Thesis Submitted for the Degree of Doctor of Medici

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Publications Relating to Thesis

Papers

Khan SQ, Quinn P, Davies JE, Ng LL. N-terminal-pro-B type natriuretic peptide is better than TIMI risk score at predicting death following acute myocardial infarction. *Heart* (2008) 94(1):40-3.

Khan SQ, Kelly D, Quinn P, Davies JE, Ng LL. Myotrophin is a more powerful predictor of major adverse cardiac events following acute coronary syndrome than N terminal pro B type natriuretic peptide. *Clinical Science* (Lond) (2007) 112(4):251-6.

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Khan SQ, Kelly D, Quinn P, Davies JE, Ng LL. Prognostic value of myeloperoxidase in patients with acute myocardial infarction. *Heart* (2007) 93(7):826-31.

Abstracts

Khan SQ, Bhandari SS, Quinn P, Ng LL. Myeloperoxidase in the prediction of major adverse coronary events in unselected patients following acute myocardial infarction comparison with NT-BNP. *Acute Cardiac Care* (2006) suppl 2(8) A43.

Khan SQ, Kelly D, Quinn P, Ng LL. Myeloperoxidase aids prognostication when utilised with NT-BNP in patients with acute ST segment myocardial infarction. *European Heart Journal* (2006) suppl 1 A3858.

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Abstract

Risk stratification of myocardial infarction using cardiac peptides
Author: Dr. Sohail Q. Khan

Introduction: NTproBNP is an established marker of adverse outcome following an AMI. We investigated the utility of the TIMI risk score, Cardiotrophin-1 (CT-1) an inflammatory cytokine, myotrophin a 12 kD protein which modulates NFκB activity and MPO an inflammatory marker in combination with NTproBNP at predicting adverse outcome following AMI.
Method: We recruited 596 patients (mean age 64.4 ± 12.5, 74.3% STEMI, 25.7% NSTEMI) presenting with AMI. Serial blood measurements were taken at 0-24, 25-48, 49-72, 73-96, 97-120 hours after the onset of chest pain. Patients were TIMI risk scored and stratified into low (0 to 2), intermediate (3 to 7) and high risk (>8) groups. The concentrations of CT-1, myotrophin, MPO and NTproBNP were measured using a non-competitive chemiluminescent immunoassay. AMI patients were compared with age and sex matched control subjects. We studied 473 patients with STEMI for the TIMI study. We studied 504 post AMI patients for the CT-1 and MPO study. We studied 596 AMI patients for the myotrophin study. All patients were followed-up for death, recurrent MI, heart failure and MACE (death, MI and need for urgent revascularisation).
Results: Mortality was 8.9% and was related to higher TIMI risk scores ($p=0.029$ for trend). Higher NTproBNP levels were also related to increased mortality (median [range] fmol/ml, survivors 700.2[0.3-11485.3] vs. dead 5781.3[1.4-10835.9], $p<0.0001$). In a multivariate binary logistic regression model, independent predictors of mortality were NTproBNP levels in the first 24 hours (OR 4.21, $p<0.001$) along with drug therapies. The receiver-operating curve for NTproBNP in the first 24 hours yielded an area under the curve (AUC) of 0.79, for TIMI risk score the AUC was 0.67.
CT-1 was raised in patients with death or heart failure. Using a multivariate binary logistic model CT-1 (HR 1.5, $p=0.034$) and NTproBNP (HR 2.1, $p=0.05$) predicted death or heart failure independently of established clinical risk factors. The receiver-operating curve for CT-1 yielded an area under the curve (AUC) of 0.62; for NTproBNP the AUC was 0.77; the logistic model combining the 2 markers yielded an AUC of 0.84.
Myotrophin was raised in patients with death, death or heart failure and MACE. In Cox analysis myotrophin (HR 5.07, $p=0.007$) and NTproBNP (HR 7.15, $p=0.019$) independently predicted death. However myotrophin was better at predicting death or heart failure (HR 2.35 $p=0.043$) and MACE (HR 1.69, $p=0.05$).
Median MPO was raised in patients experiencing death, death or MI, death or HF and MACE. In Cox analysis median MPO predicted death (HR 13.05, $p=0.014$) and death or non-fatal MI (HR 5.07, $p=0.015$). MPO had predictive power in both below and above median NTproBNP levels (log rank 5.60, $p=0.020$, log rank 5.12, $p=0.024$, respectively).
Conclusion: After an AMI, NTproBNP is superior to TIMI risk scoring at predicting mortality.
After an AMI, combined levels of NT-proBNP with CT-1 are more informative at predicting death or heart failure. Combined levels of NT-proBNP with myotrophin are more informative at predicting death or heart failure and MACE. NT-proBNP and MPO are more informative at predicting death and death or reinfarction.

A multimarker approach with NTproBNP is more informative and may be useful for risk stratification in AMI patients.

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Abbreviations and Acronyms

ACE	Angiotensin converting enzyme
ACS	Acute coronary syndrome
AMI	Acute myocardial infarction
ANP	Atrial natriuretic peptide
BNP	Brain natriuretic peptide
BSA	Bovine serum albumin
CNP	C-type natriuretic peptide
ECG	Electrocardiogram
FCS	Foetal calf serum
LBBB	Left bundle branch block
LVEF	Left ventricular ejection fraction
LVSD	Left ventricular systolic dysfunction
LVWMI	Left ventricular wall motion index
MAE	Methyl acridinium ester
MI	Myocardial infarction
MPO	Myeloperoxidase
NS	Non significant
NSTEMI	Non-ST-elevation myocardial infarction
NTproBNP	N terminal of proBNP
PMH	Past medical history
ROC	Receiver operating characteristic
STEMI	ST-elevation myocardial infarction

Chapter 1:

Introduction

1.1 Ischaemic heart disease

Ischaemic heart disease can manifest itself in many different ways with stable angina at the milder end of the spectrum and myocardial infarction (MI) and death at the extreme end of the spectrum. In-between these two scenarios is the diagnosis of silent ischaemia and unstable angina. Unstable angina and MI are terms that have become grouped together and are now encompassed under the heading of "acute coronary syndromes" (ACS). It is becoming evident that there is a common pathological basis behind the ACS, namely the rupture of an atherosclerotic plaque with different degrees of superimposed thrombosis, (1,2) vasoconstriction and distal embolisation; (3) it is also becoming evident that unstable angina and MI are merely different clinical presentations that result from this common underlying mechanism. (Figure 1.1)

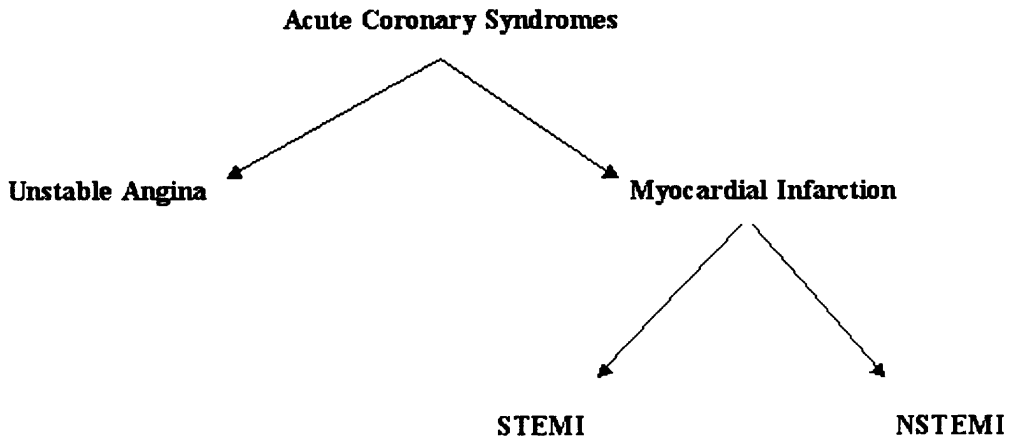

Figure 1.1. Breakdown of ACS consisting of unstable angina and MI. MI itself can be defined as ST- segment elevation MI (STEMI) or non-ST segment elevation MI (NSTEMI)

1.2 Mortality from AMI

Restoring blood flow promptly in an occluded coronary artery using fibrinolytic agents and aspirin reduces mortality in myocardial infarction with ST elevation and this has been borne out in large randomised control trials and large meta analyses (4,5,6) with greatest benefit being derived for those patients being treated early (7). Other effective treatments such as β-blockers, angiotensin II converting enzyme (ACE) inhibitors and aldosterone antagonists have also improved the prognosis of MI. ACE inhibitors improve mortality, (8,9) however this is more pronounced in patients with signs of heart failure or patients suffering from anterior wall MI (10). In the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS), aldosterone blockade with eplerenone reduced the mortality rates in patients with left ventricular systolic dysfunction and heart failure after acute myocardial infarction (11). In ISIS –1 the use of atenolol in patients with AMI reduced mortality compared with those who received placebo therapy with most of the benefit being derived in the first 24 hours (12). Timolol has also been shown to be beneficial at reducing death and reinfarction if given between 7 and 28 days (13). However it has been suggested that early introduction of a beta blocker (metroprolol) although beneficial at reducing reinfarction and ventricular fibrillation may actually increase the risk of cardiogenic shock especially if given within 24 hours. (14) Despite the availability of all these new pharmacological therapies, there still remains a sizeable mortality in patients who have an MI. (15) The aim in the future will be to better identify those patients who are at risk so therapy can be tailored to their needs.

1.3 Risk stratification

The aim of risk stratification is to be able to predict prognosis in patients with AMI/ACS in the hope to improve patient management and thereby reducing patient morbidity and mortality. Risk stratification also has financial implications in that it improves the cost effectiveness of patient care and expensive treatments such as revascularisation whether that is percutaneously or by CABG can be tailored to those who will derive most benefit. Also there is little point in placing patients at risk of an invasive procedure if the risk of the actual procedure outweighs any benefit that may be gained by the procedure. Currently the risk stratification of ACS patients is based on clinical history, examination findings, electrocardiographic changes, systolic dysfunction on echocardiography and markers of myocardial injury, in particular cardiac troponins.

1.4 Troponin in risk stratification

Cardiac troponin T and troponin I are now the preferred markers of myocardial necrosis as they are more specific than creatine kinase (CK) or its isoenzyme MB (CK-MB). Troponin T and I are exclusively expressed in cardiac myocytes making them extremely specific for myocardial damage and they are now used as the gold standard diagnostic test for the diagnosis of myocardial infarction (16). In patients with myocardial infarction troponin levels are seen to rise as early as 3 hours into the onset of the event (17) and stay elevated for a period extending up to 2 weeks. Recently the cardiac troponins have been used as markers of risk in patients with ACS. In patients with unstable angina elevations of troponin I, defined as cTnI levels greater than 3.1 ng/mL, were associated with a 2.5-fold increase in the relative risk of death or nonfatal MI both at 30 days and at 1 year (18). This was also borne out in a

paper by Hamm et al who investigated the prognostic value of serum troponin T in patients with unstable angina (19). In 298 suspected acute MI patients cardiac death or AMI was significantly greater in those with TnT peak values of greater than 0.20 microgram/l as compared with patients with levels below this (20). In a study of 1404 symptomatic patients with unstable angina or non-Q-wave myocardial infarction. Antman et al, found patients with a cardiac troponin I level of at least 0.4ng/ml had a significantly higher mortality rate at 42 days post-infarct compared with patients with a troponin I level below 0.4ng/ml (21). There is much published data on the use of troponin for risk stratification; however the majority of this is for unstable angina and NSTEMI with little data on risk stratification of STEMI. Whereas cardiac troponins are extremely specific for myocardial necrosis, they do not discriminate between ischaemic and non-ischaemic aetiologies of myocardial injury. Clinicians must therefore determine whether a patient's presenting symptoms are consistent with ACS.

1.5 Electrocardiogram in risk stratification

The electrocardiogram (ECG) may also be used in the risk stratification of patients with ACS. The categorization of MI into STEMI, NSTEMI or left bundle branch block MI itself can give useful information about the prognosis of patients who present with these various ECGs. Terkelsen (22) in a selected cohort study from Denmark investigated the prognostic significance of these different categories of AMI. The associated 1-year mortality was 31, 21, and 55%, respectively for NSTEMI, STEMI, and LBBB MI. The more favourable outcome observed in patients with STEMI was probably as a result of the well defined pathway of treatment of these patients compared with NSTEMI and LBBB MI. Similar trends are seen in

other studies for example the GRACE (23) registry; this large registry involving collaboration of centres from 14 different countries showed that the presenting ECG could be used as a tool for risk stratification. The associated 6-month mortality was 6.2, 4.8 and 3.6% respectively for NSTEMI, STEMI, and unstable angina which they defined as chest pain and ECG changes at rest but with no enzyme rise. The reason for the large discrepancy in the mortality may be due to the inclusion and exclusion criteria used and the fact that patients in the GRACE study were recruited after 24 hours and needed to give informed consent leading to natural exclusion of high risk patients.

The ECG may also be used in NSTEMI as a marker of risk prognostication. Results of the TIMI III registry (24) showed that patients with greater than 1mm of ST segment deviation had 11% incidence rate of mortality or new MI at one year, compared to 6.8% in patients with isolated T wave changes. The 30-day incidence of death or myocardial reinfarction in a retrospective analysis of the presenting ECGs of patients enrolled in Global Use of Strategies To Open Occluded Arteries in Acute Coronary Syndromes (GUSTO-IIb) was 5.5% in patients with T-wave inversion, 9.4% in those with ST-segment elevation, 10.5% in those with ST-segment depression, and 12.4% in those with ST-segment elevation and depression (25). The ECG may be beneficial if it is combined with another risk stratification modality such as troponin (26). It must be remembered however that an absence of ECG changes does not exclude the diagnosis of ACS.

1.6 Left ventricular systolic function in risk stratification

The assessment of left ventricular systolic function whether that be via echocardiography or ventriculography has a useful role in the risk stratification of

patients after an AMI. The assessment of ejection fraction as a surrogate marker of left ventricular impairment has been known about for many years and found to be particularly useful (27). As the ejection fraction falls cardiac mortality increases (27). This is also true for the assessment of ejection fraction by other modalities. In one study a predischarge radionuclide ejection fraction of less than 40% was found to be particularly useful (28). The measurement of ejection fraction is superior to other clinical variables in multivariate models with greater predictive power (29). Radionuclide exercise ventriculography along with two-dimensional echocardiography have been used as non-invasive methods for assessing medium and long-term prognosis in post AMI patients (30). A combined approach increases the ability to risk stratify patients into low, medium and high risk groups. Interestingly the addition of cardiac catherisation to this algorithm did not increase the predictive power. Radionuclide ventriculography and assessment of ejection fraction can offer prognostic information on adverse events after an AMI but their predictive powers stop at prediction of death and heart failure. To date there have not been any studies demonstrating the benefit of estimating ejection fraction and the prediction of recurrent nonfatal MI (31). With this in mind and the fact that it is usually a timely procedure the utility of risk stratification using the ejection fraction short of performing an echocardiogram has not become mainstream and is usually used as a research tool.

1.7 Clinical history and examination in risk stratification

Clinical factors still have a role to play in trying to determine the prognosis of patients following an AMI. Killip's (32) initial description of short-term mortality in

250 AMI patients was based on good clinical observation. He found that the mortality of patients increase as the clinical signs of heart failure increased.

Killip class can be thought of as a "poor mans echocardiogram" as it gives a clinical picture of the degree of left ventricular systolic impairment following an acute myocardial infarction.

- Killip class I- individuals with no clinical signs of heart failure.
- Killip class II - individuals with rales in the lungs, an S_3 gallop, and elevated jugular venous pressure.
- Killip class III - individuals with frank pulmonary edema.
- Killip class IV - individuals in cardiogenic shock.

As the Killip grading increases so does the mortality rate. In Killip's original description the mortality rates were found to be Killip class I=6%, Killip class II= 17%, Killip class III= 38%, Killip class IV=81%. It must be emphasised that this data is historic and predates the use of thrombolyis and other drug therapies. Despite this the Killip grade is usually found to be an independent predictor of adverse outcome following an AMI in multivariate statistical modelling showing its clinical value. Other factors which can easily be gauged are age of patient, heart rate and systolic blood pressure and indeed all of these have been shown to be useful tools for risk stratifying patients. (15,29)

1.8 TIMI and GRACE score in risk stratification

Scoring systems have been developed to aid the clinician in making decisions; although accurate in predicting mortality they are either cumbersome to use (15) or unweighted. (33) Recently the TIMI risk score for STEMI, a bedside scoring system,

has been developed and is probably the most widely used scoring system for risk assessment of STEMI. (34) The TIMI risk score was developed by identifying prognostic information from a multivariable analysis of the Intravenous nPA for Treatment of Infarcting Myocardium Early II (InTIME II) trial and found 10 clinical variables, which accounted for 97% of the predictive capacity of the model. The TIMI score has been found to be useful at predicting mortality when investigated in a population of STEMI patients (35) and also in predicting mortality in patients with right ventricular infarction. (36) Along with the TIMI score for STEMI there has been the development of a risk score for NSTEMI, which has also been validated. This scoring system assigns 1 point for each clinical and biochemical variable with a maximum score of 7. It has good prognostic ability particularly in the early setting of a NSTEMI. (37) A unifying scoring system incorporating all acute coronary syndromes has also been developed-the GRACE score (38). All scoring systems have been shown to be superior to clinical judgment in conferring additional prognostic information after an acute coronary syndrome (39). However in some studies the GRACE score has been shown to be superior in head to head comparisons of the different scoring systems with a greater area under the curve showing greater predictive power (40).

TIMI Score for STEMI
Age
Killip class >1
HR >100
Anterior AMI
Systolic <100
History of diabetes, hypertension or previous AMI
Time to treatment > 4 hours
Weight < 67

Table 1.1. Summary of the factors used in TIMI score for STEMI

TIMI Score for NSTEMI
Age> 65
3 risk factors for coronary artery disease
Prior use of aspirin
ST- segment deviation on ECG
Severe angina in preceding 24 hours
Elevated biomarkers (Troponin)
Significant coronary stenosis

Table 1.2. Summary of the factors used in TIMI score for NSTEMI

GRACE score
Age
Prior history of heart failure
Prior history of AMI
Resting heart rate
ST-segment Depression
Systolic blood pressure
Serum creatinine
Elevated cardiac enzymes
In hospital PCI

Table 1.3. Summary of the factors used in GRACE score for risk stratification of ACS

1.9 BNP and NTproBNP

1.9.1. Introduction

B-type natiuretic peptide or BNP as it is more commonly known is a 32 amino acid peptide with is released primarily from the ventricular myocytes in response to stretch or strain (41,42). BNP is produced from its precursor preproBNP, which is a 108 amino acid peptide (43). During production of the active hormone there is also production of the inactive peptide from the N-terminus namely N terminal proBNP (NTproBNP); this is a larger peptide molecule consisting of 76 amino acids. The function of the active molecule is to cause vasodilation, natiuresis, diuresis. BNP also acts to relax vascular smooth muscle cells and counteracts both the sympathetic (44) and renin angiotensin aldosterone systems. (45) These responses are via its actions on

natiuretic receptors. There are active clearance mechanisms for BNP which is broken down in the blood stream by neutral endopeptidases and then removed via the kidney. NTproBNP on the other hand is not acted upon via neutral endopeptidases and is merely excreted via the kidney. The active breakdown and excretion mechanism mean that the half-life of BNP is much shorter than NTproBNP (20minutes vs. 120minutes). For this reason some would argue that NTproBNP is the more discerning marker as it is present in the blood stream for longer periods of time and increases in its concentration are proportionally greater. (46) Also, of the currently available commercial assays NTproBNP has a lower coefficient of variation. (47) There has been much debate about and many comparisons of the two peptides in various disease states, suffice to say that both have been intensively investigated. I will focus briefly on the utility of the markers in heart failure and then move on to describing work done in acute coronary syndromes.

