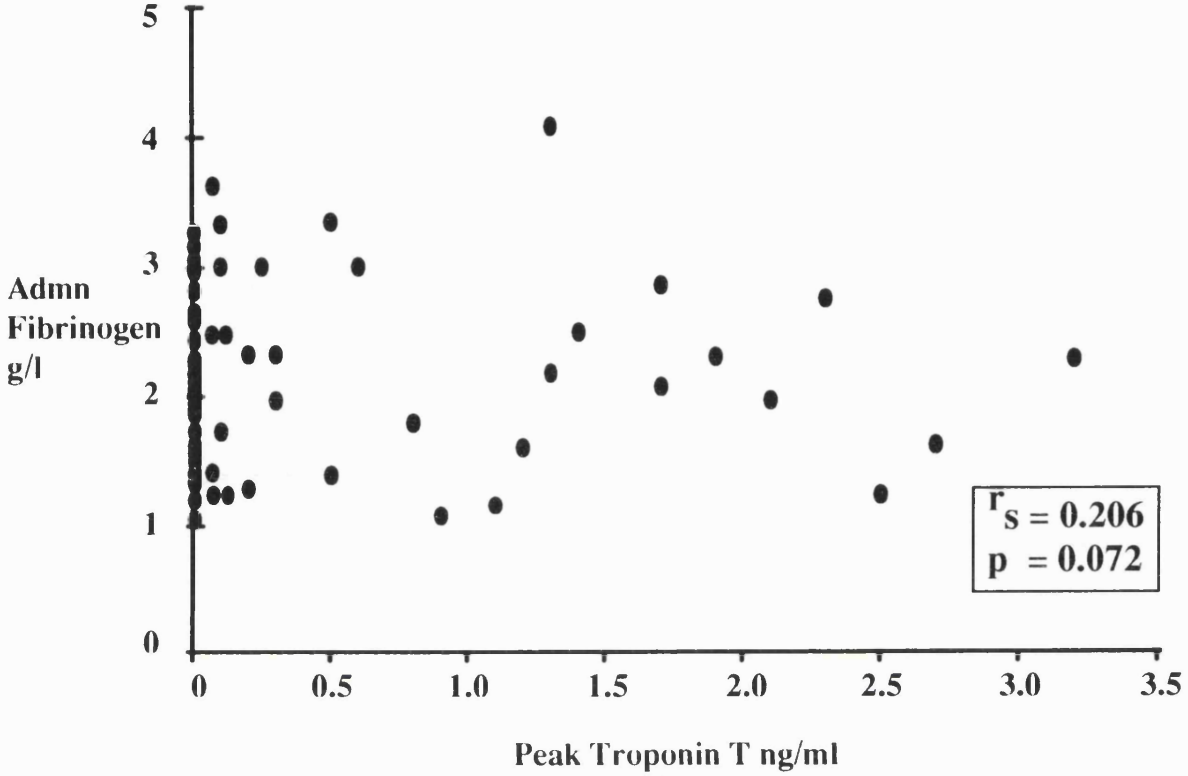
(a) Admission Fibrinogen and admission cardiac Troponin T concentrations.




(b)Admission Fibrinogen and diagnostic cardiac Troponin T concentrations



(c)Admission Fibrinogen and peak cardiac Troponin T concentrations




Significant differences were then tested between patient groups against the admission $F_{1+2}$ and Fibrinogen concentrations according to their cardiac Troponin T values at the three time points, dichotomised according to the two discriminant levels. These results are summarised in Table 8.2. Again none of the results achieved conventional statistical significance.

Table 8.2. Differences between admission $F_{1+2}$ and Fibrinogen concentrations and Troponin T status at different time points and using different discriminant levels.

| Variable | Number | Mean TnT (ng/ml) | Mean F1+2 (ng/ml) | Significance p value | Mean Fibrinogen (g/l) | Significance p value |
|---|---|---|---|---|---|---|
| Admission cTnT ≥0.1ng/ml | 17 | 0.24 | 40.8 (7.2) | 0.96 | 2.12 (.2) | 0.40 |
| Admission cTnT ≤0.1ng/ml | 79 | 0.01 | 39.9 (3.4) | | 1.94 (.1) | |
| Admission cTnT ≥0.2ng/ml | 9 | 0.50 | 34.3 (8.1) | 0.48 | 2.37 (.2) | 0.08 |
| Admission cTnT ≤0.2ng/ml | 87 | 0.01 | 40.3 (3.2) | | 1.95 (.1) | |
| Diagnostic cTnT ≥0.1ng/ml | 28 | 0.72 | 41.2 (5.2) | 0.47 | 2.24 (.2) | 0.08 |
| Diagnostic cTnT ≤0.1ng/ml | 68 | 0.01 | 38.8 (3.8) | | 1.90 (.1) | |
| Diagnostic cTnT ≥0.2ng/ml | 24 | 0.80 | 37.1 (4.8) | 0.96 | 2.22 (.2) | 0.16 |
| Diagnostic cTnT ≤0.2ng/ml | 72 | 0.01 | 40.7 (3.7) | | 1.92 (.1) | |
| Peak cTnT ≥0.1ng/ml | 33 | 0.60 | 42.4 (4.9) | 0.33 | 2.22 (.1) | 0.07 |
| Peak cTnT ≤0.1ng/ml | 63 | 0.01 | 38.8 (3.9) | | 1.88 (.1) | |
| Peak cTnT ≥0.2ng/ml | 27 | 0.90 | 37.7 (4.9) | 0.89 | 2.19 (.2) | 0.14 |
| Peak cTnT ≤0.2ng/ml | 69 | 0.01 | 40.6 (3.8) | | 1.92 (.1) | |

Legend. cTnT= cardiac Troponin T; admission $F_{1+2}$ and Fibrinogen concentrations are presented as means with standard errors in parentheses. Differences between values were tested using the Chi - squared statisitical test.

## Discussion.

In patients admitted with chest pain who have a final diagnosis of unstable angina, there is evidence of activation of coagulation on admission to the CCU compared to normal healthy controls. This is in agreement with and confirms the findings of Merlini et al (1994). There did not however appear to be any significant differences between the activation of coagulation in the cTnT positive versus the cTnT negative unstable angina groups.

There are a number of possible explanations for these findings. Alexopoulos et al (Alexopoulos et al,1991) studied Fibrin D-dimer and plasminogen activator inhibitor levels

both peripherally and from the coronary sinus in patients with unstable angina and rest pain in the 24 hours prior to sampling. They were unable to demonstrate any differences between levels of these coagulation markers in the unstable angina group compared to controls and concluded that the amount of thrombus in the coronary artery maybe too small to significantly elevate markers of increased coagulation activation. The same may be true in this study; whilst unstable angina per se has now been shown to be associated with increased prothrombin fragment 1+2 activation, peripheral sampling for this marker may be unable to detect small differences in local thrombin activation in coronary arteries. As activation of coagulation would have been suppressed once antithrombotic therapy was instituted, only the admission coagulation factors were studied. Another more interesting hypothesis exists.

What the presence of cardiac Troponin T in the serum of patients with unstable angina actually represents at a pathophysiological level has not been accurately defined. Its presence is either thought to represent release of the cytoplasmic compartment in critically ischaemic but viable myocardium, or to fragmentation and distal embolisation of intracoronary luminal thrombus causing small distal micro infarction with myocyte necrosis and enzyme release.