1.9.2. Utility of BNP and NTproBNP in heart failure

In heart failure circulating levels of both natiuretic peptides are raised with NTproBNP being more raised than its counterpart BNP (48). Plasma BNP has now been shown to be useful in helping the clinician diagnose heart failure accurately and effectively (49). In the Maisel study 1586 patients with breathlessness were studied who presented acutely short of breath to hospital and 47% of these patients were clinically determined to have heart failure. When compared with clinical findings and laboratory results, a BNP level greater than 100pg/ml was found to be more accurate at diagnosing heart failure. The negative predictive value was particularly high at 96% (at 50pg/ml). This study like others (50,51) also established the grading increase of BNP with New York Heart Association class. The area under the receiver

operating characteristic curve was extremely high at 0.91. The same is true with NTproBNP. In a head to head study comparing commercially available ELISA assays with radioimmunoassays both were found to be effective in the diagnosis of heart failure with BNP being more sensitive and NTproBNP more specific. (52) Using BNP has also shown to reduce the need for hospitalisation by a median of 3 days for patients presenting to hospital with acute breathlessness as alternative diagnosis can be considered in such patients. (53) This finding obviously has financial implications for hospitals. The levels of BNP however are known to increase with age and females have also been shown to have higher levels; however this also appears to depend on the format of the assay being used (54,55,56). The utility of BNP in heart failure screening has also been examined. Initial studies suggested that BNP was probably not a good screening tool for heart failure (57,58) but further studies have suggested otherwise. (59) Hobbs et al suggested that BNP and NTproBNP were probably equally good in detecting undiagnosed left ventricular systolic impairment. NTproBNP has also shown some promise particularly in combination with urinary NTproBNP levels (60) or abnormal ECG findings. (61) Pre discharge high BNP levels after an admission to hospital for heart failure also identifies a group of patients who are at high risk of readmission (62). Likewise NTproBNP levels pre discharge can identify a group of patients with a high rate of adverse events. (63) Treatment adjustment guided by measurement of BNP or NTproBNP may also become a possibility for the future. A small study showed (64) a reduction in a composite endpoint of death or hospitalisation for heart failure in patients whose therapy had been adjusted using levels of NTproBNP to a target of less than 200pmol/L. A larger study looking at BNP guided therapy has reported similar

findings (65). A larger study investigating NTproBNP in this respect is currently under way. (66)

1.9.2. Utility of BNP and NTproBNP in acute coronary syndromes

Levels of the natiuretic peptides have been shown to be raised following an acute myocardial infarction and are related to short and long-term mortality. (67,68,69). BNP is able to provide additional prognostic information over and above that provided by left ventricular dysfunction following an acute myocardial infarction (69). NTproBNP also has similar prognostic power and levels measured in the sub-acute phase; between days 2 and 4 have prognostic power to predict left ventricular function and 2 year survival (70). A comparison of NTproBNP and BNP as prognostic markers following an acute myocardial was also carried out by Richards and co-workers. (70) Here they investigated a large cohort of 666 patients and BNP and NTproBNP were found to give equal areas under the ROC curve for prediction of death and heart failure. Interestingly the markers also had some power towards prediction of new acute coronary syndrome. Another interesting finding was that raised levels of BNP or NTproBNP with a normal ejection fraction also conferred risk of an adverse event.

This finding has been borne out recently in patients with acute coronary syndromes. (71) Those patients who did not have clinical signs of heart failure (in Killip class 1) also demonstrated an increased risk of adverse event if they had a raised NTproBNP level. This study was similar to a sub-study of OPUS-TIMI 16 which looked at the benefit of BNP in patients with acute coronary syndromes (72). Again early (1 month and late (10 month) mortality could be predicted with the measurement of BNP. The event rates were related to quartiles of BNP, thus able to grade the degree of risk.

This study corroborated the findings of Richards et al (70) showing that recurrent AMI could also be predicted; however reinfarction prediction was not borne out by James who investigated NTproBNP in over 6000 patients who were recruited into a GUSTO IV sub-study, although the other risk prediction towards adverse events was corroborated. (73)

Other large clinical studies have also found that NTproBNP is a useful marker for risk prognostication. Heeschen (74) investigated 1791 patients as part of the PRISM trial and levels of NTproBNP >250ng/L identified a high-risk group of patients. Even patients who were troponin negative could be risk stratified on the basis of their NTproBNP result.

BNP and NTproBNP have also been shown to be raised in unstable angina. (75,76) BNP is known to be rapidly induced after an AMI (77) and the levels in the plasma probably initially reflect stored peptide; following this there is usually a peak in secretion around day 2, presumably corresponding to increased synthesis of the protein. (67)

1.9.3. Limitations of the utility of BNP and NTproBNP

We have shown that BNP and NTproBNP have good utility in ruling out a diagnosis of heart failure and ruling out a poor prognosis. The biochemical markers NTproBNP and BNP have high negative predictive values. However there are some drawbacks as previously alluded to. The levels of the peptides may be raised to high levels in other generalised disease states such as sepsis (78) and pulmonary embolism (79). Certain patient characteristics can increase circulating levels of NTproBNP and BNP. NTproBNP levels increase with deteriorating renal failure, so much so that some have classed it more as a marker of cardio-renal function. (80) Levels are also lower in

patients with a raised body mass index (81). BNP levels increase with age and are higher in females than males. (54,55)

There is also a grey area for both peptides. Although they are sensitive, they are not very specific and the specificities of about 40% in these studies give low positive predictive values, making them less useful in identification of patients with a poor prognosis.

A multimarker approach may assist in refining the risk stratification of patients following MI. Two additional peptides, which have initially been described as cardiac hypertrophy inducing factors have now been associated with the presence of heart failure, namely cardiotrophin (CT-1) and myotrophin. Also as AMI is a multifaceted disorder involving inflammation (82) the addition of an inflammatory marker-MPO may also aid with increasing the specificity.

1.10 CT-1

1.10.1. Introduction

Cardiotrophin-1 (CT-1) is a 201 amino-acid inflammatory cytokine, which belongs to the interleukin-6 family. (83) Other peptides in this family include interleukin-11, ciliary neurotrophic factor (CNTF), leukaemia inhibitory factor (LIF) and oncostatin M. CT-1 induces cardiac myocyte hypertrophy, (84) adding sarcomeres in series rather than in parallel and leading to increased cardiac myocyte size due to an increase in cell length, with little change in width. (85) CT-1 has been found to be present in a number of tissues including heart, skeletal muscle, and lung. (86)

It binds to the glycoprotein 130 (gp130) and causes heterodimerization of the leukaemia inhibitory factor receptor. (87) Activation of gp130 also leads to activation of MAPK (mitogen activated protein kinase) pathways. (88)

The actions of CT-1 are dependent on receptor binding but it has been shown to have protective effects on adult rat or human cardiomyocytes when added prior to ischaemia and at reperfusion. (89,90) This cytoprotection may be as a result of the production of heat shock proteins. (91)

CT-1 has also recently been shown to have anti-apoptotic effects in mice liver. (92) CT-1-/- mice died faster than wild-type animals after challenge with a lethal dose of the Fas agonist Jo-2. At sub lethal doses of Jo-2, all wild-type mice survived whereas CT-1-/- animals developed extensive hepatocyte apoptosis with 50% mortality at 24 hours. Pre-treatment with CT-1 however was shown to improve survival with reduced injury in both CT-1-/- and wild-type animals.

gp130 plays a crucial role in cardiac embryology as disruption of the gp130 gene proves lethal in mice resulting in an immature hypoplastic ventricular myocardium. (93)

1.10.2. Role of CT-1 in IHD

When given to rats CT-1 causes a decline in blood pressure and a reflex increase in heart rate. (94) It has been shown to be raised in patients following an AMI and unstable angina (76,95,96) and in those patients with echocardiographic heart failure compared to controls. (97) In patients with AMI CT-1 detected the presence of LV systolic dysfunction with high sensitivity (95%) and specificity (82.5%), with an area under the ROC curve of 0.94. The levels of CT-1 were further elevated in patients about 2 months after infarction. (97) However, this study was not powered to examine end-points such as death, non-fatal MI or heart failure readmissions.

Most recently CT-1 has been shown to be raised in hypertension (98,99) propagating the humoral theory of hypertension. It is unclear however whether CT-1 has a

protective or detrimental effect in AMI as it triggers hypertrophy and anti-apoptotic pathways by distinct kinase cascades. (88) (Figure 1.2). The hypertrophic response of cardiac myocytes to CT-1 is dependent on the JAK/STAT pathway, and the anti-apoptotic effects of CT-1 causes signalling via the MEK/MAPK pathway (100)

Due to its release during cardiac ischaemia, it is possible that CT-1 could contribute to cardioprotection during infarction. It may also have initial beneficial effects by causing the addition of sarcomeres in parallel causing eccentric hypertrophy at a time post AMI to help augment wall stress. In the long term however CT-1 release may also reflect the degree of LV dysfunction leading to ventricular dilatation, which is a well-known poor prognostic factor. CT-1 has already been shown to be a stable peptide and is known to remain stable for up-to 48 hours when stored at room temperature. (101)

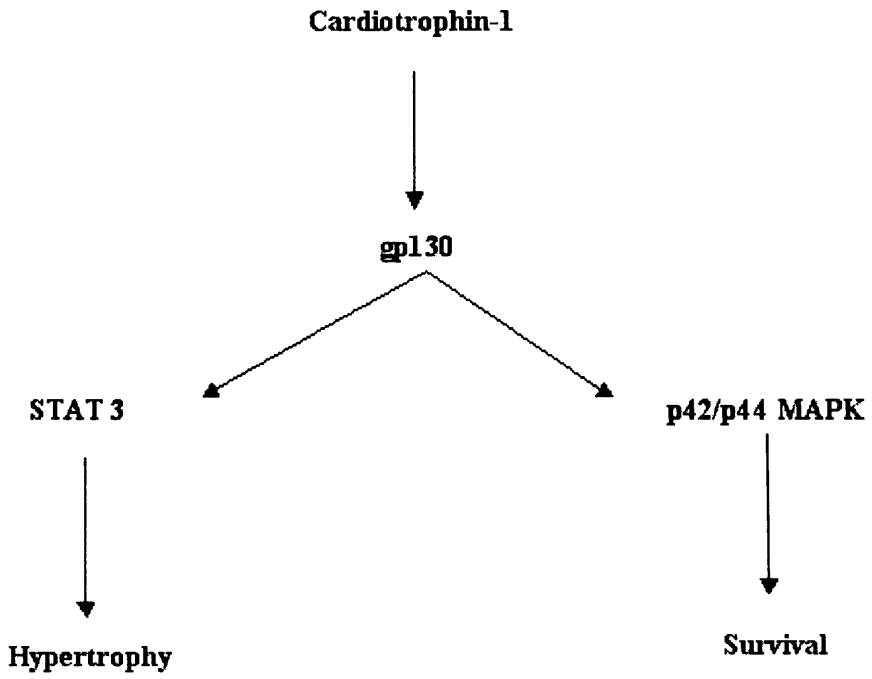

Figure 1.2. Signaling pathways of cardiotrophin-1. There is a differential pathway with activation of STAT 3 or p42/p44 MAPK causing cellular hypertrophy or survival. (gp130-glycoprotein 130, STAT 3- signal transducer and activator of transcription; MAPK- mitogen-activated protein kinase)

1.11 Myotrophin

1.11.1. Introduction

Myotrophin is a 12 kD protein initially isolated from hypertrophied hearts of spontaneously hypertensive rats. (102) The in-vitro effects of myotrophin on cultured cardiomyocytes include an increase in protein synthesis, cellular hypertrophy, gap-junction formation, increased sarcomere number, induction of early response genes such as c-myc, c-fos, c-jun, and, subsequently, of transcripts of skeletal alpha-actin, total myosin, and atrial natriuretic peptide (103). These effects are thought to be mediated via protein kinase C activation (104). Myotrophin is thought to interact with nuclear factor kappa B (NFκB) (105) disrupting the formation of the NFκB p50-p65 transactivating heterodimers while increasing the formation of repressive NFκB p50-

p50 homodimers (106). Nuclear factor kappa-B (NFκB) is a transcription factor which is known to regulate a number of inflammatory genes; for example it has been implicated as playing an important role in the pathogenesis in atherosclerosis. (107) It may also be a protective factor as there is some evidence that it may protect cells from cell death. In an experiment by Antwerp and co-workers (108) it was shown that inhibition of NFκB actually increased cell death; this survival function may be as a result of induction of anti apoptotic factors. (109)

Activated NFκB has been found in macrophages and endothelial cells in human atherosclerotic plaques but is not present in healthy vessels. (110,111) It is known that NFκB activation is necessary for myotrophin induced cardiac hypertrophy (112). Myotrophin also increases TNFα expression in cardiomyocytes. (113)

Myotrophin has a close homology with inhibitory-kappa B (I-kappa B), a regulatory peptide controlling the activity of NFκB. (111) Human myotrophin has been cloned (114) and found to be highly homologous to the rat protein with its messenger ribonucleic acid (mRNA) widely distributed in many tissues, including relatively high levels in heart and skeletal muscle.

1.11.2. Role of myotrophin in IHD

Little is known about myotrophin in heart disease. Elevated levels have also been found in human cardiomyopathic hearts (115) and although originally thought of as a cytosolic protein it has also been described in human plasma. (116) Early activation of the myotrophin system is evident in human heart failure, which is more pronounced in males. Interestingly, the levels of myotrophin appeared to be inversely related to the degree of heart failure as measured by NYHA class. (116) Although

there is evidence of increased activity of NFκB in human heart failure little is known about the role of NFκB in acute MI (117).

1.11.3. Myotrophin in other tissues

Outside of the cardiovascular system, myotrophin has been predicted to be and validated as a target of the islet-specific microRNA, miR-375. Over expression of miR-375 suppresses glucose-induced insulin secretion. Thus, myotrophin is a regulator of insulin secretion and may thereby constitute a novel pharmacological target for the treatment of diabetes (116). It is known to be increased in skeletal muscle cells where it also has hypertrophic effects. (119)

It is possible therefore that myotrophin may play a signaling role in cardiac hypertrophy. It is unclear at the moment whether myotrophin secretion would be beneficial or whether it reflects the degree of myocardial damage. The interaction of myotrophin with NFκB may have beneficial effects on the one hand due to the anti-apoptotic functions of NFκB, however due to its effects on enhancing repressive NFκB signalling myotrophin may also be detrimental.

1.12 MPO

1.12.1. Introduction

Myeloperoxidase (MPO) is a white cell enzyme which is present in the granules of the leucocyte. (120) It is involved in inflammatory mechanisms. It is stored within neutrophil and monocyte granules and is released during leukocyte activation and degranulation. (121) MPO is also present in tissue macrophages. (122)

The functional role of MPO is to catalyse the conversion of hydrogen peroxide to generate highly reactive species such as hypochlorous acid, tyrosyl radical species and nitrogen dioxide (123,124). These are part of the defense mechanism. However MPO may also be detrimental and has been shown to cause oxidation of low density lipoproteins leading to foam cell generation. (123) Immunohistochemical studies have demonstrated the presence of MPO in atheromatous plaques. (125) MPO can also activate metalloproteinases and inactivate plasminogen activator inhibitor, promoting destabilization and rupture of the atherosclerotic plaque surface. (126) Furthermore, MPO catalytically consumes endothelium-derived nitric oxide, thereby reducing nitric oxide bioavailability, leading to vasoconstriction (120) and endothelial dysfunction. (127)

1.12.2. Role of MPO in IHD

As our understanding of IHD has increased, the importance of the leucocyte as being pivotal in this process has also increased. (128) Indeed the association of the leucocyte with the extent of coronary artery disease has been known for some years. (129) Also strong evidence exists which links the existence between MPO and coronary artery disease. Genetic studies show that subjects with either total or sub-total MPO deficiency (130) or those subjects with reduced expression due to polymorphism (131,132) seem less likely to develop coronary artery disease or non-fatal myocardial infarctions or cardiac death.

This has also been shown in some case control studies. Zhang and co-workers (133) demonstrated that elevated levels of MPO were associated with coronary artery disease in patients with angiographic confirmation of coronary artery disease when compared to controls.

MPO has also been associated with endothelial dysfunction. (127)

MPO is emerging as a useful marker for prognostication in a variety of clinical settings. Recently it was shown to be of prognostic value in patients presenting with chest pain to the emergency room. (134) Here it was found to be useful as an independent predictor of early MI and major adverse cardiac events in the ensuing 30 days and at 6 months. Brennan et al study recruited all patients presenting with chest pain and the study included 23.5% of patients with a final diagnosis of AMI. MPO levels were found to be higher in patients who subsequently went on to have a myocardial infarction suggesting that there is neutophil activation which precede AMI. Baseline MPO levels were also raised in patients who went on to have a major adverse event in the next 30 days. Interestingly MPO had predictive value for adverse events in the ensuing 30-day and 6-month period in those patients who presented with chest pain and who were troponin negative.

There is also an association between myeloperoxidase levels and the risk of coronary artery disease. (133) The inflammation in acute coronary syndromes is thought to be widespread. (135)

The utility of MPO as a prognostic marker has been borne out previously in patients with acute coronary syndromes. (136) In this study, which included over 1000 patients, MPO was found to be an independent predictor of death or non-fatal MI both early within 72 hours and at 6 months. MPO did not correlate with troponin suggesting that it is not a marker of necrosis. Patients with MPO levels >350 μg/L had a markedly increased risk of an adverse cardiac event. Again, as in Brennan's study (134) MPO was further able to risk stratify those patients with a normal troponin level. Another recent study has investigated MPO levels between days 2 and 4 following an AMI. Mocatta and co-workers (137) have found that MPO levels are

raised significantly after an AMI and that patients with an above median level MPO had an increased mortality by nearly 2 fold when compared to patients with a below median MPO level at 5 years follow-up. The risk of re-infarction was not examined by this group.

These studies demonstrate the utility of MPO as a predictor of cardiovascular risk and as a marker of the vulnerable plaque.

1.13 Hypothesis

The current study is designed to test the following hypothesises:

1) The biochemical marker NTproBNP is a more sensitive and specific marker of death post AMI than current risk stratification techniques which is comprised of clinical variables.
2) Since both CT-1, myotrophin and MPO are found in higher concentrations in plasma from patients with left ventricular systolic dysfunction, we also wish to test the hypotheses that:
    a) these cardiac peptides are secreted post myocardial infarction and may be related to the degree of left ventricular systolic dysfunction;
    b) CT-1, myotrophin and MPO secreted after infarction may yield prognostic information on primary and secondary end-points. The primary endpoint is cardiovascular death and the secondary end-points are a combination of cardiovascular morbidity events such as hospital readmissions with heart failure, non-fatal MI and Braunwald Class IIIB unstable angina;

c) they may be used in combination with NTproBNP (a marker of poor prognosis, but with low positive predictive value) as part of a multimarker approach to prognostication post myocardial infarction.

1.14 Aims of the study

In this project we propose to: -

1) Investigate the prognostic utility of NTproBNP as a marker for risk stratification of AMI patients over and above clinical variables.
2) Establish the secretory patterns of CT-1, myotrophin and MPO in patients with AMI by frequent blood sampling within the first five days following the ischaemic event.
3) Compare the secretory patterns of CT-1, myotrophin and MPO to that of NTproBNP, which is known to peak within the first 24 hours and then, fall.
4) Investigate the prognostic utility of CT-1, myotrophin and MPO as markers for risk stratification of AMI patients especially in combination with NTproBNP.

Chapter 2:

Materials and Methods

2.1 Principles of immunoassay

We used the immunoassay as our main analytical technique. The principle of the immunoassay is based on the premise that antibodies have a strong binding ability to antigen. An immunoassay is similar to a radioimmunoassay however it negates the use of potentially harmful radioactive substances and has now probably become the assay of choice in determining concentration of peptides.

The procedure usually involves 2 antibodies. The first is a so-called capture antibody, which is immobilised by binding to a surface on the plate. Antigen (peptide) is then added and the antibody is then able to bind to its antigen with high specificity. A second antibody then serves as a detecting antibody by binding to a different epitope of the antigen. The antigen is then effectively "sandwiched" between two antibodies. Hence the term "sandwich antibody". The detecting antibody is usually labelled with fluorescent, chemiluminescent or even on occasions radioactive isotope as this allows detection of the signal which is usually generated by the production of luminescence under favourable reaction conditions. (138) In our experiments we have used a chemiluminescent label called methyl acridinium ester (MAE) to detect plasma concentrations of our peptides. The chemiluminescent reaction which takes place does so under alkaline conditions with the addition of dilute hydrogen peroxide and involves cleavage of the ester linkage of acridinium, which produces an unstable dioxetanone intermediate. This then further decomposes to form N-methylacridone in an excited state. The relaxation of N-methylacridone to its ground state results in the emission of light of wavelength 430nm, which is measured on commercially available luminometers. (139)

In this study biotinylated antibodies were used. Biotin is a small water-soluble molecule. The biotinylation antibody is a gentle reaction, which does not interfere

with the antibody's biological activity. (139) Biotin then has a strong affinity for streptavidin.

2.1.1 Streptavidin labelling with MAE

Streptavidin was labelled with MAE by adding 10 μg of MAE dissolved in 2 μl of anhydrous dimethylformamide to a solution containing 100 μg of recombinant streptavidin in 50 μl of 0.1 M Na_2HPO_4. This mixture was left to react in the dark for 30 minutes at room temperature. 50 μl of 10 mg/ml lysine in 0.1 M Na_2HPO_4 was added to this mixture which scavenged the unreacted MAE for 10 minutes. The solution was then gel-filtered through Sephadex G-25 and the labelled streptavidin was identified in the eluting fractions using chemiluminescent detection.

2.2 Materials

General laboratory reagents were from Sigma Chemical Company (Dorset, United Kingdom). Microlite-2 plates were from Dynex Technologies (West Sussex, U.K.). Aprotinin was from Nordic Pharma (Reading, U.K.). Methyl Acridinium Ester (MAE) was from Molecular Technology (West Sussex, U.K.). NTproBNP peptide was from Spectral Diagnostics (Toronto, Canada). Streptavidin was from Chemicon International (Harrow, U.K.).

2.2.1 Antibodies

Anti-Rabbit IgG, Goat anti-mouse IgG and Biotinylated goat anti-rabbit IgG antibodies were obtained from Sigma Chemical Company (Dorset, U.K.). Anti-Myotrophin antibody was from Medical Research Council (University of Leicester, U.K.). Mouse antibody directed against C terminal of human NTproBNP was a

generous gift from Unipath (Bedford, U.K.). Sheep Antibody directed against N-terminal of human NTproBNP was from Roche (Welwyn Garden City, U.K.). Rabbit anti-human MPO antibody and MPO standard was from Merck biosciences, (Nottingham, UK) Mouse anti-human MPO antibody was from Research diagnostics, (Flanders, New Jersey, USA). Rabbit anti-CT-1 antibody was from Peprotech Inc (Rocky Hill, NJ). Mouse monoclonal antibody was from BioVendor Laboratory Medicine (Modrice, Czech Republic). Myotrophin anti-mouse IgG was from Sigma Aldrich Company (Gillingham, UK). Monoclonal myotrophin antibody ($IgG2_b$, clone 49, was from Becton Dickinson Biosciences Pharmingen (Oxford, UK). Goat biotinylated anti-rabbit IgG was from Rockland Immunochemicals Inc. (Gilbertsville, PA, USA).

2.2.2 Stock solutions and buffers

Constituents of ILMA Buffer:

- NaH_2PO_4 1.5mmol/L
- Na_2HPO_4 8mmol/L
- NaCl 140mmol/L
- EDTA 1.0mmol/L
- BSA 1.0g/L
- Azide 0.1g/L
- Triton X 100 0.1%
- pH 7.4

Constituents of Wash Buffer B:

- Tween 0.05%
- NaCl 118.5g

- NaH_2PO_4 1.4g
- Na_2HPO_4 6.4g
- Na Azide 6.1g/L
- pH 7.3

Constituents of Wash Buffer C-Phosphate Buffered Solution (PBS):

- NaCl 137mmol/L
- KCL 2.7mmol/L
- KH_2PO_4 1.5mmol/L
- Na_2HPO_4 8mmol/L

2.3 Patient recruitment

We studied consecutive acute myocardial infarction patients. Patients were recruited from the Coronary Care Unit of the University Hospitals of Leicester NHS Trust-Leicester Royal Infirmary. The study complied with the Declaration of Helsinki and was approved by the local ethics committee; written informed consent was obtained from patients.

AMI was diagnosed if a patient had a plasma creatine kinase-MB elevation greater than twice normal or cardiac troponin I level >0.1 ng/mL in conjunction with at least one of the following, chest pain lasting >20 minutes or diagnostic serial electrocardiographic (ECG) changes consisting of new pathological Q waves or ST-segment and T-wave changes. (16)

AMI was sub-categorised into ST segment elevation myocardial infarction (STEMI) or non-ST segment myocardial infarction (NSTEMI).

2.3.1 Determination and definition of the end points

The end points in the study were observed over a 2-year period. We assessed the value of both NTproBNP and the other peptides for the prediction of the endpoint of death, hospitalisation for AMI (as defined above) and hospitalization for heart failure. Hospitalization for heart failure was defined as a hospital admission for which heart failure was the primary reason. Endpoints were obtained by reviewing the Office of National Statistics Registry and by contacting each patient. There was a minimum 60-day follow-up of all surviving patients.

2.3.2 Statistical analysis

Statistical analyses were performed on SPSS Version 14 (SPSS Inc, Chicago, Illinois). Comparisons of continuous variables were made using the Mann Whitney U test. Comparisons in the daily sampling study were performed using the general linear model with repeated measures, with correction for multiple comparisons using the Bonferroni method. To test the independent predictive power for all endpoints Cox proportional hazards analyses were conducted using peptide levels as continuous variables. We included as variables baseline patient characteristics (age, gender, eGFR, Killip class, territory of AMI, past history of myocardial infarction or heart failure, therapy with ACE inhibitors, angiotensin receptor blockers, beta-blockers, and peptide markers). Cox models were always constructed with the same variables entered simultaneously (which included variables statistically significant in univariate analyses and those variables that may have an effect on the end point on the basis of previous studies).

Levels of the peptides were normalised by log transformation. Thus hazard ratios refer to a tenfold rise in the levels of these markers. Spearman's correlations were

performed for peptide values and continuous variables. To compare the accuracy of the peptides, receiver-operating characteristic (ROC) curves were generated and the area under the curves (AUC) was calculated. Kaplan Meier survival curves were generated to visualise the relationship between the peptides and the primary and secondary endpoints. The Mantel-Cox log rank test was used to assess the significance of the stratification using medians of peptide (and log rank tests for linear trend of factor levels for stratification using ordered medians of peptide) dichotomised according to NTproBNP median levels. A two-tailed p value of less than 0.05 was deemed to be statistically significant.

2.3.3 Power calculations

2.3.3.1 TIMI sub-study

Power calculations suggested that 473 patients recruited over 24 months with a follow-up period of at least 1 month would enable median survival probabilities of 0.9 or 0.85 at 12 months in the groups stratified by the biomarker median to be distinguished with a power of 93% at $P<0.05$ (2-sided test).

2.3.3.2 CT-1 and myotrophin

For the primary end-point of cardiovascular death, we calculated that for a relative risk of 4, the number of patients that needed to be recruited in order to demonstrate a difference in mortality between supra- and infra-median CT-1 levels (with a power of 90% at $P<0.05$ with 12 months follow-up) would be 590.

2.3.3.3 MPO

Power calculations suggested that 318 patients recruited over 24 months and a follow-up period of at least 1 month, with median survival probabilities of 0.8 or 0.7 at 12 months in the groups stratified by the biomarker median, would enable the hypothesis to be tested with a power of 95% at $P<0.01$ (2-sided test). We recruited 20% more patients in case follow-up was incomplete in some cases.

2.3.4 Plasma samples

Blood samples were drawn from 596 patients on a daily basis for 5 days after the onset of chest pain for determination of plasma peptide levels. After 15 minutes bed rest, 20mL blood was collected into tubes containing EDTA and aprotinin. Blood samples were immediately immersed in ice water and then centrifuged for 15 minutes at 3000rpm at 4°C. All plasma was stored at -70°C until assayed in a blinded fashion in a single batch.

2.3.5 ECG

The ECG of each patient was analysed for the following major abnormalities: ST segment elevation, ST segment depression, pathological Q waves, inverted T waves, left bundle branch block, left ventricular hypertrophy, atrial flutter and atrial fibrillation.

2.3.6 Echocardiography

Transthoracic echocardiography was performed in patients using a Sonos 5500 instrument (Philips Medical Systems, Reigate, UK). Measurements of chamber dimensions, peak early transmitral flow velocity (*E*), atrial flow velocity (*A*), *E/A*

ratio and deceleration time of the mitral *E* wave were obtained from the digitised images. A 16-segment left ventricular wall motion index (LVWMI) based on the American Society of Echocardiography mode was derived by scoring each LV segment (1=normal, 2=hypokinesis, 3=akinesis and 4=dyskinesis (paradoxical motion), and dividing the total by the number of segments scored. (140)

Left ventricular ejection fraction (LVEF) was calculated using the biplane method of discs formula. (138) Impaired LV systolic function was defined as an EF<40% or a LVWMI >1.8.

The scanning protocol for obtaining a LVWMI consisted of obtaining the following views:- parasternal long axis, parasternal short axis at papillary valve level, apical 4 chamber and 2 chamber views. In addition the following parameters were measured: end systolic volume index (ESVI), end diastolic volume index (EDVI), LV end-systolic diameter (LVESD) and LV end-diastolic diameter (LVEDD).

2.3.7 Control subjects

Control subjects for CT-1 and myotrophin were age and gender matched and recruited from University of Leicester and had peptide measurements made on one occasion. Control subjects for myeloperoxidase were normal volunteers (257, 132 male, mean age 61.8 ± 14.3) derived from a heart failure screening study which was being performed concurrently in the community. (61) All control subjects were on no therapy, had no history of hypertension, diabetes, ischaemic heart disease, ECG or echocardiographic abnormalities (including segmental wall motion abnormalities, valvular disease, left ventricular hypertrophy).

2.4 Methods

2.4.1 Non-competitive Immunoassay for NTproBNP

Our NTproBNP assay was based on a non-competitive assay (71). Sheep antibodies were raised to the N-terminal of human NTproBNP and monoclonal mouse antibodies were raised to the C- terminal. The N-terminal IgG was affinity-purified and biotinylated. Samples of NTproBNP standards were incubated in C-terminal IgG–coated wells with the biotinylated antibody for 24 hours at 4°C. Detection was with methyl acridinium ester (MAE)–labelled streptavidin. (71) The lower limit of detection was 0.3 fmol/ml. There was no cross reactivity with atrial natriuretic peptide, BNP, or C-type natriuretic peptide. The results from this in-house assay are highly correlated ($r=0.90$, $P<0.0001$, $n=86$) to those obtained on the NTproBNP assay marketed by Roche Diagnostics Ltd. (Lewes, East Sussex, UK).

2.4.2 Non-competitive Immunoassay for CT-1

The CT-1 assay was based on a non-competitive assay. ELISA plates were coated with anti-rabbit IgG (100ng/well). The capture antibody was a rabbit anti-CT-1 antibody (100ng/100μL assay buffer, Peprotech Inc, Rocky Hill, NJ), and detection employed a biotinylated mouse monoclonal antibody (50ng/100μL assay buffer, BioVendor Laboratory Medicine, Modrice, Czech Republic). Plasma samples (50 μL) or CT-1 standards were incubated for 24 hours at 4°C. Following washes, detection was performed using methyl acridinium ester (MAE)–labelled streptavidin. Intra- and inter- assay coefficients of variation were found to be less than 10%.

2.4.3 Non-competitive Immunoassay for myotrophin

The myotrophin assay was based on an immunoluminometric non-competitive assay. ELISA plates were coated with 100 μL of anti-mouse IgG (100 ng/well, Sigma Aldrich Co., Gillingham, UK) in PBS. Wells were then blocked with 10% foetal calf serum in PBS. A specific commercial monoclonal antibody ($IgG2_b$, clone 49, Becton Dickinson Biosciences Pharmingen, Oxford, UK) served as the capture antibody. The detector antibody was a rabbit polyclonal antibody that had been previously reported by us in immunoluminometric assays of myotrophin (120), but for the current assays, was further enriched by affinity purification on a column of myotrophin peptide (LTAFEATDNQAI, corresponding to amino acids 102-113 in the C-terminal domain of human myotrophin) immobilized onto Affigel 10 (Biorad Laboratories, Hemel Hempstead, UK). Bound specific antibody was then eluted using 0.1 M glycine-HCl (pH 2.4) and rapidly neutralized with Tris base. 100 μL of immunoluminometric assay buffer containing 10ng of the Becton Dickinson monoclonal antibody was pipetted into the ELISA wells, followed by 50 μL of plasma samples and standards. Plates were incubated overnight at 4°C. After washes, the detector affinity purified rabbit antibody (20ng/100μL) was pipetted into the wells and plates were incubated at room temperature for 3 hours. Following washes, a goat biotinylated anti-rabbit IgG (Rockland Immunochemicals Inc., Gilbertsville, PA, USA, previously pre-adsorbed with human, rabbit, mouse serum proteins) at a dilution of 1:200000 was incubated within the wells for 1 h, followed by MAE-labeled streptavidin for another 1½ hours. Chemiluminescence was elicited with sequential injections of H_2O_2 in nitric acid, followed by sodium hydroxide containing cetyl trimethylammonium bromide, as described (114). Intra and inter-assay coefficients of variation were found to be less than 10%.

2.4.4 Non-competitive Immunoassay for MPO

The MPO assay was based on a non-competitive assay. Capture antibody was 100ng of a monoclonal anti-MPO (Research Diagnostics Inc., Flanders, NJ) coated onto ELISA plates, and detection was with a rabbit anti-MPO antibody (Merck Biosciences Ltd., Nottingham, UK). Samples or MPO standards were incubated for 24 hours at 4°C. Following washes, detection was performed using sequential incubations with biotinylated goat anti-rabbit IgG and methyl acridinium ester (MAE)–labelled streptavidin. Intra and inter-assay coefficients of variation, which were found to be less than 10%.

2.4.5 Reading of plates

Chemiluminescence was measured using a DYNEX MLX luminometer (Dynex Technologies, UK). The injectors were primed before use. The chemiluminescence reaction was initiated by the first injection of 100µl of 100mmol/l of HNO_3 containing 0.05% hydrogen peroxide. A second injection followed 4 seconds later, which consisted of 100µl of 250mmol/L NaOH containing 0.25% cetyl triethlammonium bromide. Chemiluminescence was measured in the subsequent 2 seconds. The results of the reaction were calculated as a mean of the duplicate measurements, and expressed in relative light units (RLU).

2.4.6 Fitting standard curves

Standard curves were fitted using the quadratic equation on Fig P regression analysis (Biosoft Cambridge UK). The concentration of the peptide in the patient's samples was interpolated from the standard curve. Intra and interassay coefficients of variation were assessed to ensure reliability and validity of the assay.

2.4.7 Assay coefficients

Intra and inter assay coefficients of variation respectively were as follows NTproBNP 2.3% and 4.8%, CT-1 3.5% and 7%, myotrophin 7.8% and 9%, MPO 4.6% and 8.7%.

2.4.8 Cross-reactivity

There was no cross-reactivity within the above peptides or with peptides previously demonstrated to be elevated in AMI such as atrial natriuretic peptide, brain natriuretic peptide, C-type natriuretic peptide, or leukaemia inhibitory factor.

2.4.9 TIMI scoring for STEMI

Patients were TIMI risk scored for STEMI as described: - age 65-74 2 points, age >75 3 points, history of diabetes mellitus, hypertension or angina, 1 point, systolic blood pressure <100mmHg, 3 points, heart rate >100, 2 points, Killip class II-IV, 2 points, weight <67kg, 1 point, anterior ST- segment elevation MI or left bundle branch block, 1 point, time to treatment > 4 hours, 1 point (32) and grouped into low (TIMI score 0-2), intermediate (3 to 7) and high risk (>8) groups.

2.4.10 Determination of eGFR

The estimated GFR (eGFR) of these subjects was calculated from the simplified formula derived from the Modification of Diet in Renal Disease (MDRD) study, recently validated in patients with HF. (141)

Chapter 3:

Results

3.1 Study population

The whole study population consisted of 596 patients, 473 with ST-segment elevation AMI (STEMI) and 123 with non-ST segment elevation AMI (NSTEMI). Thrombolytic reperfusion therapy was performed in 325 (68.7%) of the eligible 473 patients. The mean age was 64.4 ± 12.5 years with 455 (76%) of the patient cohort enrolled being male. 104 (17.4%) had a past history of myocardial infarction, 287 (48.1%) had a past history of hypertension, 122 (20.4%) had a history of diabetes mellitus, 187 (37.3%) had hypercholesterolaemia and 244 (41%) were current or ex-smokers. The site of infarction was anterior territory in 255 (42.7%) of the patients.

The drug history prior to admission showed that 175 (29.3%) were on aspirin, 163 (27.3%) on a beta-blocker, 129 (21.6%) on an ACE inhibitor, 158 (26.5%) on a statin. During the primary hospitalisation, 324 (54.4%) patients demonstrated clinical signs of heart failure. Of those the majority were classified as Killip class II (n=282, 47.3%); the remaining patients were classified Killip class III (n=36, 6.1%) or IV (n=6, 1.0%). The serum creatinine levels on admission to hospital averaged 98.9 ± 13.2 μmol/L, the eGFR 68.3 ± 19.5 ml/min/1.73m^2 surface area, the peak creatine kinase levels averaged 1243 ± 1372 IU/L and troponin I, 20.6 ± 32.2 ng/ml. During the median follow up period of 301 days (range 0-645) a total of 58 (9.7%) patients died, 60 (10.1%) were readmitted with an acute myocardial infarction and 45 (7.5%) were readmitted with heart failure.

	AMI Patients
Age (in years)	64.4 ± 12.5
Male Sex	455 (76%)
Thrombolysed (%)	68.7
Previous Medical History	
Myocardial infarction	104 (17.4%)
Angina Pectoris	108 (18.1%)
Hypertension	287 (48.1%)
Diabetes mellitus	122 (20.4%)
Hypercholesterolaemia	187 (31.3%)
Obesity	99 (16.6%)
Current/Ex-Smokers	244 (40.3%)
Diagnosis	
ST-segment elevation AMI (%)	473 (74.3)
Non-ST-elevation AMI (%)	123 (25.7)
Territory of Infarct	
Anterior or LBBB	255 (40.0%)
Inferior/Other	341 (60.0%)
Killip Class on Admission	
I	267 (44.7%)
II	282 (47.3%)
III	36 (6%)
IV	6 (1%)
Biochemical Markers	
Peak CK (IU/L)	1243 ± 1372
Peak Troponin I (ng/ml)	20.6 ± 32.2
Creatinine μmol/l	98.9 ± 13.2
eGFR ml/min/1.73m^2	68.3 ± 19.5
Peptide Markers Day 1	
Plasma NTproBNP (fmol/ml)	911(0.3-11779.3)
Plasma CT-1 (fmol/ml)	1.13(0.03-556.3)
Plasma myotrophin (fmol/ml)	1459(509-16921)
Plasma MPO (ng/ml)	60.3(6-35440)

Table 3.1. Characteristics of patients in the study. Values are means (SD), median (range) or numbers (percentage)

3.2 TIMI score for STEMI

3.2.1 Study population for STEMI

The cohort of STEMI patients and their demographics are shown in table 3.2. This included all the STEMI patients within the larger cohort of 596 patients.

During follow-up, 42 (8.9%) patients died. Echocardiographic data was available for 399 (84.4%) of the 473 patients and performed at a median of 3.5 days (range 2-5) after presentation with AMI. 30 echocardiograms were unanalysable and 44 patients did not receive an echocardiogram.