A recent study (Kruger et al, 1994) showed that in patients with multi vessel coronary disease undergoing rapid atrial pacing to ischaemia, this short lasting ischaemia was associated with cytoplasmic myoglobin but not cTnT release. All the patients experienced typical chest pain and ST segment changes during the rapid pacing period. Their study would support the hypothesis that cTnT in the serum represents myocyte necrosis from distal embolisation of thrombus. If the fragmenting intracoronary thrombus was mainly platelet rich, one would expect to see evidence of cTnT in the circulation, but there may not be a significant difference in the levels of thrombin present compared to those patients without an intraluminal thrombus. This findings of this study would support such a hypothesis and may have important clinical implications.

A significant number of studies (Hamm et al, 1992., Katus et al, 1991., Seino et al, 1993., Ravkilde et al,1993), have now shown that conventional therapy with heparin, aspirin or

both do not protect the cTnT positive unstable angina subgroup from adverse cardiac events. The need for newer improved antithrombotic regimens for this subgroup has been acknowledged (Chesebro et al, 1992) as this thesis and others have shown they form approximately one third of admissions with unstable angina (Hamm et al, 1992., Katus et al, 1991., Seino et al, 1993., Ravkilde et al,1993).

The lack of difference and lack of correlations in the prothrombin fragment 1+2 and fibrinogen concentrations in relation to cTnT status in this study, would suggest that more specific inhibitors of platelet activation may be more appropriate for this subgroup.

## Chapter Nine

## The role of Lipoprotein (a) concentrations in patients admitted with unstable angina in relation to cardiac Troponin T levels.

### Introduction

The findings in the previous chapter indicate that although in patients with unstable angina there is evidence of increased activation of coagulation on admission, there were no significant differences between the cardiac Troponin T (cTnT) positive and negative groups, that might help explain the long term adverse prognosis of the former group. Another possible mechanism was therefore examined.

Lipoprotein (a) (Lp(a)) was first described over thirty years ago by Berg (Berg et al, 1963). It is a cholesterol-rich plasma lipoprotein with pre-β mobility on lipoprotein electrophoresis (Dahlen et al, 1972). The Lp(a) lipid composition is similar to that of low-density lipoprotein (LDL), but the protein composition is greater, consisting of both apolipoprotein B-100 and a specific hydrophilic glycoprotein (a) named apolipoprotein (a), bound together by a disulphide linkage (Gaubatz et al, 1983).

Apolipoprotein (a) has close structural homology with plasminogen (Mclean et al,1987), with both genes clearly linked on the long arm of chromosome 6 (Frank et al, 1988). There is evidence to suggest that the close homology of Lp(a) with plasminogen results in competitive inhibition of the fibrinolytic properties of plasminogen (Karadi et al, 1988), supporting the concept that defective fibrinolysis is a thrombogenic risk factor in patients with coronary disease.

Very little data exists on the role of Lp(a) in patients admitted with unstable angina. Qui and coworkers (Qui et al, 1991) found no differences between the Lp(a) concentrations on admission between patients with myocardial infarction, unstable angina and normals, whilst Oshima et al (1991) documented transient rises in Lp(a) concentrations in patients admitted with unstable angina.

No data exists however, examining the role of Lipoprotein (a) concentrations in patients admitted with unstable angina in relation to cardiac Troponin T levels.

The purpose of this study was therefore to examine the role of this prothrombotic marker in my unstable angina patient cohort to see if patients with higher concentrations of Lp(a) were more likely to be in the cardiac Troponin T positive sub group.

## Methods.

Samples for Lipoprotein (a) estimation were taken on admission to the Coronary Care Unit along with samples for total cholesterol, triglyceride, apolipoprotein A1 and apolipoprotein B. Sample handling and measurement were as described in the methods chapter.

Differences between the two groups were tested using the non parametric Mann Whitney statistical test for ordinal variables and the Chi-squared statistical test for categorical variables. Correlations were sought between the admission Lp(a) concentration and both the diagnostic cTnT and the peak cTnT concentrations in the unstable angina group, using the Rank Spearman statistical test.

## Results

Admission Lipoprotein (a) concentrations were available for 167 patients with a final diagnostic classification of unstable angina. The baseline demographics are summarised in Table 9.1. 56 patients had cTnT concentrations above the discriminant level of 0.2mcg/litre at the diagnostic time period.

Admission Lipoprotein (a) concentrations were significantly higher in the cTnT positive unstable angina group compared to the cTnT negative group:- median and interquartile ranges 22.25 mg/dl (6.25, 32.0) versus 6.0 mg/dl ( 2.22, 14.8), $p = 0.0004$ (Tables 9.1 and 9.2, Figure 9.1).

Table 9.1. Baseline demographics

| Variable | cTnT -ve | cTnT +ve | Significance |
|---|---|---|---|
| Number | 111 | 56 | - |
| Age(years) | 59.6 | 63.8 | 0.0294 |
| Male sex | 74% | 66% | 0.2943 |
| FHIHD(<55) | 22% | 12% | 0.1214 |
| Hypertension | 27% | 27% | 0.9736 |
| Diabetes Mellitus | 13% | 21% | 0.1910 |
| Current Smoker | 28% | 36% | 0.3038 |
| Previous MI | 38% | 35% | 0.7892 |
| CCSC 3 or 4 | 17% | 14% | 0.6399 |
| Accelerated Angina | 43% | 45% | 0.8637 |
| Rest Pain | 43% | 37% | 0.4780 |
| Abnormal ECG on admission (ST or T) | 40% | 52% | 0.5221 |
| Median time to CCU from worst pain (hrs) | 4.97 | 5.00 | 0.3283 |
| Median Admission Lp (a)(mg/dl) | 6.00 | 22.25 | 0.0004 |
| Mean Admission Total Cholesterol (mmol/l) | 6.26 | 6.43 | 0.8216 |
| Median Admission Triglyceride (mmol/l) | 1.60 | 1.60 | 0.7384 |
| Mean Admission Apolipoprotein A1 (g/l) | 1.35 | 1.42 | 0.3622 |
| Mean Admission Apolipoprotein B (g/l) | 1.1 | 1.1 | 0.7370 |
| Admission TnT mcg/l | 0.01 | 0.10 | 0.0001 |
| Diagnostic TnT mcg/l | 0.01 | 0.80 | 0.0001 |
| Peak TnT mcg/l | 0.01 | 1.06 | 0.0001 |

Legend:- FHIHD = premature family history of heart disease; CCSC = Canadian Cardiac society angina classification before admission; Accelerated angina = increasing angina in the 48 hours before admission; Rest pain = rest pain in the 48 hours before admission; Abnormal ECG on admission = ST or T wave changes on the admission electrocardiogram; TnT= cardiac Troponin T.

Figure 9.1. Boxplots of median, range and interquartile ranges of admission Lipoprotein (a) concentrations in unstable angina patients according to cardiac Troponin T status.

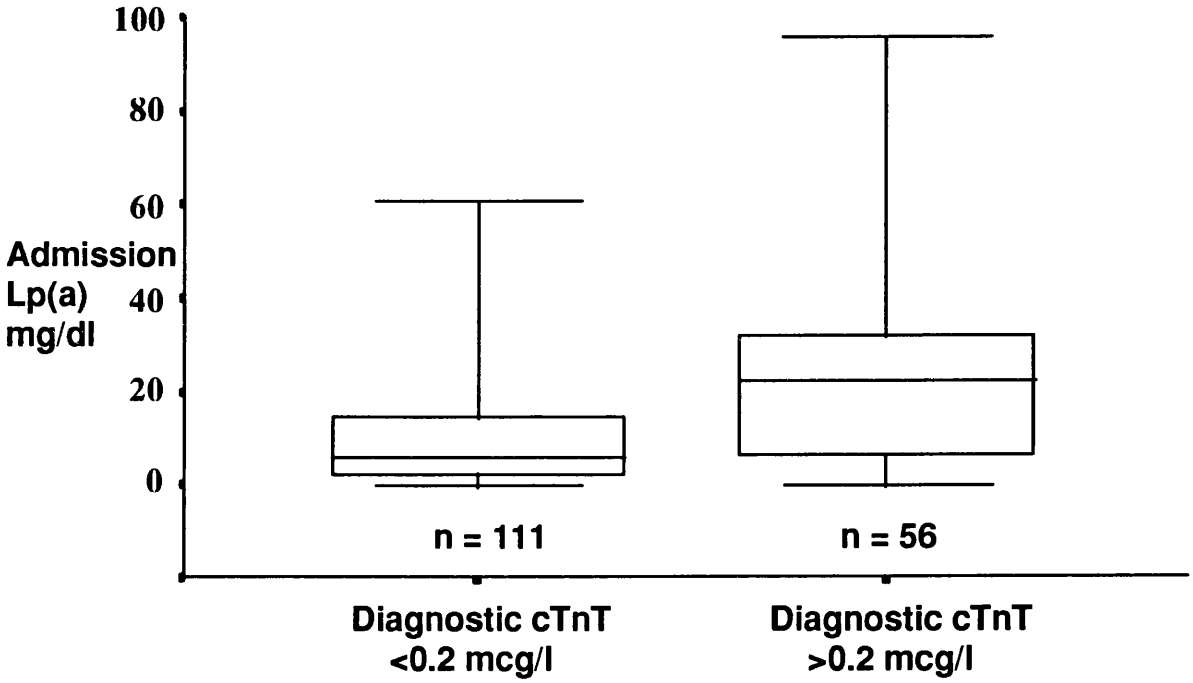



Table 9.2 Median, range and interquartile ranges of admission Lipoprotein (a) concentrations in unstable angina patients according to cardiac Troponin T status.

| Cardiac Troponin T status | Number | Median admission Lp(a) | Range | 25% ile | 75% ile |
|---|---|---|---|---|---|
| cTnT +ve | 111 | 22.25 mg/dl | 0 - 98.0 | 6.25 | 32.00 |
| cTnT - ve | 56 | 6.0 mg/dl | 0 - 62.6 | 2.22 | 14.80 |

Figure 9.2. Scatter plot, rank spearman correlation and significance between admission Lp(a) and diagnostic cTnT concentrations.

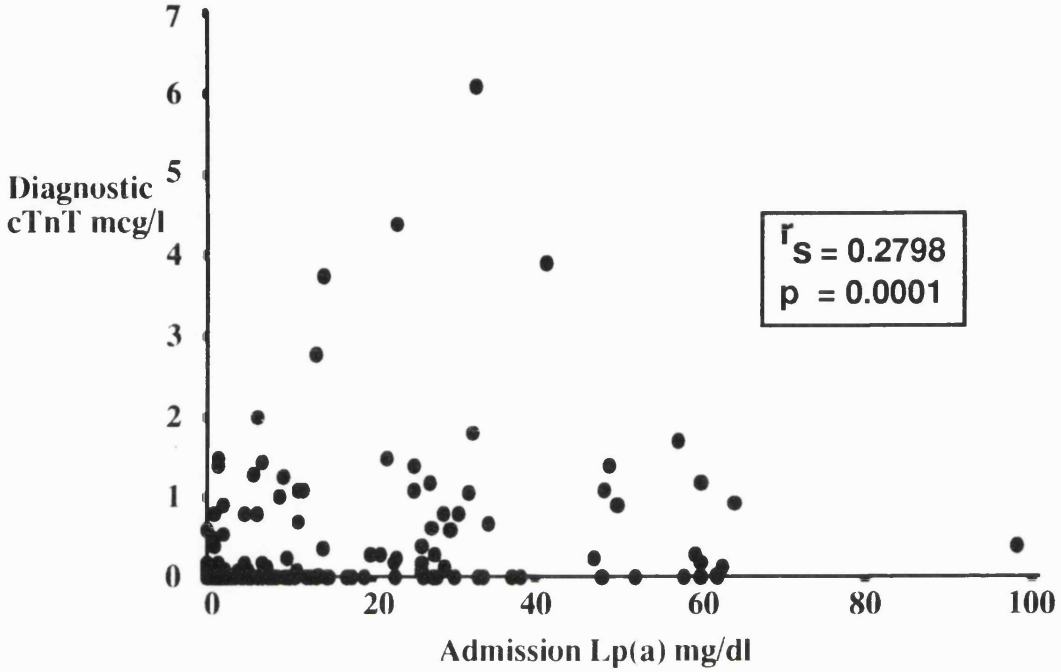
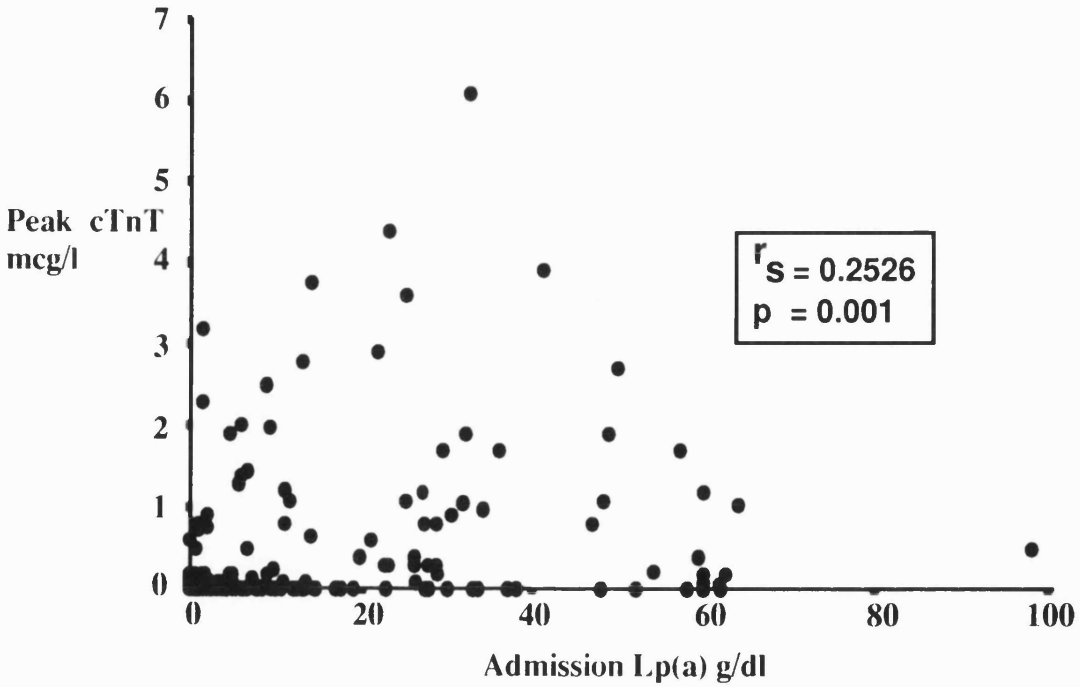



Figure 9.3. Scatter plot, rank spearman correlation and significance between admission Lp(a) and peak cTnT concentrations.



Significant correlations were also found between admission Lp(a) concentrations and both the diagnostic and peak cTnT concentrations in the unstable angina population, Lp(a) and diagnostic cTnT $r_s$ = 0.2798, p = 0.0001 and Lp(a) and peak cTnT $r_s$ = 0.2526, p = 0.001 (Figures 9.2 and 9.3).