	STEMI Patients
Number	473
Age (in years)	63.7 ± 12.3
Male Sex	352
Previous Medical History	
Myocardial infarction	61 (12.9)
Angina Pectoris	64 (13.5)
Hypertension	213 (45.0)
Diabetes mellitus	95 (20.1)
Hypercholesterolaemia	133 (28.1)
Obesity	65 (13.7)
Current/Ex-Smokers	170 (30.9)
Thrombolytic	322 (68.5)
Territory of Infarct	
Anterior	180 (38.5)
Inferior	220 (46.5)

Other/undetermined	73 (15.0)
Killip Class on Admission	
I	255 (53.9)
II	185 (39.1)
III	25 (5.3)
IV	3 (0.6)
Peak CK (I/U)	1442.7 ± 1412.8
Peak Troponin I (ng/ml)	26.6 ± 35.7
Creatinine (μmol/l)	99.1 ± 24.1
Systolic blood pressure (mmHg)	131.4 ± 23.4
Heart rate (bpm)	99.1 ± 29.1

Table 3.2. Characteristics of patients in the study. Values are means (SD) or numbers (percentage)

3.3 NTproBNP levels in STEMI

NTproBNP was significantly elevated in patients who died compared to survivors (median [range] fmol/ml, survivors 700.2[0.3-11485.3] vs. dead 5781.3[1.4-10835.9], $p<0.0001$).

3.4 Relationship between NTproBNP and echocardiographic parameters

For the STEMI population the mean LVWMI was 1.53 (range 1.08-2.83). The LVWMI score was significantly higher in patients who died compared to survivors (median, [range], 1.83 [1.06-2.83] vs. 1.5 [1.0-2.85], $p=0.002$). The LVWMI score in

those subjects with anterior AMI was higher than in those with inferior AMI (1.8 [1.08-2.75] vs. 1.4 [1.00-2.83], p<0.001). NTproBNP correlated with LVWMI (r=0.342, p<0.0001),

3.5 TIMI score for STEMI and NTproBNP as predictors of death

Mortality was 8.9% and was related to higher TIMI risk scores (p=0.029 for trend, figure 3.1). When clinical and demographic characteristics were entered into a multivariate binary logistic model (we included as variables baseline patient characteristics as part of the TIMI score along with gender, prior history of AMI, LVWMI, post-AMI drug therapies, coronary revascularisation and peptide markers including troponin I and peak CK) NTproBNP (OR 4.21, 95% CI: 1.96-9.07, p<0.001) and post-AMI treatment with beta blockers (OR 0.24, 95% CI: 0.1-0.56, p=0.001) and angiotensin converting enzyme inhibitors/angiotensin receptor blockers (OR 0.29, 95% CI: 0.12-0.72, p=0.007) were the only independent predictors of death (table 3.3). This was also confirmed on the Cox proportional hazards model with the independent predictors of death being NTproBNP (HR 3.82, 95% CI: 1.89-7.78, p<0.001) and post-AMI treatment with beta blockers (HR 0.27, 95% CI: 0.12-0.57, p=0.001) and angiotensin converting enzyme inhibitors/angiotensin receptor blockers (HR 0.33, 95% CI: 0.16-0.71, p=0.004) (table 3.4).

Variable	Odds Ratio	95% CI	p value
NTproBNP	4.21	1.96-9.07	<0.001
β blockers post AMI	0.24	0.1-0.56	0.001
ACE inhibitors/AIIRB	0.29	0.12-0.72	0.007
Prior history of AMI	2.05	0.5-8.36	NS
LVWMI	1.75	0.50-6.08	NS
coronary revascularisation	0.57	0.16-1.99	NS
Peak CK	1.00	1.00-1.00	NS
Gender	0.45	0.17-1.19	NS
TIMI score	2.89	0.71-11.78	NS

Table 3.3. Multivariate binary logistic regression model of predictors of death

Variable	Hazards Ratio	95% CI	p value
NTproBNP	3.82	1.89-7.78	<0.001
β blockers post AMI	0.27	0.12-0.57	0.001
ACE inhibitors/AIIRB	0.33	0.16-0.71	0.004
Prior history of AMI	2.45	0.95-7.86	NS
LVWMI	1.68	0.59-4.81	NS
coronary revascularisation	0.82	0.26-2.25	NS
Peak CK	1.0	1.0-1.0	NS
Gender	0.47	0.20-1.09	NS
TIMI score	2.22	0.47-10.30	NS

Table 3.4. Multivariate Cox proportional hazards regression model of predictors of death

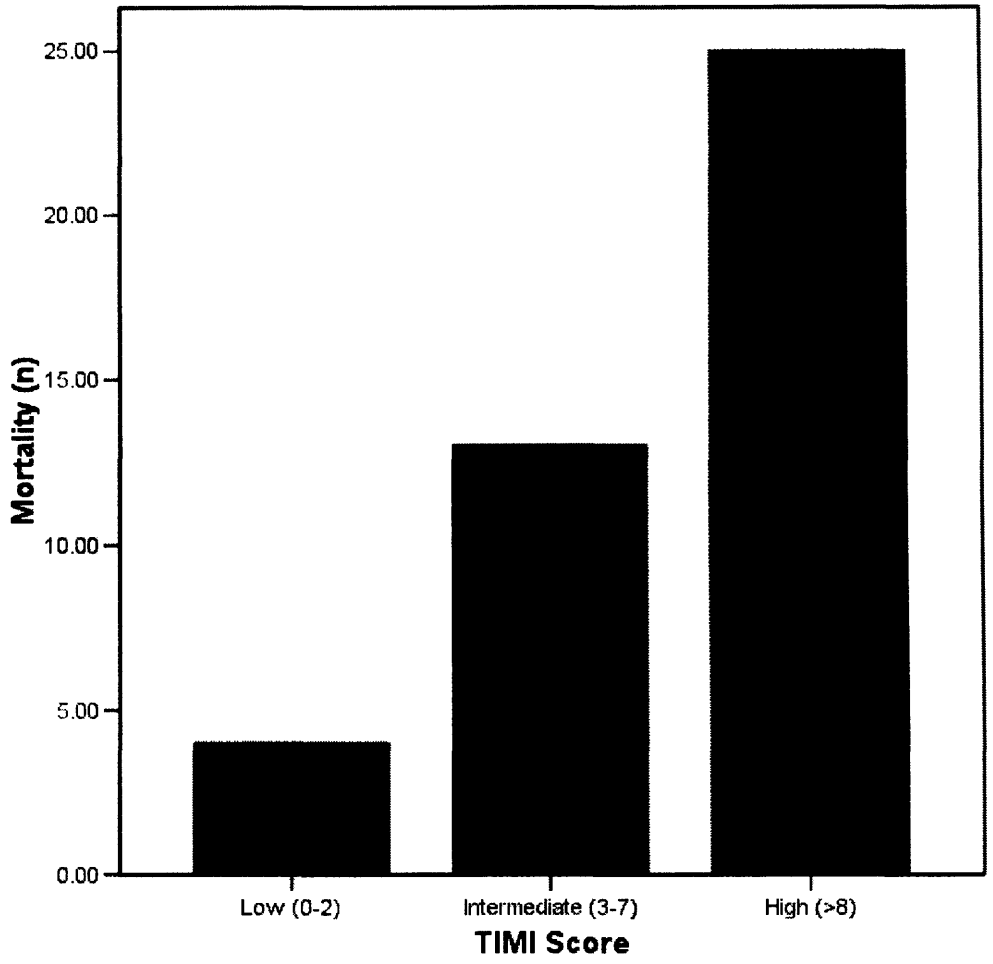

Figure 3.1. Bar chart showing relationship between higher TIMI score and increased mortality. There were 142, 179 and 152 patients in the low, intermediate and high TIMI risk groups respectively

3.6 Kaplan-Meier survival curve for TIMI

The Kaplan-Meier survival curve revealed a significantly better clinical outcome in patients with NTproBNP below the median compared with those with NTproBNP above the median (log rank 15.06, p=0.0001, figure 3.2). There was also a grading of mortality on the Kaplan-Meier survival curve related to whether the patients were in low, intermediate or high TIMI risk groups (log rank 29.86, p=0.0001, figure 3.3).

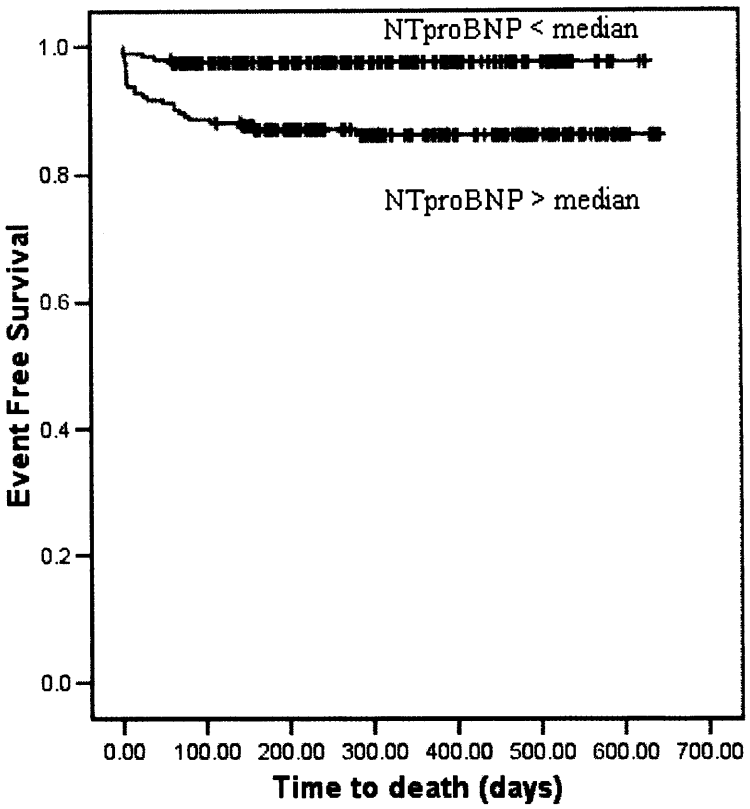

Figure 3.2. Kaplan-Meier Curve: Time to death related to serum NTproBNP

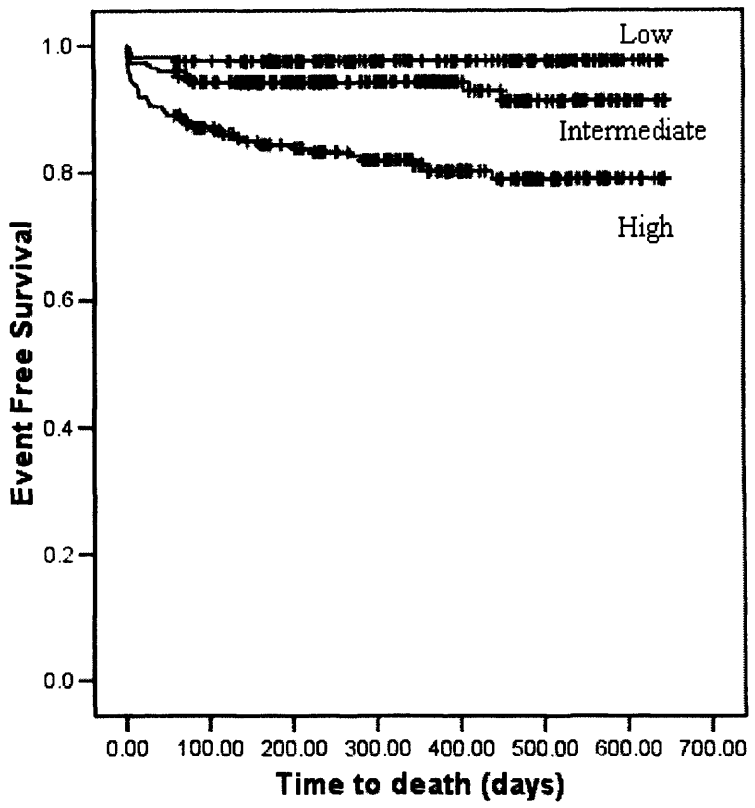

Figure 3.3. Kaplan-Meier Curve: Time to death related to low, intermediate or high TIMI risk groups

3.7 Receiver operating characteristic curve for TIMI and NTproBNP

The receiver operating characteristic curve for NTproBNP in the first 24 hours yielded an area under the curve (AUC) of 0.79 (95% CI: 0.70-0.88, $p<0.001$). For TIMI risk score the AUC was 0.67 (95% CI: 0.58-0.76, $p=0.001$, figure 3.4). The combination of TIMI score and NTproBNP did not significantly improve risk prediction for mortality. When NTproBNP above the median with or without the clinical presence of heart failure post AMI was investigated there was no improvement in the predictive power of the ROC curve. No difference was noted with regards to whether NTproBNP was measured early (within first 24 hours) or late (72-96 hours) after an infarct at predicting death (OR for NTproBNP 72-96 hours, 6.25,

95% CI: 1.92-20.34, p=0.002). Also measurement of NTproBNP within the first 12 hours performed as well as measurements taken between 13-24 hours.

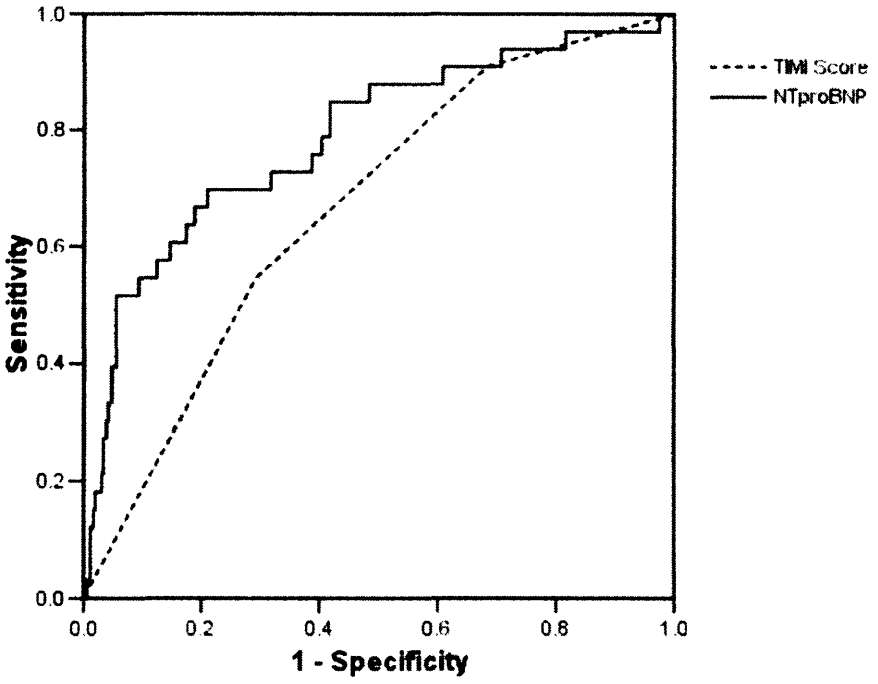

Figure 3.4. Receiver operating characteristic curve comparing NTproBNP and TIMI score for prediction of mortality

3.8 Time course of NTproBNP secretion after AMI

The data is presented as median (range) in fmol/ml. The table shows the plasma NTproBNP levels at the different 24 hour time points after AMI.

Time (Hours)	0-24	25-48	49-72	73-96	97-120
Number of patients	470	378	369	380	233
Median (fmol/ml)	911.2	1367.5	1064.2	944.7	1130.3
Range (fmol/ml)	0.3-11779	0.3-12175	0.3-10646	0.3-10325	0.3-9832

Table 3.5. Plasma NTproBNP levels at the different 24 hour time points after AMI

Using the repeated measures general linear model procedure, there was a statistically significant change in NTproBNP secretion over the 5 days. Figure 3.5 illustrates the time course of plasma NTproBNP showing significant changes with day of sampling ($P<0.001$), with peak levels on day 2 ($P<0.001$ and 0.02 compared to day 1 and day 3 respectively using the Bonferroni correction).

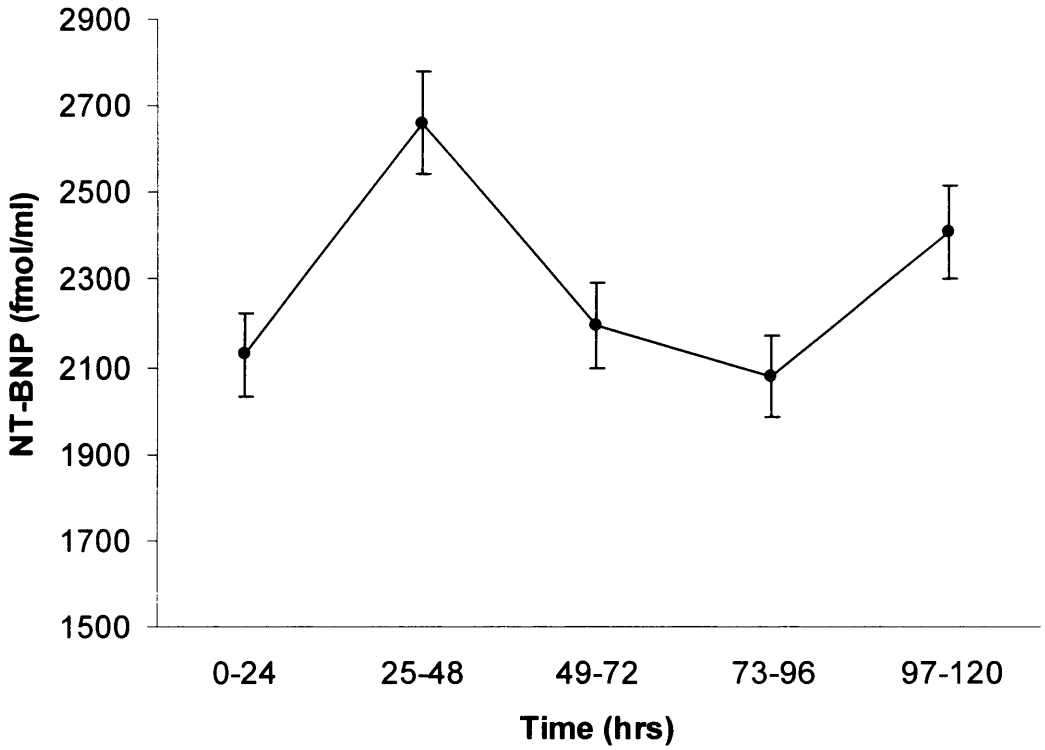

Figure 3.5. Plasma profile of plasma NTproBNP in patients following AMI

The plasma concentration of NTproBNP was significantly higher in AMI patients compared to age and sex matched controls over all time points ($P<0.0001$, over all time points).

3.9 NTproBNP levels (univariate analysis)

Plasma NTproBNP obtained over the 5 days was significantly higher in patients who died (Table 3.6). The box plot shows the statistically significant difference in NTproBNP levels in controls, patients who survived and those that died in the first 24 hours (Figure 3.6). NTproBNP levels were significantly raised in patients readmitted with HF compared to event-free survivors on days 1 and 4, with a trend to increased levels on the other 3 days (Table 3.7). There were significant differences in patients

who re- infarcted on days 1 and 3 (Table 3.8). There were significant differences in those patients who experienced MACE on days 1, 4 and 5 (Table 3.9).