## Discussion.

The findings of this study are interesting but are not easy to explain. The significant correlations between the admission Lp(a) concentrations and both the diagnostic and peak cTnT concentrations suggest that higher Lp(a) levels are associated with the extent of myocardial damage in this acute iscaemic syndrome.

Two possible explanations are examined. The first is that Lipoprotein (a) significantly biases the homeostatic thrombogenesis/thrombolysis system in unstable angina patients inducing a more prothrombotic state at the site of the plaque injury.

In an in vitro study, Brandstrom et al (1988) found that Lp(a) had a weak inhibitory effect on the fibrin stimulated tissue plasminogen activator mediated activation of gluplasminogen to plasmin. Several other groups have now shown that Lp(a) in vitro competes for fibrin binding (Hajjar et al, 1989., Loscalzo et al, 1990) and is capable of interacting with cell plasminogen receptors with an affinity comparable with that of plasminogen (Gonzales-Gronow et al, 1989., Miles et al, 1989). Lipoprotein (a) may therefore have the potential to cause a more prothrombotic state at the site of plaque rupture, and the cTnT concentrations reflect distal myocte necrosis from embolising thrombus. An alternative explanation is that Lp(a) concentrations have an affect on the plaque composition.

From animal and in vitro studies (Gianturco et al, 1983., Lawn et al, 1992), Lipoprotein (a) appears to induce foam cell formation and significant lipid accumulation in atherosclerotic lesions which may lead to a softer plaque. When these plaques rupture in acute coronary syndromes, a more severe plaque injury may result (Fuster et al, 1992). The correlations between admission Lp(a) concentrations and subsequent cTnT

concentrations may therefore reflect an appropriate thrombogenic state in response to a deeper plaque injury. Either explanation is possible, as is of course a combination of the two.

This study shows that higher admission Lipoprotein (a) concentrations are found in patients with unstable angina who have evidence of the cardiac specific protein Troponin T in their serum as compared to those who are negative for this marker. Whether this is associated with a subsequent adverse outcome and what the exact pathophysiological explanation of these findings are remain to be elucidated, but would seem worthy of further study.

## Chapter Ten

## Discussion of Thesis findings

The purpose of this thesis was to undertake a detailed examination of the biochemical marker cardiac Troponin T to assess its potential usefulness in routine clinical practice as a marker of myocardial damage.

In Chapter three the cardiac specificity of this marker was tested in a human model of skeletal muscle injury and compared with other biochemical markers of myocyte damage. The study concluded that only cTnT was able to exclude myocardial damage with 100% specificity at the optimised decision threshold. Two of the Royal marines however, had cTnT concentrations below the discriminant level of 0.2 mcg/l but above the detection limit of 0.05 mcg/l (Figure3.2, lower graph, page36)

It was thought that there may be some cross-reactivity due to non specific absorption of skeletal muscle TnT to the assay tubes in the presence of severe skeletal muscle injury (Katus et al, 1991). With creatine kinase levels above 5000 U/l in muscle injury, Troponin T may be detected. Sequential dilutions of such samples shows a non-specific binding curve for Troponin T with disappearance of detectable Troponin T when CK reaches 5000 U/L, but with linear decreases in the CK and CK-MB concentrations in accordance with predicted values. It is now thought that this non-specific binding phenomenon is in fact due to the binding of skeletal Troponin T to the tube wall and subsequent detection within the assay system by the second antibody, which is not cardiac specific (Dr Hugo Katus, personal communication). Boehringer Mannheim have overcome this problem by developing a cardiac Troponin T specific second monoclonal antibody for the assay (Muller-Bardoff et al, 1994).

Neither of the two Royal Marines with detectable cTnT concentrations however had CK values greater than 5000 U/L. Data exists from deaths during extraordinary endurance marathons that there was evidence of cTnT in the serum of these people despite normal coronary anatomy on post mortem (Dr Paul Kerr, Boehringer Mannheim, Germany, personal communication). It is possible therefore, that in cases of extreme oxygen debt

frank cardiac ischaemia with release of cytoplasmic cTnT may result and is the possible hypothesis put forward to explain these Royal Marine findings.

Other data has begun to appear that cTnT may be present in patients with renal failure (Hafner, 1994). In a preliminary study of 115 unselected patients with biochemical evidence of renal impairment (manuscript in preparation), cTnT above 0.2 mcg/l was detectable in 65. There was no consistent relationship between elevated cTnT and either CK, CK-MB, creatine or creatine clearance. Cardiac Troponin T is not cleared across haemodialysis. Examination of the patients records however did show some interesting differences. Most of the patients with end stage renal disease did not have any detectable cTnT in their serum. A significant number who had acute renal failure as part of a multi-system failure in an ITU setting did have detectable levels of cTnT, which may be due to sepsis induced direct myocardial damage. This raises the possibility that this marker may have a place in prognostic stratifications in such patients and will be the subject of further study.

Some of the patients with chronic renal diseases did have detectable cTnT in their serum and this may reflect ongoing occult cardiac damage. It is well known that patients with renal and other metabolic diseases, as well as those with connective tissue and auto immune diseases may have evidence of cardiac involvement. Whether cTnT can be detected in these patients and be shown not only to represent cardiac damage but also to play some role as a marker of disease activity will be the subject of further study.