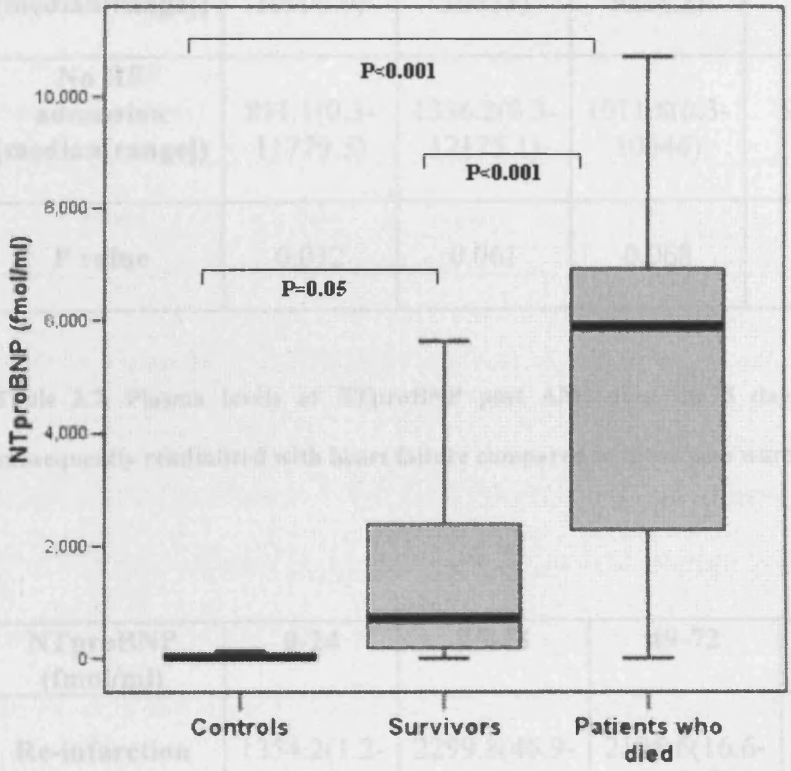

Figure 3.6 Box plot of NTproBNP in the first 24 hours and relationship between controls, survivors and patients who died

NTproBNP (fmol/ml)	0-24	25-48	49-72	73-96	97-120
Death (median[range])	5896.1(1.4-10835.9)	6573.9(20.1-11906.5)	6165.9(22.4-9854.8)	5247(10.3-10326)	5818(9-9451.3)
Survivors (median[range])	729.7(0.3-11779.3)	1236.3(0.3-12175.1)	988.6(0.3-10646)	854.1(0.3-10224.3)	1011.2(0.3-9832)
P value	<0.001	<0.001	<0.001	<0.001	<0.001

Table 3.6. Plasma levels of NTproBNP post AMI over the 5 days in patients who subsequently died compared to survivors

NTproBNP (fmol/ml)	0-24	25-48	49-72	73-96	97-120
HF admission (median[range])	2491.9(0.3-10904.9)	2817.7(0.3-10713)	1993.3(0.3-9228.3)	3011.7(146.9-10224.3)	1750.4(153.0-9598.3)
No HF admission (median[range])	811.1(0.3-11779.3)	1336.2(0.3-12175.1)	1011.8(0.3-10646)	910.2(0.3-10326)	1118.9(0.3-9832)
P value	0.012	0.061	0.068	0.003	0.334

Table 3.7. Plasma levels of NTproBNP post AMI over the 5 days in patients who were subsequently readmitted with heart failure compared to those who were not

NTproBNP (fmol/ml)	0-24	25-48	49-72	73-96	97-120
Re-infarction (median[range])	1354.2(1.2-11779)	2299.8(46.9-10126.1)	2195.6(16.6-10646)	1418.5(2.14-8712.4)	2029.2(2.6-9635.2)
No Re-infarction (median[range])	808.4(0.3-11200.2)	1332.2(0.3-12175.1)	987.7(0.3-10250.4)	906.2(0.3-10326)	1055.1(0.3-9832)
P value	0.053	0.074	0.005	0.164	0.349

Table 3.8. Plasma levels of NTproBNP post AMI over the 5 days in patients who subsequently re-infarcted compared to those who did not

NTproBNP (fmol/ml)	0-24	25-48	49-72	73-96	97-120
MACE (median[range])	1295.3(0.3-11779)	1846.7(0.3-11906)	1376.6(0.3-10645)	1402.1(2.14-10325)	2029.2(2.64-9635.2)
No MACE (median[range])	769.1(0.3-11200.2)	1264.8(0.3-12175.1)	975.6(0.3-10250.4)	834.9(0.3-10224.3)	947.7(0.3-9832)
P value	0.006	0.057	0.083	0.033	0.011

Table 3.9. Plasma levels of NTproBNP post AMI over the 5 days in patients with MACE

Significant differences in NTproBNP levels were noted between males and females ($P<0.001$ over all time points with higher levels in females) and those with a Killip class above 1 ($P<0.002$ over all time points, higher levels in patients in Killip class II, III, IV vs. Killip class I). Levels of NTproBNP were also graded and increased as the Killip grade increased ($P<0.001$, Kruskal-Wallis, figure 3.7). NTproBNP levels were also higher in patients with a past history of AMI (days 1, 4 and 5, $P<0.04$), hypertension (all time points, $P<0.036$), HF (all time points, $P<0.001$) or diabetes (day 1, $P=0.026$). Plasma NTproBNP levels were also higher in STEMI vs. NSTEMI patients (day 1, $P=0.006$), and those with anterior site of AMI (days 1 and 4 $P<0.006$). When we looked specifically at patients in Killip class I, NTproBNP levels were significantly higher in those who died in this group compared to those who did not ($n=13$ for day 1, $P=0.001$).

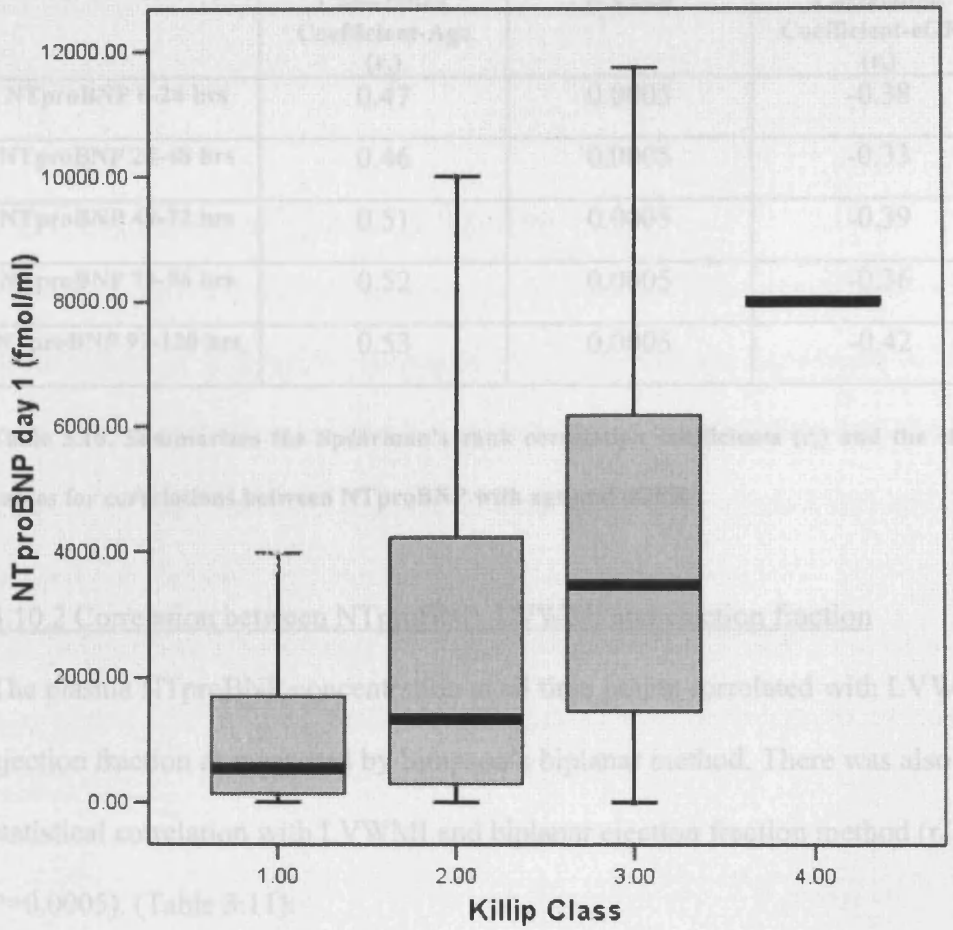

Figure 3.7. NTproBNP levels on day 1 in patients with differing Killip grades

3.10 NTproBNP correlations

3.10.1 Correlation between NTproBNP, age and eGFR

Plasma NTproBNP had a positive correlation with age and negative with eGFR (Table 3.10).

	Correlation Coefficient-Age (r_s)	P-Value	Correlation Coefficient-eGFR (r_s)	P-Value
NTproBNP 0-24 hrs	0.47	0.0005	-0.38	0.0005
NTproBNP 25-48 hrs	0.46	0.0005	-0.33	0.0005
NTproBNP 49-72 hrs	0.51	0.0005	-0.39	0.0005
NTproBNP 73-96 hrs	0.52	0.0005	-0.36	0.0005
NTproBNP 97-120 hrs	0.53	0.0005	-0.42	0.0005

Table 3.10. Summarizes the Spearman's rank correlation coefficients (r_s) and the significance values for correlations between NTproBNP with age and eGFR

3.10.2 Correlation between NTproBNP, LVWMI and ejection fraction

The plasma NTproBNP concentration at all time points correlated with LVWMI and ejection fraction as measured by Simpson's biplanar method. There was also a statistical correlation with LVWMI and biplanar ejection fraction method ($r_{S=}$ -0.43, P=0.0005). (Table 3.11).

	Correlation Coefficient-LVWMI (r_s)	P-Value	Correlation Coefficient-EF (r_s)	P-Value
NTproBNP 0-24 hrs	0.34	0.0005	-0.33	0.0005
NTproBNP 25-48 hrs	0.36	0.0005	-0.31	0.0005
NTproBNP 49-72 hrs	0.33	0.0005	-0.29	0.0005
NTproBNP 73-96 hrs	0.42	0.0005	-0.36	0.0005
NTproBNP 97-120 hrs	0.32	0.0005	-0.32	0.0005

Table 3.11. Summarizes the Spearman's rank correlation coefficients (r_s) and the significance values for correlations between NTproBNP with LVWMI and biplanar ejection fraction

No statistically significant correlations were observed between NTproBNP levels and troponin or peak CK.

3.11 Determinants of NTproBNP secretion

Univariate general linear model analyses were conducted to identify the independent determinants of NTproBNP secretion. Factors statistically significant on univariate analysis (males vs. females, past history of MI, hypertension, diabetes or heart failure, Killip class, age, territory and site of infarction) were entered into the univariate general linear model.

The independent determinants of NTproBNP secretion identified by the model were age, gender and site of infarction. The findings from the analyses are presented in table 3.12

Variable	P-Value
eGFR	0.062
Male gender	0.002
Age	0.0001
Site of infarction	0.005
Past history of heart failure	0.070
Killip class 1 vs. Killip class >1	0.082
Past history of hypertension	0.059
Past history of diabetes	0.467
STEMI vs. NSTEMI	0.073

Table 3.12. Univariate determinants of NTproBNP secretion

3.12 Patient characteristics for CT-1

The demographic features of the patient population are shown in Table 3.13. 504 of the available 596 patients had CT-1 levels measured. Median length of follow-up was 336 days with a range of 0–645 days. Of the patients enrolled, 153 (52.6 %) received thrombolysis during the index admission; no patient was lost to follow-up. During follow-up, 56 (11.1%) patients died, 34 (6.7%) were readmitted with heart failure there were 52 (10.3%) re-infarctions and 131 (61.9%) MACE. Echocardiographic data was available for 252 (86.6%) of the 291 patients and done at a median of 3.5 days (range 2-5) after presentation with AMI. 22 echocardiograms were not analysable and 17 patients did not receive an echocardiogram.

	Controls	AMI Patients
Number	47	291
Age (in years)	61.8 ± 13.7	64.0 ± 12.9
Male Sex	30	227
Previous Medical History		
Myocardial infarction	None	41 (14.1)
Angina Pectoris	None	54 (13.5)
Hypertension	None	125 (44.3)
Diabetes mellitus	None	63 (20.6)
Hypercholesterolaemia	None	87 (28.1)
Obesity	None	42 (15.4)
Current/Ex-Smokers	None	101 (36.7)
ST-elevation AMI	None	220 (75.6)

Thrombolytic	None	153 (52.6)
Territory of Infarct		
Anterior	N/A	125 (43.0)
Inferior	N/A	140 (48.1)
Other/undetermined	N/A	26 (8.9)
Killip Class on Admission		
I	N/A	143 (49.1)
II	N/A	119 (40.9)
III	N/A	25 (8.6)
IV	N/A	4 (1.4)
Peak CK (IU)	N/A	1251.9 ± 1401.1
Peak Troponin I (ng/ml)	N/A	20.4 ± 31.2
Creatinine (μmol/l)	N/A	102.2 ± 35.2

Table 3.13. Characteristics of patients and controls in the study. Values are means (SD) or numbers (percentage)

3.13 Time course of CT-1 secretion after AMI

The data is presented as median (range) in fmol/ml. The table shows the plasma CT-1 levels at the different 24-hour time points after AMI.

Time (Hours)	0-24	25-48	49-72	73-96	97-120
Number of patients	385	291	285	300	181
Median (fmol/ml)	1.13	0.85	0.79	0.82	1.13
Range (fmol/ml)	0.03-556.3	0.03-462.5	0.03-450.4	0.03-530.2	0.03-93.5

Table 3.14. Plasma CT-1 levels at the different 24 hour time points after AMI

Using the repeated measures general linear model procedure the time course of secretion of CT-1 was measured revealing a significant difference over the 5 days ($P<0.0001$) and is shown in figure 3.8. The plasma concentration of CT-1 was significantly higher in AMI patients compared to age and sex matched controls over days 1 and 5 ($P<0.002$).

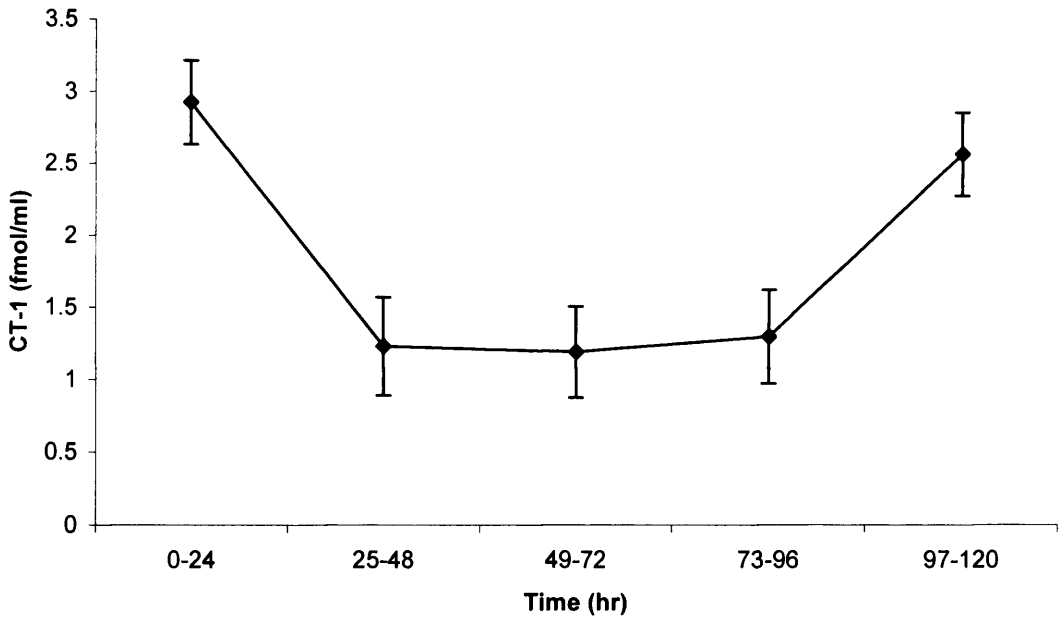

Figure 3.8. Plasma profile of plasma CT-1 in patients following AMI

3.14 CT-1 levels (univariate analysis)

CT-1 was higher in patients following AMI compared to control subjects (median [range] fmol/ml 0.77[0.03-457.1] vs. 0.73[0.20-1.78], p=0.001). Plasma CT-1 obtained over the 5 days was significantly higher in patients who died on day 2 but not on the other days (Table 3.15). The box plot shows the statistically significant difference in CT-1 levels in controls, patients who survived and those that died between 24 and 48 hours (Figure 3.9). CT-1 levels were significantly raised in patients readmitted with HF compared to event-free survivors on days 2 and 3, with a trend to increase on day 1 (Table 3.16). CT-1 was also significantly elevated in patients who had a combined endpoint of death or heart failure on days 2 and 3 (Table 3.17). There were no significant differences in patients who re- infarcted, died or re-infarcted or had a MACE (Table 3.18-3.20).

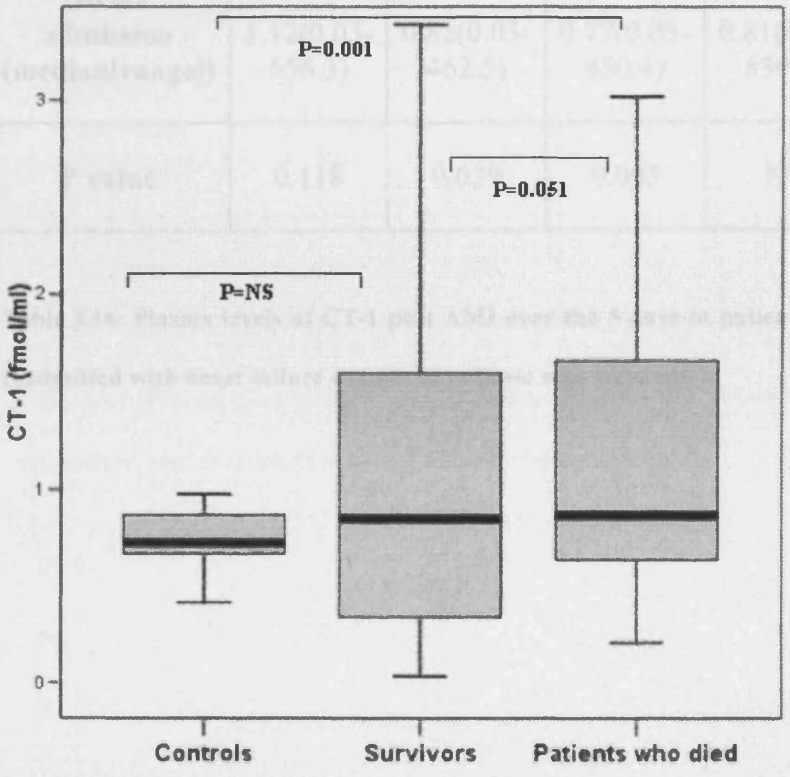

Figure 3.9 Box plot of CT-1 plasma levels between 24 and 48 hours and relationship between controls, survivors and patients who died, NS=non-significant

CT-1 (fmol/ml)	0-24	25-48	49-72	73-96	97-120
Death (median[range])	1.11(0.08-304.9)	0.90(0.21-294.1)	0.86(0.12-287.4)	0.66(0.03-27)	0.85(0.09-4.06)
Survivors (median[range])	1.13(0.03-556.3)	0.84(0.03-462.5)	0.78(0.03-450.4)	0.85(0.03-530.2)	1.14(0.03-93.5)
P value	NS	0.051	NS	NS	NS

Table 3.15. Plasma levels of CT-1 post AMI over the 5 days in patients who subsequently died compared to survivors

CT-1 (fmol/ml)	0-24	25-48	49-72	73-96	97-120
HF admission (median[range])	1.62(0.11-404.3)	0.94(0.05-392.2)	1.20(0.03-23.8)	0.95(0.03-21.0)	1.14(0.03-21.3)
No HF admission (median[range])	1.12(0.03-556.3)	0.82(0.03-462.5)	0.77(0.03-450.4)	0.81(0.03-530.2)	1.12(0.03-93.5)
P value	0.118	0.039	0.005	NS	NS

Table 3.16. Plasma levels of CT-1 post AMI over the 5 days in patients who were subsequently readmitted with heart failure compared to those who were not

CT-1 (fmol/ml)	0-24	25-48	49-72	73-96	97-120
Death or HF admission (median[range])	1.31(0.08-404.3)	0.93(0.05-392.2)	1.10(0.03-287.4)	0.75(0.03-27.0)	0.96(0.03-21.3)
No Death or HF admission (median[range])	1.12(0.03-556.3)	0.82(0.03-462.5)	0.77(0.03-450.4)	0.86(0.03-530.2)	1.14(0.03-93.5)
P value	NS	0.016	0.007	NS	NS

Table 3.17. Plasma levels of CT-1 post AMI over the 5 days in patients who were subsequently died or were readmitted with heart failure compared to those who were not

CT-1 (fmol/ml)	0-24	25-48	49-72	73-96	97-120
AMI admission (median[range])	1.07(0.05-211.3)	0.39(0.03-5.33)	0.72(0.03-210.4)	0.54(0.03-91.0)	1.33(0.03-5.13)
No AMI admission (median[range])	1.22(0.03-556.3)	0.79(0.03-453.3)	0.74(0.03-432.2)	0.78(0.03-530.2)	1.19(0.03-48.0)
P value	NS	NS	NS	NS	NS

Table 3.18. Plasma levels of CT-1 post AMI over the 5 days in patients who were subsequently readmitted with re-infarction compared to those who were not

CT-1 (fmol/ml)	0-24	25-48	49-72	73-96	97-120
Death or AMI (median[range])	1.09(0.05-304.9)	0.78(0.05-294.1)	0.76(0.03-287.4)	0.58(0.03-91.6)	1.17(0.03-5.13)
No Death or AMI (median[range])	1.26(0.03-556.3)	0.77(0.03-453.3)	0.72 (0.03-432.1)	0.79(0.03-530.2)	1.21(0.03-48.0)
P value	NS	NS	NS	NS	NS

Table 3.19. Plasma levels of CT-1 post AMI over the 5 days in patients who died or reinfarcted compared to those who did not

CT-1 (fmol/ml)	0-24	25-48	49-72	73-96	97-120
MACE (median[range])	1.16(0.03-556.3)	0.88(0.03-294.1)	0.73(0.03-287.4)	0.75(0.03-530.3)	1.15(0.03-5.13)
No MACE (median[range])	1.20(0.03-479.1)	0.67(0.03-453.3)	0.73(0.03-432.1)	0.74(0.03-424.7)	1.29(0.03-48.0)
P value	NS	NS	NS	NS	NS

Table 3.20. Plasma levels of CT-1 post AMI over the 5 days in patients who subsequently experienced MACE compared to those who did not

CT-1 did not differ significantly according to gender, smoking status, the presence or absence of hypertension, previous MI diagnosis, hypercholesterolemia, previous heart failure admission or whether a patient received thrombolyis or not. Plasma CT-1 levels were higher in STEMI vs. NSTEMI patients (day 4 and 5, $P<0.01$), and those with anterior site of AMI (days 1 2 and 3 $P<0.033$). Plasma CT-1 levels were also

higher in patients with a prior history of diabetes mellitus (day 1, P<0.030) and in those with a Killip class above 1 (day 3, p=0.016, higher levels pts in Killip II, III, IV vs. Killip class I).