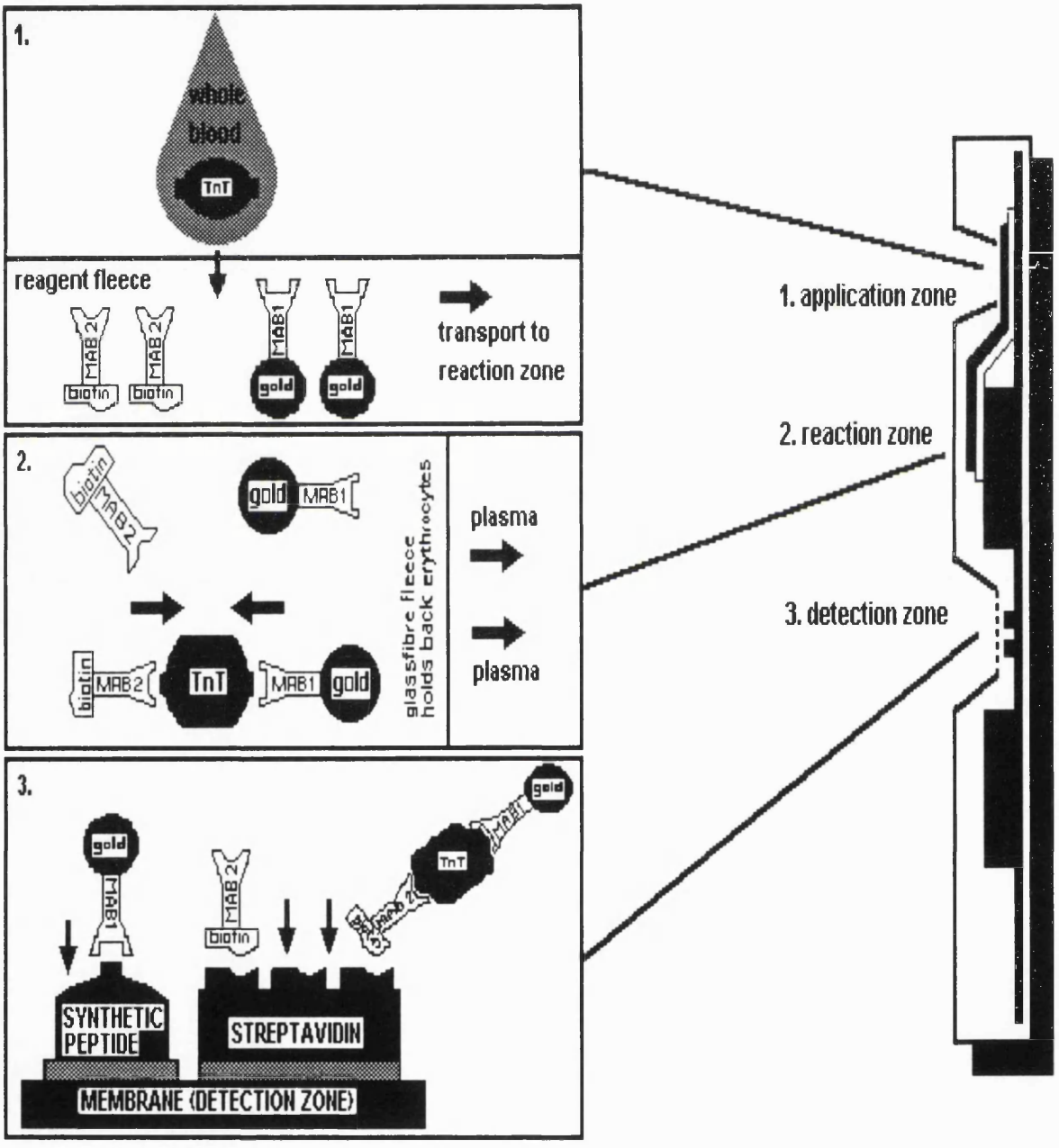
In Chapter four the potential role of cTnT measurement in the differential diagnosis of suspected ischaemic myocardial damage was examined. The study concluded that a single measurement of cTnT 12-24 hours from admission or 12-48 hours from onset of chest pain will confirm or exclude myocardial damage. The assay has a number of limitations however. In a direct comparison of identical patients cTnT offered no advantage over CK-MB, or even CK in the unthrombolysed case when sample timing was accurate and patients with symptoms of more than 12 hours duration on admission are excluded. It was noted however that this represented an ideal situation which may not be achieved in routine clinical practice. The cTnT assay requires an ES analyser (Boehringer Mannheim

Diagnostics, Lewes) for its measurement. These analysers are expensive and are not routinely available. The quantitative assay also takes about two hours from sample collection to result. In order to achieve optimal clinical usefulness for patient management a more rapid cTnT assay system may be required which can produce diagnostically valid results within a short time frame and on a "stat" basis. A dry chemistry system requiring no instrumentation using whole blood as the primary sample material has been developed (Trop T, Boehringer Mannheim Diagnostics, Lewes). The system is shown diagrammatically in Figure 10.1.

150 microlitres of sample material is pipetted onto the sample port using a semi-automatic pipette. The reaction zone contains two monoclonal antibodies (mabs) specific for different epitopes of the cardiac isoform of the troponin molecule (figure10.1, section 1). One mab is gold labelled and the other biotinylated. The two mabs dissolve in the blood and bind to any troponin present to produce a double antibody troponin complex (figure10.1, section 2). The sample spreads by capillary action along the test strip and through a fleece where erythrocytes (if present) are separated from the serum or plasma to the detection zone (figure10.1, section 3). This comprises an immobilised streptavidin reaction zone which binds the double antibody sandwich with development of a red colour. The gold labelled antibody is present in excess. Unbound gold labelled antibody binds to a second control line comprising a synthetic peptide with sequence homology to the epitope of cardiac troponin recognised by the antibody. Binding to this synthetic peptide creates a red coloured control line to confirm successful sample application and sample diffusion.

This technique, referred to as Gold Linked Optical Read Immuno Assay (GLORIA) reaction, is complete in twenty minutes and reads as follows : no lines visible, unsuccessful reaction; 1 line visible, negative test with successful reaction; 2 lines visible, positive test with successful reaction. In a preliminary evaluation of 197 unselected patients admitted with chest pain considered cardiac in origin (P.Collinson, P.Stubbs et al, Annals of Clinical Biochemistry in press), the performance of this rapid assay compared to the quantitative ELISA assay were compared.

Figure 10.1 Diagram of the Trop T rapid assay system.



When all measurements of the rapid assay were undertaken by a single operator, the manafacturers quoted, and the required accuracy, of a diagnostic cut-off of 0.2mcg/l was achieved by the rapid assay. Interestingly, there was a reciprocal relationship between time to first positive and cTnT value suggesting that the assay could be made semi-quantitative.

In a similar European based trial, more routine testing of the rapid assay by multiple operators found the diagnostic cut-off for the rapid assay to be closer to 0.3 mcg/l (Dr W.Gerhardt, personal communication), indicating that some experience with the assay may be required to achieve the optimal diagnostic level. An international multicentre study is underway to evaluate the potential usefulness of this rapid assay in triaging patients admitted to emergency departments with chest pain. Whilst the rapid assay will allow cTnT measurement in hospitals that do not have the quantitative analyser a number of important caveats exist.

A major limiting factor for all biochemical markers of myocyte injury that could be used for early patient triage is that their appearance in the circulation is time dependent. Patients with severe cardiac symptoms may present very early to casualty when insufficient time has elapsed for biochemical markers to appear in the serum (Bakker et al, 1993). Such patients presenting within two to three hours from the onset of the chest pain will not have any detectable cTnT in their serum despite obvious ECG evidence of myocardial infarction. ST segment elevation can occur very early in the course of myocardial infarction before any significant elevation of any biochemical marker (Timmis, 1990), and is present in the admission ECG of at least half of patients with a final diagnosis of myocardial infarction (Norris et al, 1994).

There is very little need for early biochemical markers in this group. Single estimation of any biochemical marker in the casualty setting similarly has little or no place in the decision to admit or send home patients presenting with chest pain (Timmis, 1994). As shown in figure 4.2 page 45 even cTnT, which appears in the circulation slightly earlier than other routinely measured biochemical markers of myocyte damage (Katus et al,

1991), was only found above the diagnostic cut-off in at most 50% of patients on admission to the CCU with a final diagnosis of myocardial infarction. The study also showed that cTnT reaches maximal efficiency some twelve hours after the onset of chest pain and included all patients with a final diagnosis of myocardial infarction irrespective of the ECG findings. The precise role of cTnT measurement if only used when the ECG is not diagnostic requires further study. At the present state of knowledge, the decision to admit patients with chest pain and non diagnostic ECG's should remain clinically based.

Cardiac Troponin T has a long diagnostic window and so is useful as a "catch-all" marker for confirming myocardial damage. This long diagnostic window does however have a disadvantage in the clinical setting. If a patient is discharged a week or so following a myocardial infarction and represents to casualty a few days later with further chest pain, then cTnT may still be present in the circulation. To accurately time the myocardial damage therefore a marker with a shorter diagnostic window such as CK or CK-MB is also required. This has the added advantage of using these other markers to classify the cTnT positive patients into myocardial infarction or unstable angina.

The finding of cTnT in patients with unstable angina explains the paradox shown in chapter four of a completely specific marker for myocardial damage being only 90% specific for myocardial infarction. The presence of this marker in the unstable angina patients appeared to be associated with an adverse prognosis and this was therefore studied and the results presented in chapter six.

This was a single centre observational study with a median of approximately three year follow up. The setting for the study was a District General Hospital without on site angiographic facilities and a conservative strategy for unstable angina patients being practised for the most part. This enabled me to study what was in essence the natural history of this unstable angina sub group. The study concluded that the presence of cardiac Troponin T at a diagnostic cut-off of 0.2mcg/l in the serum of patients admitted with unstable angina, when measured at a practical time point in the routine clinical environment, identified a high risk subgroup that had a higher frequency of cardiac death. The presence of cTnT also identified a subgroup that had a higher frequency of coronary

revascularisation, cardiac death or coronary revascularisation and cardiac death or readmission with non-fatal myocardial infarction as first event on long term follow up.

In the regression model for each endpoint, which compared Troponin T status with clinical and ECG variables, the presence of cTnT fared well for the endpoints need for coronary revascularisation and cardiac death or revascularisation as first event. When Dr Braunwald published his unstable angina classification (Braunwald, 1989) , he justified the need for such a classification by commenting that:

"patients with unstable angina can be described by using information obtained not only from the clinical examination and routine electrocardiogram but also from a variety of specialised tests, including coronary arteriography, left ventriculography, continuously recorded (Holter) electrocardiography and perfusion scintigraphy. Characterisation of patients by all of these methods can lead to the classification of unstable angina into an almost infinite number of subgroups. Such fine classification is almost as useless to the clinician as is the lumping of all patients into a single category".

His own classification however (Table1.3, page 10) potentially allows 54 different subgroups and its heavy reliance on rest pain, which is almost universal in patients admitted to hospitals in routine clinical practice (Murphy et al, 1992), markedly limits its practical application in the routine UK clinical environment. The significance of rest pain as a predictor of future cardiac events can also be questioned as in the study described in chapter six it did not achieve statistical significance in any of the endpoints studied..

In patients admitted with unstable angina this study showed that the presence of accelerated angina in the 48 hours before admission or cTnT presence was the best predictor for the endpoints coronary revascularisation or cardiac death or revascularisation as first event. This simple combination accounted for 61% of all subsequent cardiac deaths, 86% of all subsequent revascularisations, and 77% of all cardiac deaths or revascularisations as first events. Interestingly, although the discriminant of 0.2mcg/l appeared to be the best predictor of the endpoints cardiac death and cardiac death or revascularisation as first event, the higher the cTnT discriminant the higher the need for

revascularisation (Table 6.14, page 70). Presumably this reflects a deeper plaque injury and/or may reflect a continuing prothrombotic state.

This study concluded that the routine measurement of cTnT has a place in routine clinical practice in patients admitted with unstable angina and will aid in risk stratification. The marker can therefore crossover from being a diagnostic tool to being a patient management tool in this condition. The question then arises about what management stategy should be adopted in this unstable angina subgroup. No study has tackled this difficult problem yet. From my study it appears that this group benefit from revascularisation but whilst the type of procedure will be determined by the anatomy, the ideal timing for the intervention remains to be elucidated. Some answers may come from the GUSTO investigators. Part of the GUSTO 11(a) protocol examined cTnT concentrations in patients admitted with chest pain without ST segment elevation on the admission ECG and tested an early intervention versus an early conservative strategy.

A number of other studies examining different therapeutic strategies in unstable angina are also measuring cTnT concentrations (Dr Uppenkamp, Boehringer Mannheim, Germany, personal communicaton). All of the studies however will be retrospective and although they may provide some data there is a need for a prospective study to test different management protocols, particularly ones which are feasible in routine clinical practice. Management protocols for the optimum treatment for patients admitted with chest pain who are cTnT negative are also required.

At the present time most patients admitted with chest pain are given the benefit of the doubt and occupy expensive CCU beds waiting for enzymes and receiving oral and intravenous drug therapy according to local practice. This population may form at least 50% of CCU bed occupancy (Pozen et al, 1984). If no cTnT is detected 12-24 hours from admission then no detectable myocardial damage has occured and these patients form a lower or no risk group depending on the final diagnostic classification. The potential exists therefore to move this group to a general ward and to investigate them earlier. An example of a possible protocol would be to stop all therapy besides aspirin once the cTnT result is known and to exercise test them. The exercise test protocol used could be a

submaximal or a modified Bruce protocol to 75% of predicted heart rate. Those with negative treadmill tests could be discharged for out patient review, whilst those with positive tests could receive a number of different treatment strategies, ranging from intensive medical treatment to early invasive investigation.

The event rates for the cTnT negative group for all of the endpoints studied in chapter six show that Troponin status should not be used as a sole discriminator of risk. Other factors such as extent of disease may play a part as well as other systemic factors. From the findings in chapter nine, Lipoprotein(a) may have a role as well as factors like reactivation of a prothrombotic state when heparin therapy is discontinued (Theroux et al, 1992). A number of studies appear to be justified to see what other factors could improve even further the impressive prediction of future events picked up by the simple combination of cTnT and accelerated angina.

The remaining chapters of the thesis focussed on attempting to explain why the presence of cTnT in unstable angina is associated with an adverse outcome. In chapter seven a detailed study of the coronary angiographic and culprit lesion morphological findings in unstable angina in relation to cTnT concentrations was undertaken. The findings of this study suggest that the adverse prognosis of the cTnT positive unstable angina group compared to the cTnT negative unstable angina group is not explained by any major differences in the extent or severity of coronary artery disease, or by the location or morphological findings of the culprit lesion during the repair phase of plaque disruption. The findings of the study are open to a number of criticisms. By restricting the study to stabilised unstable angina patients in the repair phase of the syndrome, I may have missed significant differences between the cTnT positive and cTnT negative unstable angina groups. It has recently been reported (Kondo et al, 1994) that intracoronary mural thrombus on early coronary angiography could be identified in over 50% of patients admitted with unstable angina who were cardiac Troponin T (cTnT) positive. No intracoronary thrombus was seen in the cTnT negative group. This study was small however and the findings remain to be confirmed. It does suggest however, that early angiography and examination of the culprit lesion morphology might show significant

differences. Because of the conservative management strategy practiced by the physicians and lack of on-site angiographic facilities it was not possible to study these patients any earlier.

The study group was also selected in that almost all the patients were referred because of positive evidence of continuing ischaemia on follow up investigation, either exercise treadmill testing or persantin thallium scintigraphy or for some other reason such as young age. A more definitive study would therefore be to examine the coronary anatomy and culprit lesion morphology both whilst inpatients and again during follow up in patients admitted with cTnT +ve and cTnT -ve unstable angina. Another criticism of the study is the sample size. Correlations were observed between both mean luminal diameter, plaque area and cTnT concentrations that might have achieved conventional statistical significance if the sample size had been larger. Although the differences between the morphological findings were small, it is difficult to know if they are meaningful or not, as no data exists that quantifies level of risk to degree of variation of a morphological variable. In defence of the study, the finding of an eccentric stenosis in the culprit vessel in 70% of the unstable angina patients studied is in agreement with other much larger studies (Ambrose et al, 1985, Ambrose et al, 1988).

A more recently published large study (Bar et al, 1994) has also shown that the early coronary angiographic and plaque morphological findings in patients admitted with unstable angina do not predict clinical outcome. Taken with the findings of Hamm et al (1992) who did not identify any significant differences in early visual coronary angiographic findings between their cTnT +ve and cTnT -ve unstable angina groups, on the available data to date it does not appear that what you see on a coronary angiogram in unstable angina will predict those patients who are going to experience an adverse outcome. Evidence for the role of a more prothrombotic state in the cTnT +ve unstable angina group was examined in chapter eight.