3.15 CT-1 correlations

There was no correlation of CT-1 with age, troponin I, peak CK, eGFR, creatinine, or biplanar ejection fraction. There was a weak correlation of CT-1 with LVWMI ($r_{s=}$ 0.125, p= 0.049).

There was some weak statistically significant positive correlation between CT-1 on day 1 and NTproBNP on days 2 and 3 ($r_{s=}$ 0.12, P=0.04 and $r_{s=}$ 0.12, P=0.035, respectively).

3.16 Determinants of CT-1 secretion

Univariate general linear model analyses were conducted to identify the independent determinants of CT-1 secretion. Factors statistically significant on univariate analysis (past history of diabetes, Killip class, territory and site of infarction) were entered into the univariate general linear model. The independent determinants of CT-1 secretion on day 3 were Killip class and site of infarction identified by the model.

3.17 Relationship between CT-1 and echocardiographic parameters

For the whole population mean LVWMI was 1.52 (range 1.08-2.75) and EF was 38% (range 12-49%). The LVWMI score in those subjects with anterior AMI was higher than in those with inferior AMI (1.7 [1.08-2.75] vs. 1.4 [1.00-2.60], p<0.0001). However LVEF was no different between the two groups (39 [12-68] vs. 40 [13-65])

%, p=0.45). There was a weak correlation of CT-1 with LVWMI (r= 0.125, p= 0.049).

3.18 CT-1 and NTproBNP as predictors of death

Binary logistic regression was performed to test for independent predictors of death by one or more of the peptide factors. Demographic and clinical factors were entered into the model as standard predictors; these included age, sex, serum creatinine, Killip class, territory of AMI and whether the patient received thrombolysis or not and peptide markers (including troponin I). NTproBNP (OR 4.73, 95% CI: 1.39-16.13, p=0.013) and male gender (OR 0.28, 95% CI: 0.10-0.85, p=0.026) independently predicted death (Table 3.21). This was also confirmed on the Cox proportional hazards model with the independent predictor of death being NTproBNP (HR 4.65, 95% CI: 1.44-15.0, p=0.01 and male gender (HR 0.38, 95% CI: 0.15-0.97, p=0.04) (Table 3.22).

Variable	Odds Ratio	95% CI	p value
NTproBNP	4.73	1.39-16.13	0.013
Male gender	0.28	0.10-0.85	0.026
CT-1	1.60	0.80-3.28	NS
age	1.04	0.98-1.10	NS
Serum creatinine	18.5	0.40-928.6	NS
Killip class	2.81	0.79-9.93	NS
Peak CK	1.00	1.00-1.00	NS
Territory of AMI	0.72	0.25-2.07	NS
Thrombolysis	0.66	0.23-1.91	NS
Troponin I	1.01	0.98-1.05	NS

Table 3.21. Multivariate binary logistic regression model of predictors of death

Variable	Hazards Ratio	95% CI	p value
NTproBNP	4.65	1.44-15.0	0.01
Male gender	0.38	0.15-0.97	0.04
CT-1	1.31	0.82-2.10	NS
age	1.04	0.99-1.09	NS
Serum creatinine	21.34	0.97-467.9	NS
Killip class	2.66	0.91-7.77	NS
Peak CK	1.00	1.00-1.00	NS
Territory of AMI	0.58	0.24-1.42	NS
Thrombolysis	0.86	0.36-2.01	NS
Troponin I	1.01	0.98-1.05	NS

Table 3.22. Multivariate Cox proportional hazards regression model of predictors of death

3.19 CT-1 and NTproBNP as predictors of death or non-fatal MI

Binary logistic regression was performed to test for independent predictors of death or non-fatal MI, by one or more of the peptide factors. Demographic and clinical factors were entered into the model as standard predictors; these included age, sex, serum creatinine, Killip class, territory of AMI and whether the patient received thrombolysis or not and peptide markers (including troponin I). NTproBNP (OR 3.83, 95% CI: 1.59-9.2, p=0.003) independently predicted death or non-fatal MI (Table 3.23). This was also confirmed on the Cox proportional hazards model with the independent predictors of death or non-fatal MI being NTproBNP (HR 2.73, 95% CI: 1.37-5.44, p=0.004, Table 3.24).

Variable	Odds Ratio	95% CI	p value
NTproBNP	3.83	1.59-9.20	0.003
Male gender	0.78	0.29-2.10	NS
CT-1	0.94	0.50-1.76	NS
Age	1.02	0.98-1.07	NS
Serum creatinine	15.27	0.41-470.3	NS
Killip class	1.54	0.61-3.88	NS
Peak CK	1.00	1.00-1.00	NS
Territory of AMI	0.84	0.36-1.99	NS
Thrombolysis	1.35	0.50-3.66	NS
Troponin I	1.01	0.99-1.03	NS

Table 3.23. Multivariate binary logistic regression model of predictors of death or non-fatal MI

Variable	Hazards Ratio	95% CI	p value
NTproBNP	2.73	1.37-5.44	0.004
Male gender	0.79	0.39-1.63	NS
CT-1	0.96	0.61-1.51	NS
Age	1.03	0.99-1.06	NS
Serum creatinine	10.26	0.86-122.1	NS
Killip class	1.83	0.89-3.75	NS
Peak CK	1.00	1.00-1.00	NS
Territory of AMI	0.69	0.36-1.30	NS
Thrombolysis	1.33	0.70-2.33	NS
Troponin I	1.01	0.99-1.02	NS

Table 3.24. Multivariate Cox proportional hazards regression model of predictors of death or non-fatal MI

3.20 CT-1 and NTproBNP as predictors of death or heart failure

Binary logistic regression was performed to test for independent predictors of death or heart failure, by one or more of the peptide factors. Demographic and clinical factors were entered into the model as standard predictors; these included age, sex, serum creatinine, Killip class, territory of AMI and whether the patient received thrombolysis or not and peptide markers (including troponin I). CT-1 (OR 1.84, 95% CI: 1.06-3.22, p=0.031), NTproBNP (OR 2.40, 95% CI: 1.11-5.17, p=0.026) and age (OR 1.08, 95% CI: 1.03-1.12, p=0.001 independently predicted death or heart failure (Table 3.25). This was also confirmed on the Cox proportional hazards model with the independent predictors of death or heart failure being CT-1 (HR 1.45, 95%

CI: 1.03-2.06, p=0.034), NTproBNP (HR 2.06, 95% CI: 1.00-4.31, p=0.05) and age (HR 1.08, 95% CI: 1.04-1.12, p=0.001) Table 3.26).

Variable	Odds Ratio	95% CI	p value
NTproBNP	2.40	1.11-5.17	0.026
CT-1	1.84	1.06-3.22	0.031
Male gender	0.56	0.25-1.31	NS
age	1.08	1.03-1.12	0.001
Serum creatinine	6.91	0.31-159.6	NS
Killip class	2.11	0.88-5.04	NS
Peak CK	1.00	1.00-1.00	NS
Territory of AMI	0.80	0.36-1.79	NS
Thrombolysis	1.32	0.50-3.63	NS
Troponin I	1.01	0.99-1.03	NS

Table 3.25. Multivariate binary logistic regression model of predictors of death or heart failure

Variable	Hazards Ratio	95% CI	p value
NTproBNP	2.06	1.00-4.31	0.05
CT-1	1.45	1.03-2.06	0.034
Male gender	0.73	0.37-1.45	NS
age	1.08	1.04-1.12	0.001
Serum creatinine	4.82	0.36-118.1	NS
Killip class	1.86	0.85-4.08	NS
Peak CK	1.00	1.00-1.00	NS
Territory of AMI	0.75	0.38-1.79	NS
Thrombolysis	1.33	0.70-2.33	NS
Troponin I	1.01	0.99-1.02	NS

Table 3.26. Multivariate Cox proportional hazards regression model of predictors of death or heart failure

3.21 CT-1 and NTproBNP as predictors of MACE (death, MI, need for urgent revascularisation)

Binary logistic and Cox regression analysis was performed to test for independent predictors of MACE by one or more of the peptide factors. Demographic and clinical factors were entered into the model as standard predictors; these included age, sex, serum creatinine, Killip class, territory of AMI and whether the patient received thrombolysis or not and peptide markers (including troponin I). No variables were found to independently predict MACE in binary or Cox regression models.

3.22 Kaplan-Meier survival curve for CT-1 (death or heart failure)

The Kaplan-Meier survival curve revealed a significantly better clinical outcome for death or heart failure in patients with CT-1 below the median compared with those with CT-1 above the median (log rank 5.79, p=0.016, figure 3.10). This was also true for NTproBNP (log rank 20.24, p<0.0001, figure 3.11).

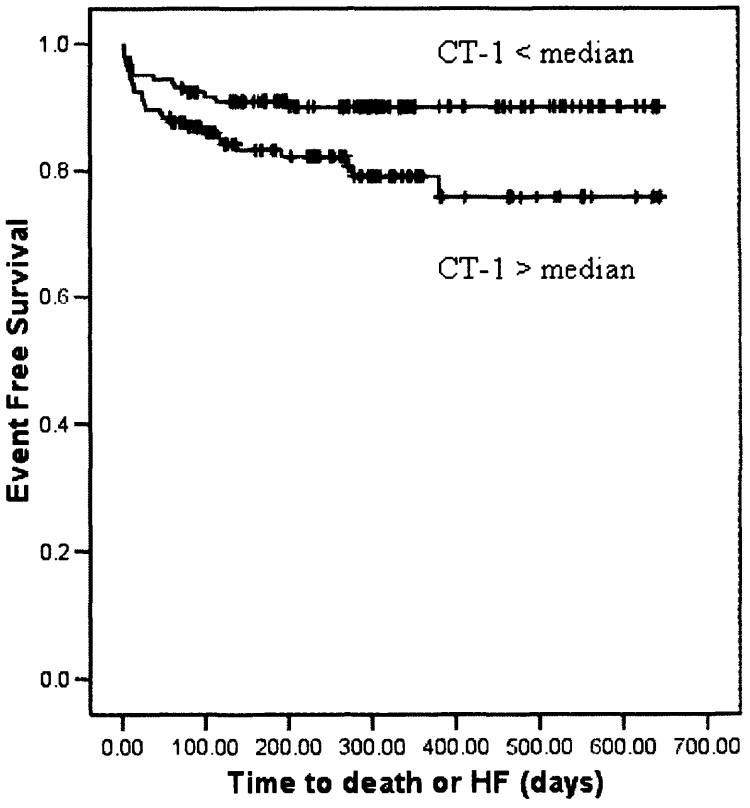

Figure 3.10. Kaplan-Meier Curve: Time to death or heart failure related to serum CT-1

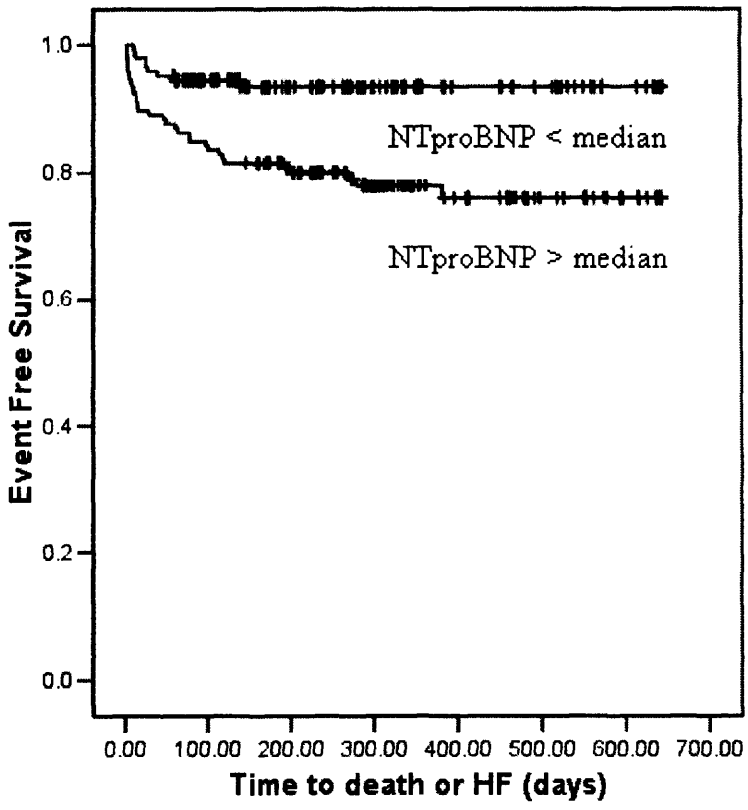

Figure 3.11 Kaplan-Meier Curve: Time to death or heart failure related to serum NTproBNP

3.23 Receiver-operating characteristic curve for CT-1 and NTproBNP (death or heart failure)

The receiver-operating curve for CT-1 yielded an area under the curve (AUC) of 0.62 (95% CI: 0.53-0.70, p=0.017); for NTproBNP the AUC was 0.77 (95% CI: 0.69-0.86, p<0.001). The logistic model combining the 2 markers yielded an AUC of 0.84 (95% CI: 0.78-0.91, p<0.001), which exceeded that of either peptide alone (figure 3.12).

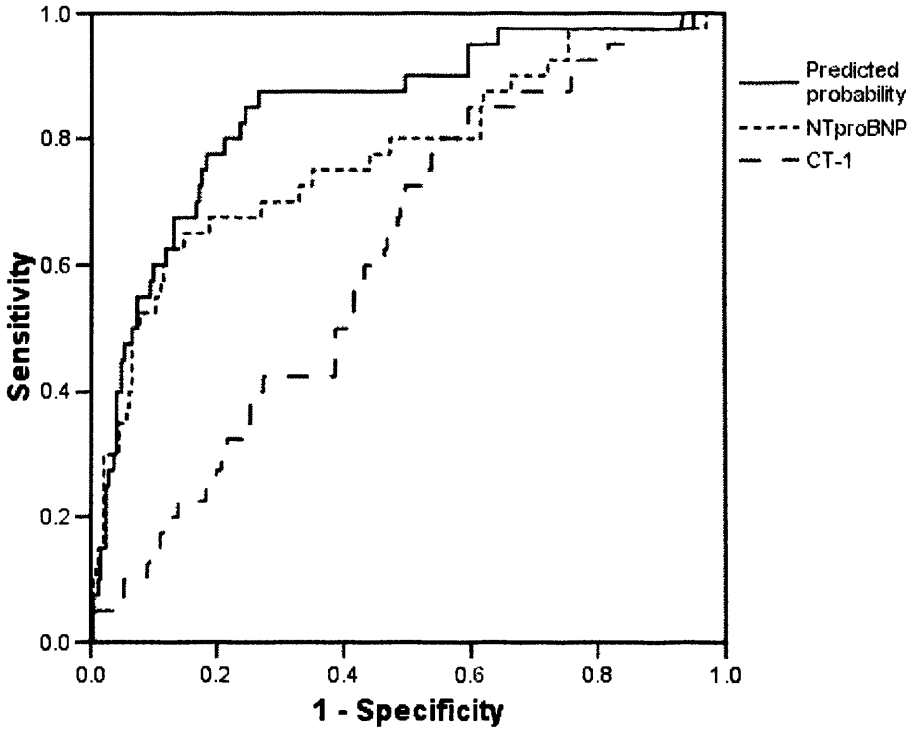

Figure 3.12. Combined receiver operating characteristic curve comparing NTproBNP, CT-1 and the combined predicted probabilities of death or heart failure

3.24 Patient characteristics for myotrophin

The demographic features of the patient population are shown in Table 3.27.All 596 patients had myotrophin levels measured. Median length of follow-up was 355 days with a range of 0–645 days. Of the patients enrolled, 65.5% of the STEMI patients received thrombolysis during the index admission. No patient was lost to follow-up. During follow-up, 28 patients died, 27 were readmitted with AMI, 73 patients required urgent revascularisation and there were 28 readmissions with heart failure. Echocardiographic data was available for 297 (83.6%) of the 356 patients and performed at a median of 3.5 days (range 2-5) after presentation with AMI. 36 echocardiograms were unanalysable and 22 patients did not receive an echocardiogram.

	Controls	AMI Patients
Number	40	356
Age (in years)	60.4 ± 11.6	63.0 ± 12.8
Male Sex	25	276 (77.7)
Previous Medical History		
Myocardial infarction	None	51 (14.4)
Angina Pectoris	None	57 (16.1)
Hypertension	None	153 (43.1)
Diabetes mellitus	None	71 (20.0)
Hypercholesterolaemia	None	105 (29.6)
Obesity	None	43 (12.1)
Current/Ex-Smokers	None	125 (35.2)

ST-elevation AMI	None	287 (80.8)
Thrombolytic	None	188 (53)
Territory of Infarct		
Anterior	N/A	146 (41.1)
Inferior	N/A	142 (40.0)
Other/undetermined	N/A	66 (18.6)
Killip Class on Admission		
I	N/A	136 (38.3)
II	N/A	178 (50.1)
III	N/A	35 (9.9)
IV	N/A	6 (1.7)
Peak CK (I/U)	N/A	1313.5 ± 1453.9
Peak Troponin I (ng/ml)	N/A	22.4 ± 33.2
Creatinine (μmol/l)	N/A	101.0 ± 28.2

Table 3.27. Characteristics of patients and controls in the study. Values are means (SD) or numbers (percentage)

3.25 Time course of myotrophin secretion after AMI

The data is presented as median (range) in fmol/ml. The table shows the plasma myotrophin levels at the different 24-hour time points after AMI.

Time (Hours)	0-24	25-48	49-72	73-96	97-120
Number of patients	468	377	367	378	231
Median (fmol/ml)	1458.7	1419	1400.9	1412.3	1482.4
Range (fmol/ml)	509.0-16921.7	567.5-16830.5	567.5-16585	184.9-17073	567.5-15616.6

Table 3.28. Plasma myotrophin levels at the different 24-hour time points after AMI

Using the repeated measures general linear model procedure, there was a statistically significant change in myotrophin secretion over the 5 days. Figure 3.13 illustrates the time course of plasma myotrophin showing significant changes with the day of sampling ($P<0.01$).