Activation of coagulation was assessed by the measurement of prothrombin fragment 1+2 ($F_{1+2}$ ) and Fibrinogen concentrations in patients with unstable angina. The study concluded that in patients admitted with chest pain who have a final diagnosis of unstable

angina, there is evidence of activation of coagulation on admission to the CCU compared to normal healthy controls. There did not however appear to be any significant differences between the activation of coagulation in the cTnT positive versus the cTnT negative unstable angina groups. In acute coronary syndromes, plaque rupture triggers platelet aggregation and activation of the coagulation system (Fuster et al, 1988). In unstable angina thrombosis may occur and is usually non occlusive (Fuster et al, 1992). The prothrombin fragment 1+2 is released during the conversion of prothrombin to thrombin (Merlini et al, 1994). The most important role of the generated thrombin is that it leads to the formation and polymerisation of Fibrin, which stabilises the platelet mass and allows the arterial thrombus to resist dislodgement by the high intravascular pressure and shear forces. Thrombin will also promote further platelet aggregation (Fuster et al, 1988). If the hypothesis that the presence of cTnT in the serum of patients with a final diagnosis of unstable angina represents embolising thrombus causing distal myocyte necrosis is correct, then clearly successful stabilisation of the platelet mass is not occuring in this unstable angina sub group.

A significant correlation was found between admission $F_{1+2}$ and Fibrinogen concentrations in the whole unstable angina group confirming that activation of the coagulation system was taking place. No significant correlations or differences however were found between the cTnT status or concentrations and the coagulation variables in any of the cTnT time points studied. It may be that the differences are too small to be detected on peripheral sampling and coronary sinus sampling is required to accurately study differences in coagulation activation. The prognostic role of the finding of thrombus on early angiography in patients with unstable angina has also recently become unclear. Angiographic and angioscopic detection of thrombus in patients with unstable angina has been said to be almost exclusively found in patients with angina at rest (Chesebro et al, 1992). The finding of thrombus in the culprit vessel in patients with unstable angina is thought to reflect a deeper plaque injury and associated with an adverse prognosis when compared to patients who have no thrombus visible. Two recent studies have questioned this view.

The first from Dr Braunwalds group (Ahmed et al, 1993) studied the early visual and morphological angiographic findings in unstable angina patients and compared these findings with his proposed unstable angina classification. There was no difference between the incidences of intracoronary thrombus between his three classification groups. Patients in class 1 i.e. no rest pain, had a 17% incidence of intracoronary thrombus as compared to the same incidence found in patients with class 111 unstable angina i.e. acute pain at rest. Bar et al (1994) have also demonstrated that the presence or absence of thrombus is not related to clinical outcome. The reason for this may be that angiography or angioscopy can only comment on whether mural thrombus is present or not, and not on its stability. It is the labile intraluminal thrombus rather than the mural thrombus that may determine the degree of future risk. In a post mortem study examining the cardiac pathological findings in patients admitted with unstable angina who died (Falk et al, 1985), evidence of fragmented thrombotic material in the distal intramyocardial circulation were found suggesting a failure of plaque clot stabilisation and repair and continued thrombus formation. In experimental models of endothelial injury, labile thrombi, which are mainly platelet rich, are only apparent for between 10-20 minutes before being dislodged by the flowing blood (Badimon et al, 1986). The transient nature of these thrombi makes it impossible to systematically study their presence in unstable angina patients using angiography or angioscopy.

Another limiting factor of angiographic morphological studies is the requirement to maximally dilate the coronary vessel using intracoronary nitrates, which removes the ability to assess the role of locally mediated vasoconstriction in this syndrome (Maseri,1986). If the presence of cardiac Troponin T in the circulation therefore represents myocyte necrosis from fragmenting labile platelet thrombi, it may be more appropriate to study variations in platelet activation rather than coagulation activation in unstable angina patients and will be the subject of further study.

The overall conclusions of the study in chapter eight are that there does not appear to be evidence on peripheral sampling of any differences in coagulation activation on admission between the cTnT positive and cTnT negative unstable angina groups. The presence of

cTnT in the serum may therefore be a result of embolising platelet thrombus, reflecting a failure of plaque stabilisation. If correct, this would be an important finding, as it is becoming increasingly clear that aspirin does not protect this unstable angina subgroup from subsequent cardiac events (Hamm et al, 1992., Katus et al, 1991., Seino et al, 1993., Ravkilde et al,1993). Newer more specific inhibitors of platelet activation may be a more appropriate therapeutic option.

In chapter nine the potential role of one of the systemic risk factors for thrombogenesis was examined. The role of Lipoprotein (a) concentrations in patients admitted with unstable angina in relation to cTnT levels were studied. This study showed that higher admission Lipoprotein (a) concentrations are found in patients with unstable angina who have evidence of the cTnT in their serum as compared to those who are negative for this marker. The study provides the first *in vivo* evidence of a significant role for Lipoprotein (a) in the pathogenesis of this acute coronary syndrome. It is also the first demonstration of a risk factor that may play a role in the pathogenesis of cTnT release in unstable angina. The possible explanations for these findings were discussed at the end of chapter nine. With the findings in chapter eight of a lack of evidence of increased activation of coagulation in the cTnT positive unstable angina group compared to the cTnT negative group and the discussion above, it may be that the thrombogenic role of Lipoprotein (a) is a more plausible hypothesis. Lipoprotein (a) may be interfering with homeostatic attempts to stabilise the ruptured plaque by interfering with fibrin deposition (Hajjar et al, 1989, Loscalzo et al, 1990) thus allowing continuing labile platelet thrombus formation. The therapeutic options available to lower Lipoprotein (a) concentrations however, are very limited. Concentrations are not affected by diet or by most standard lipid lowering agents (Uterman, 1989). Drugs such as nicotinic acid will lower Lp(a) levels (Carlson et al, 1989) but there is only limited data to show that this may limit future cardiac risk (Dahlen GH, 1988). Whether significant sustained lowering of Lp(a) concentrations acutely in unstable angina is possible is unknown. Whether Lp(a) concentrations are associated with a subsequent adverse outcome and what the exact pathophysiological explanation of these findings are remain to be elucidated, but would seem worthy of further study.

**Should cardiac Troponin T positive unstable angina patients be reclassified as myocardial infarctions ?**

This question has been debated amongst investigators for about two years now. Because the presence of cTnT in the circulation in unstable angina represents myocardial damage, should these patients be reclassified as non-q wave myocardial infarctions. Examination of the event free survival curves for the endpoint cardiac death of patients with a final classification of myocardial infarction or cTnT positive unstable angina who did not undergo subsequent revascularisation (Figure 10.2), shows that these curves run practically parallel with no difference in mortality on long term follow up. The cTnT positive unstable angina group therefore have evidence of cardiac damage and do as badly as myocardial infarction patients. If these two criteria are used, reclassifying the cTnT positive unstable angina group as non-q wave myocardial infarctions would appear appropriate. A number of problems exist however.