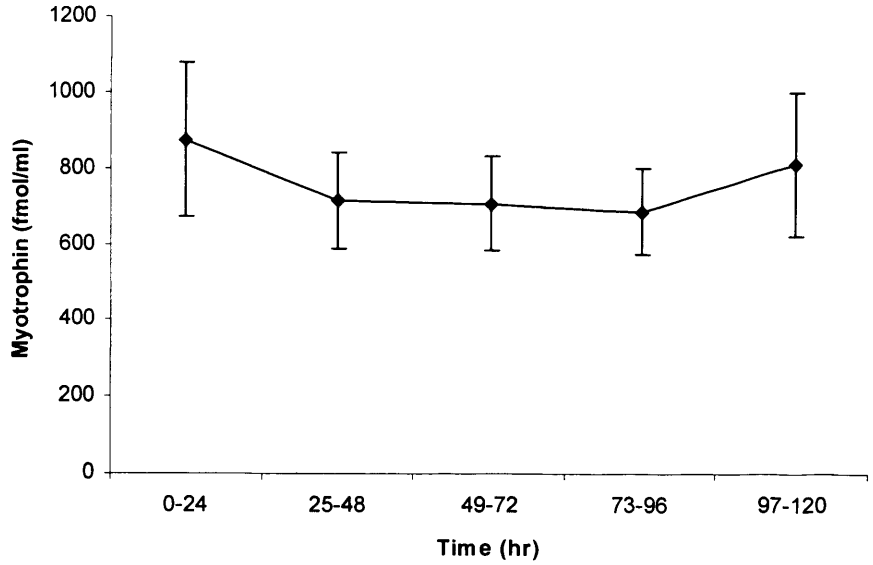

Figure 3.13. Plasma profile of plasma myotrophin in patients following AMI

The plasma concentration of myotrophin was significantly higher in AMI patients compared to age and sex matched controls over all time points ($P<0.015$).

3.26 Myotrophin levels (univariate analysis)

Levels in AMI patients were significantly higher than those observed in the control subjects (Median [Range], fmol/ml, 405.7; [51.8– 7445.5]; vs. 348.1; [34.1–3982.9]; $p=0.044$). Plasma myotrophin was significantly higher in patients who died on day 2 (Table 3.29) therefore all further analysis was carried out using day 2 samples. The box plot shows the statistically significant difference in myotrophin levels in controls, patients who survived and those that died between 24 and 48 hours (Figure 3.14). Myotrophin levels were significantly raised in patients readmitted with HF compared to event-free survivors on days 1 and 3, with a trend to increased levels on day 2 (Table 3.30). Plasma myotrophin levels were also raised in patients who had a combined endpoint of death or heart failure (Table 3.31), death or re-infarction (Table 3.33) or the combined endpoint of death, MI or need for urgent revascularisation (defined as MACE, Table 3.34). There were no significant differences in myotrophin levels in patients who re- infarcted (Table 3.32).

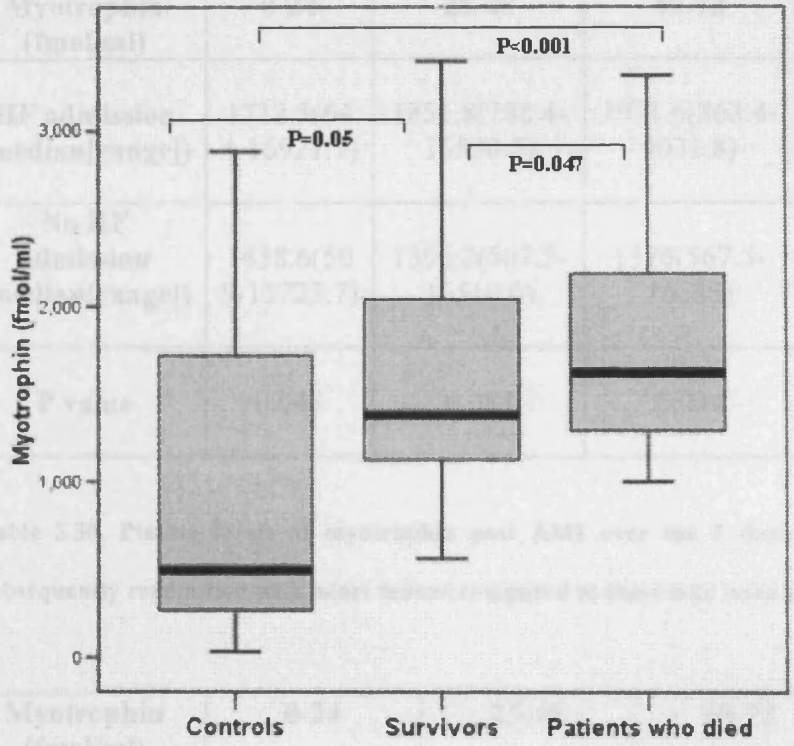

Figure 3.14 Box plot of myotrophin plasma levels between 24 and 48 hours and relationship between controls, survivors and patients who died, NS=non-significant

Myotrophin (fmol/ml)	0-24	25-48	49-72	73-96	97-120
Death (median[range])	1411.4(550-9421)	1632.2(1006.2-6790.5)	1601.1(967.7-9465.3)	1483.8(194.9-4424.4)	1655.7(567.5-4203.6)
Survivors (median[range])	1463.8(509-16921.7)	1390.3(567.5-16830.5)	1376.8(567.5-16585)	1400.8(447.6-17073.3)	1459.3(567.5-15616.6)
P value	NS	0.047	NS	NS	NS

Table 3.29. Plasma levels of myotrophin post AMI over the 5 days in patients who subsequently died compared to survivors

Myotrophin (fmol/ml)	0-24	25-48	49-72	73-96	97-120
HF admission (median[range])	1712.5(644-16921.7)	1851.8(788.4-16830.5)	1971.6(863.4-9031.8)	1580.4(643.1-8903.5)	1859.8(567.8-9151.6)
No HF admission (median[range])	1438.6(509-15723.7)	1396.2(567.5-16510.6)	1376(567.5-16585)	1402.4(194.9-17073.3)	1471.3(567.5-15616.6)
P value	0.043	0.085	0.044	NS	NS

Table 3.30. Plasma levels of myotrophin post AMI over the 5 days in patients who were subsequently readmitted with heart failure compared to those who were not

Myotrophin (fmol/ml)	0-24	25-48	49-72	73-96	97-120
Death or HF admission (median[range])	1558.3(550.6-16921.7)	1672.3(788.4-16830.5)	1665.5(863.4-9456.6)	1484.2(194.9-8903.5)	1655.7(567.8-9151.6)
No Death or HF admission (median[range])	1442.9(509-15723.7)	1381.8(567.5-16510.6)	1355(567.5-16585)	1368.5(447.5-17073.3)	1450.0(567.5-15616.6)
P value	NS	0.021	0.008	NS	NS

Table 3.31. Plasma levels of myotrophin post AMI over the 5 days in patients who were subsequently died or were readmitted with heart failure compared to those who were not

Myotrophin (fmol/ml)	0-24	25-48	49-72	73-96	97-120
AMI admission (median[range])	1533.7(567.5-15707.2)	1478.7(685.8-14789.2)	1628.0(882.8-15104.3)	1436.6(518.4-15818.6)	1606.5(871.5-8243.8)
No AMI admission (median[range])	1449.3(509-16921.7)	1416.2(567.5-16830.5)	1377.7(567.5-16585.0)	1412.2(194.9-17073.3)	1481.6(567.5-15616.6)
P value	NS	NS	NS	NS	NS

Table 3.32. Plasma levels of myotrophin post AMI over the 5 days in patients who were subsequently readmitted with re-infarction compared to those who were not

Myotrophin (fmol/ml)	0-24	25-48	49-72	73-96	97-120
Death or AMI (median[range])	1512.8(550-15707.2)	1566.3(685.8-14789.2)	1596.2(882.8-15104.3)	1467.1(194.9-15818.6)	1622.3(567.5-8243.8)
No Death or AMI (median[range])	1449.3(509-16921.7)	1385.3(567.5-16830.5)	1365.5(567.5-16585)	1400.8(447.6-17073.3)	1459.3(567.5-15616.6)
P value	NS	0.048	0.021	NS	NS

Table 3.33. Plasma levels of myotrophin post AMI over the 5 days in patients who died or reinfarcted compared to those who did not

Myotrophin (fmol/ml)	0-24	25-48	49-72	73-96	97-120
MACE (median[range])	1558.2(550.6-15723.7)	1526.8(567.5-16510.6)	1589.8(567.5-16585.0)	1484.2(194.9-17073.3)	1622.3(567.5-9151.6)
No MACE admission (median[range])	1433.2(509-16921.7)	1396.2(567.5-16830.5)	1327.5(567.5-15082.7)	1348(447.6-15094.4)	1428.9(567.5-15616.6)
P value	0.068	0.038	0.004	0.048	0.044

Table 3.34. Plasma levels of myotrophin post AMI over the 5 days in patients who experienced MACE compared to those who did not

No significant differences in myotrophin levels were noted between males and females, in patients with a past history of AMI, hypertension or heart failure Significantly higher levels were found in those with a Killip class above 1 ($p<0.034$ over all time points, higher levels pts in Killip II, III, IV vs. Killip class I). Plasma myotrophin levels were also higher in STEMI vs. NSTEMI patients (days 3 and 4, $P<0.043$), and those with anterior site of AMI (days 1 and 3, $P<0.036$).

3.27 Myotrophin correlations

There was no correlation of myotrophin with age, troponin I, peak CK, eGFR, creatinine, or biplanar ejection fraction.

There was correlation of myotrophin with LVWMI days 1, 2 and 3 ($r_{s<}$ 0.12, $P<0.01$).

There was some weak statistically significant positive correlation between myotrophin on days 2, 3 and 4 and NTproBNP on days 2 and 3 ($r_{s=}$ 0.14, $P=0.011$).

3.28 Determinants of myotrophin secretion

Univariate general linear model analyses were conducted to identify the independent determinants of myotrophin secretion. Factors statistically significant on univariate analysis (Killip class, territory and site of infarction) were entered into the univariate general linear model.

The independent determinants of myotrophin secretion on day 3 as identified by the model were Killip class and site of infarction (P=0.002 and P=0.015, respectively).

3.29 Relationship between myotrophin and echocardiographic parameters

For the whole population mean LVWMI was 1.53 (range 1.08-2.75) and EF was 36% (range 9-68%). The LVWMI score in those subjects with anterior AMI was higher than in those with inferior AMI (1.69 [1.08-2.75] vs. 1.41 [1.00-2.60], p<0.0001). However LVEF was no different between the two groups (median [range] 35 [9-68] vs. 37 [13-65]) %, p=0.074). There was correlation of myotrophin with LVWMI (r= 0.155, p= 0.007) and NTproBNP correlated positively with LVWMI (r=0.373, p<0.0001) and negatively with the EF (r= -0.30, p<0.0001).

3.30 Myotrophin and NTproBNP as predictors of death

Binary logistic regression was performed to test for independent predictors of death, in the form of one or more of the peptide factors. Demographic and clinical factors were entered into the model as standard predictors; these included age, sex, serum creatinine, Killip class, territory of AMI, whether the patient received thrombolysis or not and peptide markers (including troponin I). Myotrophin (OR 4.96, 95% CI: 1.06-23.27, p=0.042), NTproBNP (OR 10.68, 95% CI: 1.74-65.66, p=0.011) and male

gender (OR 0.16, 95% CI: 0.04-0.66, p=0.011) independently predicted death (Table 3.35)

When clinical and demographic characteristics were entered into a Cox proportional hazards model, the independent predictors of death were myotrophin (HR 5.07, 95% CI: 1.56-16.53, p=0.007) NTproBNP (HR 7.15, 95% CI: 1.38-39.92, p=0.019) male gender (HR 0.23, 95% CI: 0.07-0.69, p=0.009), age (HR 1.07, 95% CI: 1.0-1.14, p=0.037) and serum creatinine (HR 336.0, 95% CI: 3.42-32973.2, p=0.013) (Table 3.36).

Variable	Odds Ratio	95% CI	p value
NTproBNP	10.68	1.74-65.66	0.011
Male gender	0.16	0.04-0.66	0.011
Myotrophin	4.96	1.06-23.27	0.042
age	1.07	0.99-1.15	NS
Serum creatinine	24.96	0.11-5467.2	NS
Killip class	1.52	0.31-7.37	NS
Peak CK	1.00	1.00-1.01	NS
Territory of AMI	0.43	0.08-2.20	NS
Thrombolysis	0.73	0.12-4.65	NS
Troponin I	1.01	0.98-1.04	NS

Table 3.35. Multivariate binary logistic regression model of predictors of death

Variable	Hazards Ratio	95% CI	p value
NTproBNP	7.15	1.38-39.92	0.019
Male gender	0.23	0.07-0.69	0.009
Myotrophin	5.07	1.56-16.53	0.007
Age	1.07	1.00-1.14	0.037
Serum creatinine	336.0	3.42-32973.2	0.013
Killip class	3.85	0.87-16.76	NS
Peak CK	1.00	1.00-1.00	NS
Territory of AMI	0.89	0.31-2.56	NS
Thrombolysis	0.85	0.20-3.54	NS
Troponin I	1.00	0.98-1.03	NS

Table 3.36. Multivariate Cox proportional hazards regression model of predictors of death

3.31 Myotrophin and NTproBNP as predictors of death or heart failure

Binary logistic regression was performed to test for independent predictors of death or heart failure, in the form of one or more of the peptide factors. Demographic and clinical factors were entered into the model as standard predictors; these included age, sex, serum creatinine, Killip class, territory of AMI, whether the patient received thrombolysis or not and peptide markers (including troponin I). Age (OR 1.07, 95% CI:1.02-1.12, p=0.005), thrombolytic therapy (OR 0.26, 95% CI: 0.08-0.75, p=0.018), serum creatinine (OR 84.38, 95% CI: 1.67-4291.4, p=0.027) and male gender (OR 0.21, 95% CI: 0.08-0.53, p=0.001) independently predicted death or heart failure (Table 3.37).

When clinical and demographic characteristics were entered into a Cox proportional hazards model, the independent predictors of death or heart failure were myotrophin (HR 2.35, 95% CI: 1.03-5.35, p=0.043) and male gender (HR 0.23, 95% CI: 0.11-0.51, p=0.001), age (HR 1.06, 95% CI: 1.02-1.10, p=0.004), Serum creatinine (HR 171.2, 95% CI: 8.15-3586.8, p=0.001), peak CK (HR 1.00, 95% CI: 1.00-1.00, p=0.027) and thrombolysis (HR 0.38, 95% CI: 0.15-0.99, p=0.048) (Table 3.38).

Variable	Odds Ratio	95% CI	p value
NTproBNP	0.96	0.45-2.07	NS
Male gender	0.21	0.08-0.53	0.001
Myotrophin	2.27	0.80-6.44	NS
age	1.07	1.02-1.12	0.005
Serum creatinine	84.38	1.67-4291.4	0.027
Killip class	2.11	0.75-5.88	NS
Peak CK	1.00	1.00-1.00	NS
Territory of AMI	1.03	0.45-2.38	NS
Thrombolysis	0.26	0.08-0.75	0.018
Troponin I	1.01	0.99-1.03	NS

Table 3.37. Multivariate binary logistic regression model of predictors of death or heart failure

Variable	Hazards Ratio	95% CI	p value
NTproBNP	0.86	0.45-1.67	NS
Male gender	0.23	0.11-0.51	0.001
myotrophin	2.35	1.03-5.35	0.043
Age	1.06	1.02-1.10	0.004
Serum creatinine	171.2	8.15-3586.8	0.001
Killip class	2.16	0.91-5.14	NS
Peak CK	1.00	1.00-1.00	0.027
Territory of AMI	0.92	0.46-2.84	NS
Thrombolysis	0.38	0.15-0.99	0.048
Troponin I	0.99	0.99-1.02	NS

Table 3.38. Multivariate Cox proportional hazards regression model of predictors of death or heart failure

3.32 Myotrophin and NTproBNP as predictors of death or non-fatal MI

Binary logistic regression was performed to test for independent predictors of death or non-fatal MI, in the form of one or more of the peptide factors. Demographic and clinical factors were entered into the model as standard predictors; these included age, sex, serum creatinine, Killip class, territory of AMI, whether the patient received thrombolysis or not and peptide markers (including troponin I). The only independent predictor of death or non-fatal MI was NTproBNP (OR 3.53, 95% CI: 1.56-7.98, p=0.002) (Table 3.39).

When clinical and demographic characteristics were entered into a Cox proportional hazards model, the only independent predictors of death or non-fatal MI were

NTproBNP (HR 2.74, 95% CI: 1.31-5.72, p=0.007) and Serum creatinine (HR 28.96, 95% CI: 1.38-608.9, p=0.03) (Table 3.40).

Variable	Odds Ratio	95% CI	p value
NTproBNP	3.53	1.56-7.98	0.002
Male gender	0.81	0.34-1.93	NS
Myotrophin	2.06	0.80-5.33	NS
age	1.01	0.97-1.05	NS
Serum creatinine	23.26	0.60-897.6	NS
Killip class	1.17	0.50-2.74	NS
Peak CK	1.00	1.00-1.00	NS
Territory of AMI	0.85	0.40-1.79	NS
Thrombolysis	1.58	0.67-3.82	NS
Troponin I	1.00	0.99-1.02	NS

Table 3.39. Multivariate binary logistic regression model of predictors of death or non-fatal MI

Variable	Hazards Ratio	95% CI	p value
NTproBNP	2.74	1.31-5.72	0.007
Male gender	0.74	0.35-1.56	NS
Myotrophin	1.99	0.92-4.30	NS
age	1.01	0.98-1.05	NS
Serum creatinine	28.96	1.38-608.9	NS
Killip class	1.33	0.63-2.80	NS
Peak CK	1.00	1.00-1.00	NS
Thrombolysis	1.61	0.75-3.45	NS
Troponin I	1.00	0.99-1.01	NS

Table 3.40. Multivariate Cox proportional hazards regression model of predictors of death or non-fatal MI

3.33 Myotrophin and NTproBNP as predictors of MACE (death, MI, need for urgent revascularisation)

Binary logistic regression was performed to test for independent predictors of MACE, in the form of one or more of the peptide factors. Demographic and clinical factors were entered into the model as standard predictors; these included age, sex, serum creatinine, Killip class, territory of AMI, whether the patient received thrombolysis or not and peptide markers (including troponin I). Myotrophin (OR 2.05, 95% CI: 1.01-4.20, p=0.049) was the only independent predictor of MACE (Table 3.41).

When clinical and demographic characteristics were entered into a Cox proportional hazards model, the only independent predictor of MACE was again myotrophin (HR 1.69, 95% CI: 1.00-2.89, p=0.05, Table 3.42. The Kaplan-Meier survival curve

revealed a significantly better clinical outcome in patients with myotrophin below the median compared with those with myotrophin above the median (log rank 7.63, p=0.006, figure 3.15).