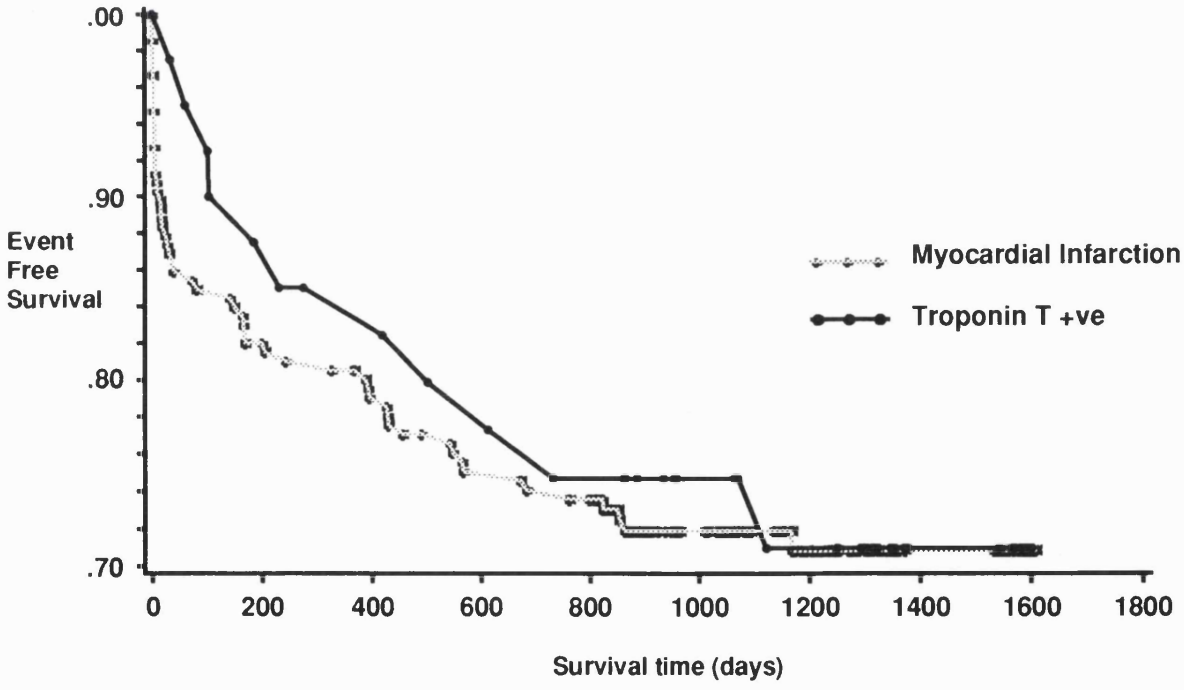
Accurately distinguishing between unstable angina and non-q wave infarction is a grey area. One of the continuing confounding problems is the WHO biochemical criteria. The criteria require that for the diagnosis of myocardial infarction biochemical markers of myocyte damage must exceed twice their upper reference limit. No strict criteria however are applied to the *frequency* of sampling. The literature is therefore littered with publications using different sampling protocols for different biochemical markers of myocyte damage and therefore variations in the final diagnostic classifications. This is especially true for biochemical markers that have a significant cytoplasmic fraction. Most of these markers will maximally appear in the circulation within the first 24 hours of myocardial damage. The more frequently one samples within this time period the more likely one would be to meet the WHO biochemical criteria for myocardial infarction and the more one would shift patients from a diagnosis of unstable angina to non-q wave infarction.

This conundrum will continue until there is an internationally agreed standardised sampling interval for each biochemical marker. The diagnostic classifications used in this thesis were

based on the daily concentrations of the routinely available biochemical markers Creatine Kinase, Aspartate Transaminase and Hydroxybutyrate Dehydrogenase.

If the concentrations of these markers did not exceed twice their upper reference limit on this sampling protocol, then myocardial infarction was not diagnosed. A more frequent sampling protocol of creatine kinase within the first 24 hours, would have moved a significant percentage of patients with a final diagnosis of unstable angina, particularly the cTnT positive group as the early release of this marker into the serum in acute coronary syndromes represents the cytoplasmic compartment fracton, over to a final diagnosis of non-q wave myocardial infarction.

Figure 10.2 Survival curves for the endpoint cardiac death for myocardial infarction and cTnT positive unstable angina patients who did not receive revascularisation.




I feel that the diagnostic classifications used in this thesis are justified and valid however, as the biochemical sampling protocol used represents routine UK clinical practice and

compared to a multiple sampling strategy is feasible in nearly all units admitting patients with acute coronary syndromes.

This thesis concludes that the presence of cardiac Troponin T in the circulation is a sensitive and specific marker of myocyte damage. The diagnostic cut off is 0.2 micrograms per litre. When used to confirm myocardial infarction in patients admitted with chest pain, the marker is maximally efficient when measured at any time between 12 and 24 hours from admission. When present in patients with unstable angina it will identify a sub group that are at higher risk of subsequent cardiac events and will aid physicians in risk stratification in this acute coronary syndrome. In patients admitted to hospital with chest pain, the routine measurement of cardiac Troponin T appears to be justified.

**Future Studies**

The findings of this thesis have raised a number of questions which are either currently under study or are hoped to be studied in the future. The studies include:

1. The comparison of cardiac Troponin T with other newer markers of myocardial damage in acute coronary syndromes. Studies are underway comparing a newer more sensitive CK-MB assay as well as Troponin I assays.

2. A management protocol has been formulated to test the safety and feasiblity of rapid mobilisation of patients admitted with chest pain who are negative for cardiac Troponin T in their serum the day following admission. It is hoped to test this strategy in the near future.

3. I would like to redo the activation of coagulation on admission study in patients with unstable angina, measuring coagulation markers from the coronary sinus. This would require taking patients with good clinical histories and non diagnostic ECG's from casualty direct to the catheter laboratory. Measurements from the coronary sinus would be made before angiography or initiation of antithrombotic therapy. It may also be possible to measure platelet activation but this would require a very exacting protocol. An attempt will be made to formulate such a protocol. The immediate angiography would also allow a more detailed appraisal of the culprit lesion morphology.

4. A prospective trial is also required to identify the best management of patients with unstable angina who are positive for cardiac Troponin T. Centres with on site invasive facilities could test a protocol comparing maximal medical therapy with early intervention, whilst centres without on site invasive facilities could test a protocol comparing maximal medical therapy versus early referral for invasive investigation. These should ideally be multi-centre randomised studies which would require significant resources.

5. The lipoprotein(a) study results warrant further research. Outcome and angiographic data have been recorded on these patients and examination of plaque morphology as well as the cardiac event rates on follow up will be the next analysis undertaken.

6. Current research examining the pathophysiology for the presence of cardiac Troponin T in renal failure will be continued and extended to examine its potential role as a marker of cardiac involvement in other metabolic and auto-immune conditions. Three particular studies will be undertaken if possible. The first will be to examine the frequency of the finding of cardiac Troponin T in a number of these conditions. The second will be to try and confirm that the cTnT is coming from the heart. The cardiac involvement in these conditions may be patchy, and so it may be better to compare Positron Emission Tomography findings in matched patients who are positive or negative for the presence of the marker than attempt cardiac biopsy. The third study will be to assess whether the marker mirrors disease activity by measuring cTnT concentrations in patient undergoing treatment for acute flare ups of their disease.

7. It would also be interesting to assess the prognostic role of the presence of cardiac Troponin T in patients with sepsis induced multi-system failure in an ITU setting. An attempt will be made to set up a blinded study of the value of daily cTnT measurement in ITU for such patients and whether its presence correlates with other prognostic variables measured.

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## Supervisors statement

**M.D thesis of Dr P.J. Stubbs**

**Cardiac Troponin T and myocardial damage**

I can confirm that, apart from the exceptions stated under "acknowledgements", all the work described in this thesis has been undertaken solely by Dr Stubbs at his own initiative. Specifically, the collection of data in the described cohort of patients being admitted to CCU was not an on-going enterprise of my Unit, but was initiated by Dr Stubbs himself to enable the research he describes to be undertaken. He has organised all aspects of this work and completed the follow-up which allows the thesis to contain a presentation of prognostic results.

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## Presentations

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