Variable	Odds Ratio	95% CI	p value
NTproBNP	1.05	0.74-1.51	NS
Male gender	0.94	0.48-1.84	NS
myotrophin	2.05	1.01-4.20	0.05
Age	1.00	0.98-1.03	NS
Serum creatinine	1.10	0.07-17.62	0.03
Killip class	1.50	0.84-2.69	NS
Peak CK	1.00	1.00-1.00	NS
Territory of AMI	1.20	0.70-2.03	NS
Thrombolysis	0.63	0.34-1.15	NS
Troponin I	1.00	0.99-1.01	NS

Table 3.41. Multivariate binary logistic regression model of predictors of MACE

Variable	Hazards Ratio	95% CI	p value
NTproBNP	0.95	0.61-1.31	NS
Male gender	0.91	0.51-1.57	NS
myotrophin	1.69	1.00-2.89	0.05
Age	1.00	0.98-1.02	NS
Serum creatinine	1.54	0.16-14.51	NS
Killip class	1.45	0.89-2.37	NS
Peak CK	1.00	1.00-1.00	NS
Territory of AMI	1.11	0.73-1.70	NS
Thrombolysis	0.71	0.43-1.17	NS
Troponin I	1.00	0.99-1.01	NS

Table 3.42. Multivariate Cox proportional hazards regression model of predictors of MACE

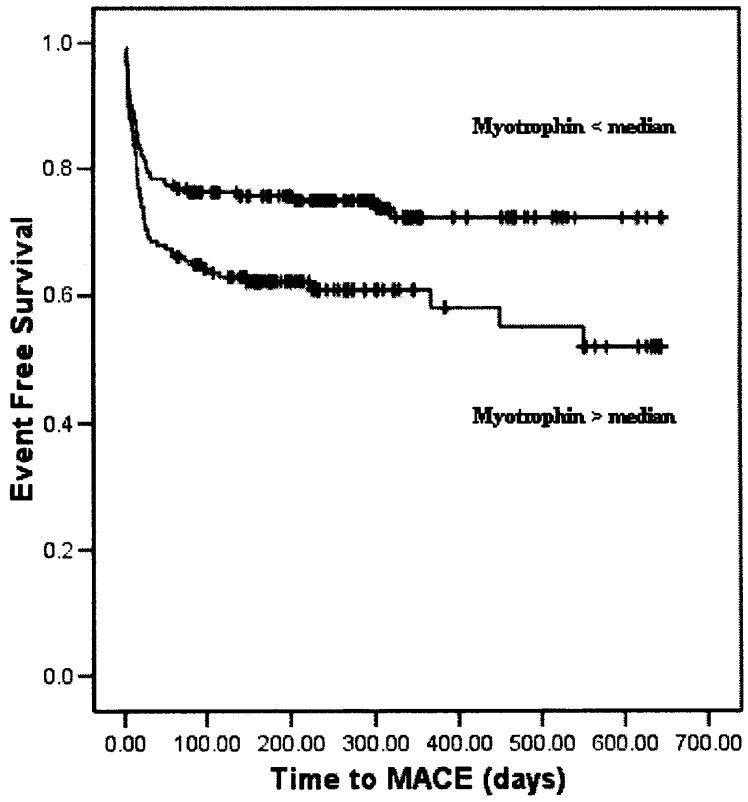

Figure 3.15. Kaplan-Meier Curve: Time to MACE related to above and below plasma myotrophin

3.34 Survival curve for myotrophin and NTproBNP (death)

A positive myotrophin and NTproBNP was associated with a significantly higher rate of the death than having one raised peptide level or two low levels of peptide (log rank 18.07, P=0.0001).

3.35 Survival curve for myotrophin and NTproBNP (death or heart failure)

A positive myotrophin and NTproBNP was associated with a significantly higher rate of the death or heart failure than having one raised peptide level or two low levels of peptide (log rank 5.23, P=0.07).

3.36 Combined Kaplan-Meier survival curve for myotrophin and NTproBNP (MACE)

There was a grading to MACE which increased as the levels of myotrophin or NTproBNP increased. A positive myotrophin and NTproBNP (i.e. both above their respective median values) was associated with a significantly higher rate of MACE than having either one peptide level above their medians, or both peptides below their medians (log rank 3.93, p =0.048, figure 3.16).

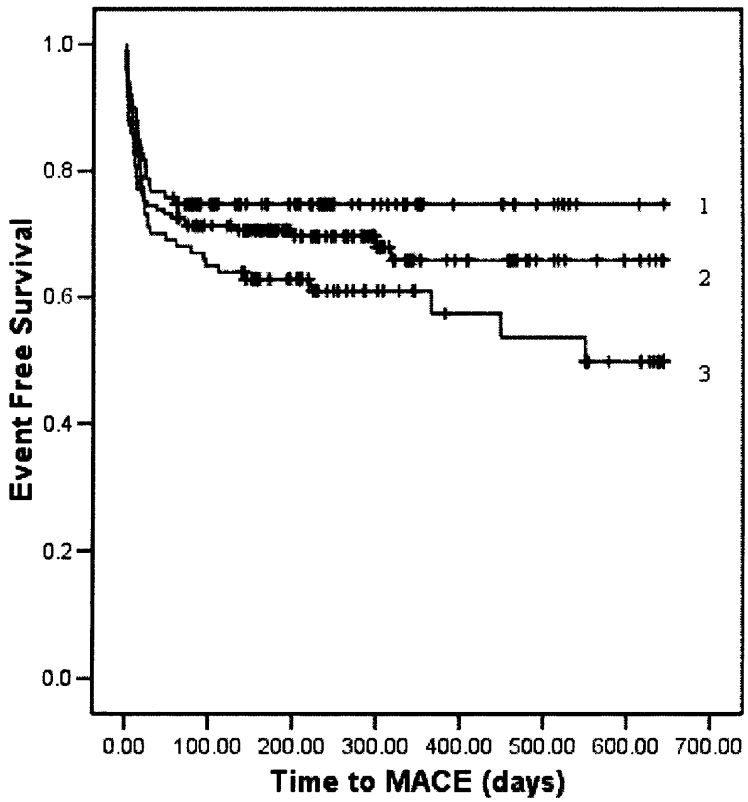

Figure 3.16. Kaplan-Meier Curve: Time to MACE related to above and below median plasma myotrophin and NTproBNP levels. 1) below median myotrophin and NTproBNP, 2) below median myotrophin or NTproBNP, 3) above median myotrophin and NTproBNP

3.37 Receiver operating characteristic curve for myotrophin and NTproBNP (MACE)

When patients were examined for one or more raised myotrophin or NTproBNP peptide levels, the receiver characteristic operating curve for NTproBNP yielded an area under the curve (AUC) of 0.56 (95% CI: 0.49-0.62, p=0.091); for myotrophin the AUC was 0.60 (95% CI: 0.54-0.67, p=0.002). The logistic model combining the 2 markers yielded an AUC of 0.62 (95% CI: 0.56-0.68, p<0.001), which exceeded that of either peptide alone (figure 3.17).

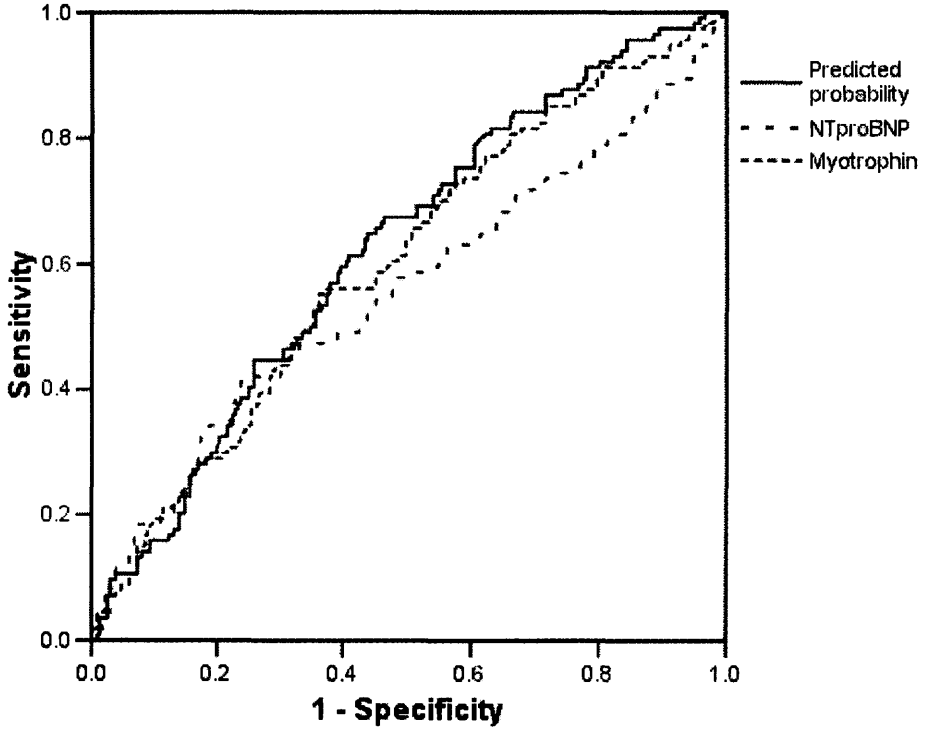

Figure 3.17. Combined receiver operating characteristic curve comparing NTproBNP, myotrophin and the combined predicted probabilities of MACE

3.38 Patient characteristics for MPO

The demographic features of the patient population are shown in Table 3.43. 384 of the 473 STEMI patients had MPO levels taken. There were 257 controls (132 male), age 61.8 ± 14.3. Median length of follow-up was 330 days with a range of 0–644 days (0 was due to death). Of the patients enrolled, 70.8 % received thrombolysis during the index admission. No patient was lost to follow-up. During follow-up, 40 (10.4%) patients died, 37 (9.6%) were readmitted with AMI, there were 23 (6.0%) readmissions with heart failure and 89 (23.2%) MACE. Echocardiographic data was available for 334 (87.0%) of the 384 patients and performed at a median of 3.5 days (range 2-5) after presentation with AMI. 39 echocardiograms were unanalysable (due to off axis apical views, and/or poor image quality) and 11 patients did not receive an echocardiogram.

	AMI Patients	Controls
Number	384	257
Age (in years)	64.0 ± 12.3	61.8 ± 14.3
Male Sex	283	
Previous Medical History		
Myocardial infarction	46 (12.0)	None
Angina Pectoris	52 (13.5)	None
Hypertension	174 (44.3)	None
Diabetes mellitus	79 (20.6)	None
Hypercholesterolaemia	108 (28.1)	None
Obesity	59 (15.4)	None

Current/Ex-Smokers	141 (36.7)	None
ST-elevation AMI	384 (100)	None
Thrombolytic	272 (70.8)	None
Territory of Infarct		
Anterior	144 (37.5)	N/A
Inferior	184 (47.9)	N/A
Other/undetermined	56 (14.6)	N/A
Killip Class on Admission		
I	212 (55.2)	N/A
II	149 (38.8)	N/A
III	20 (5.2)	N/A
IV	3 (0.8)	N/A
Peak CK (I/U)	1436.0 ± 1385.1	N/A
Peak Troponin I (ng/ml)	26.4 ± 35.1	N/A
Creatinine (μmol/l)	98.3 ± 23.8	N/A

Table 3.43. Characteristics of patients in the study. Values are means (SD) or numbers (percentage)

3.39 Time course of MPO secretion after AMI

The data is presented as median (range) in ng/ml. The table shows the plasma MPO levels at the different 24-hour time points after AMI.

Time (Hours)	0-24	25-48	49-72	73-96	97-120
Number of patients	467	375	366	377	231
Median (ng/ml)	60.3	39.6	28.6	29.4	26.9
Range (ng/ml)	6.0-35440.6	5.6-26587.2	4.9-22362.1	3.6-18992.0	6.3-22205.0

Table 3.44. Plasma MPO levels at the different 24 hour time points after AMI

Using the repeated measures general linear model procedure, there was a statistically significant change in MPO secretion over the 5 days. Figure 3.18 illustrates the time course of plasma MPO showing significant changes with day of sampling ($P<0.0001$).

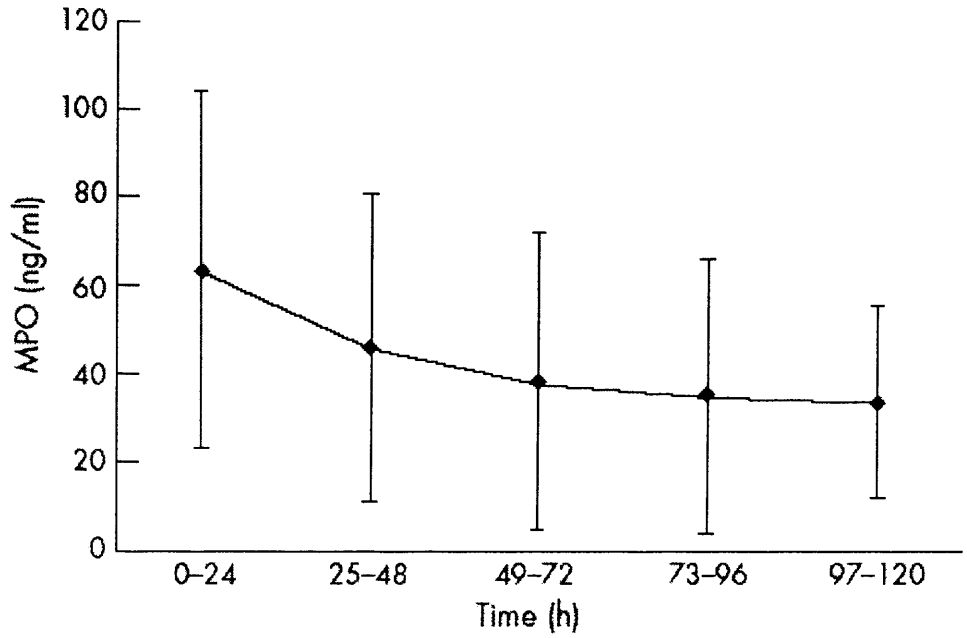

Figure 3.18. Plasma profile of plasma MPO in patients following AMI

The plasma concentration of MPO was significantly higher in AMI patients compared to age and sex matched controls over days 1 and 2 (P<0.001).

3.40 MPO levels (univariate analysis)

There were no differences in the levels of MPO in patients who died, were readmitted with heart failure or experienced MACE (Table 3.45-3.50). Plasma median MPO was raised in patients who died, who died or were re-admitted with heart failure, died or reinfarcted and those who experienced MACE. The box plot shows the statistically significant difference in median MPO levels in controls, patients who survived and those that died (Figure 3.19). There were no significant differences in MPO levels in patients who, re- infarcted or were admitted with heart failure or experienced MACE as individual endpoints (Table 3.51-3.56).

Plasma median MPO in STEMI (n=384) was raised in patients experiencing death or MI compared to survivors without recurrent MI (median [range] ng/ml, 50.6[15.3-124.1] vs. 34.5[6.6-400.2], p=0.001).

MPO did not differ significantly according to gender, smoking status, the presence or absence of diabetes mellitus, hypertension, previous MI diagnosis, hypercholesterolemia, previous statin treatment or whether a patient received thrombolyis or not. There was no correlation however between NTproBNP and MPO (r=0.068, p=0.401).

MPO(ng/ml)	0-24	25-48	49-72	73-96	97-120
Death (median[range])	60.1(13.7-157.3)	45.9(11.3-112.2)	33.9(8.8-98.4)	28.4(8.1-70.2)	40.1(7.6-107.2)
Survivors (median[range])	62.1(7.1-403.2)	35.1(5.6-397.2)	27.0(4.9-370.7)	27.8(4.0-405.2)	26.8(6.3-102.7)
P value	NS	NS	NS	NS	NS

Table 3.45. Plasma levels of MPO post AMI over the 5 days in patients who subsequently died compared to survivors

MPO(ng/ml)	0-24	25-48	49-72	73-96	97-120
HF admission (median[range])	66.4(7.1-120)	59.1(9.8-150.0)	27.7(6.5-74.1)	29.8(9.6-84.5)	35.4(22.1-61.6)
No HF admission (median[range])	61.2(7.3-403.2)	45.1(5.6-397.2)	27.4(4.9-370.7)	27.8(4.0-405.2)	26.9(6.3-107.2)
P value	NS	NS	NS	NS	NS

Table 3.46. Plasma levels of MPO post AMI over the 5 days in patients who were subsequently readmitted with heart failure compared to those who were not

MPO(ng/ml)	0-24	25-48	49-72	73-96	97-120
Death or HF (median[range])	60.6(7.1-157.3)	48.8(9.8-150.0)	32.5(6.5-98.4)	29.7(8.1-84.5)	40.1(7.6-107.2)
No death or HF (median[range])	61.9(7.3-403.2)	35.0(5.6-397.2)	27.0(4.9-370.7)	27.3(4.0-405.2)	26.3(6.3-102.7)
P value	NS	NS	NS	NS	NS

Table 3.47. Plasma levels of MPO post AMI over the 5 days in patients who subsequently died or were readmitted with heart failure

MPO(ng/ml)	0-24	25-48	49-72	73-96	97-120
AMI admission (median[range])	71.5(20.9-127.8)	40.0(16.8-124.1)	24.4(7.4-121.9)	28.0(5.3-87.6)	28.3(7.6-77.6)
No AMI admission (median[range])	60.2(7.1-403.2)	35.0(5.6-397.2)	27.6(4.9-370.7)	28.0(4.0-405.2)	26.3(6.3-107.2)
P value	NS	NS	NS	NS	NS

Table 3.48. Plasma levels of MPO post AMI over the 5 days in patients who subsequently reinfarcted compared to those who did not

MPO(ng/ml)	0-24	25-48	49-72	73-96	97-120
Death or AMI (median[range])	66.6(13.7-157.3)	43.6(11.3-124.1)	28.9(7.4-121.9)	28.8(5.3-87.6)	37.4(7.6-107.2)
No Death or AMI (median[range])	60.2(7.1-403.2)	34.1(5.6-397.2)	27.4(4.9-370.7)	27.6(4.0-405.2)	26.4(6.3-102.7)
P value	NS	NS	NS	NS	NS

Table 3.49. Plasma levels of MPO post AMI over the 5 days in patients who died or reinfarcted compared to those who did not

MPO(ng/ml)	0-24	25-48	49-72	73-96	97-120
MACE (median[range])	66.6(7.1-157.3)	46.4(9.8-150.0)	30.2(6.5-121.9)	29.7(5.3-87.6)	36.4(7.6-107.2)
No MACE (median[range])	60.2(7.3-403.2)	34.1(5.6-397.2)	27.2(4.9-370.7)	27.3(4.0-405.2)	26.2(6.3-102.7)
P value	NS	NS	NS	NS	NS

Table 3.50. Plasma levels of MPO post AMI over the 5 days in patients who experienced MACE compared to those who did not

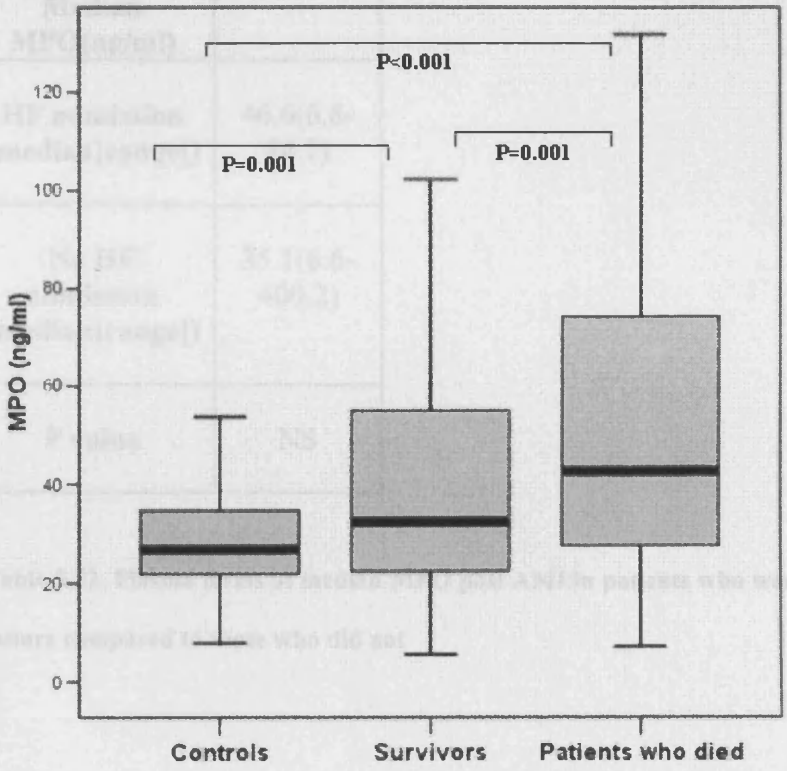

Figure 3.19 Box plot of median MPO plasma levels and relationship between controls, survivors and patients who died

Median MPO(ng/ml)	
Death (median[range])	43.4(5.3-132.1)
No Death (median[range])	34.4(6.6-400.2)
P value	0.001

Table 3.51. Plasma levels of median MPO post AMI in patients who died compared to those who did not

Median MPO(ng/ml)	
HF admission (median[range])	46.6(6.8-84.7)
No HF admission (median[range])	35.1(6.6-400.2)
P value	NS

Table 3.52. Plasma levels of median MPO post AMI in patients who were readmitted with heart failure compared to those who did not

Median MPO(ng/ml)	
Death or HF (median[range])	45.1(6.8-132.1)
No Death or HF (median[range])	34.0(6.6-400.2)
P value	0.005

Table 3.53. Plasma levels of median MPO post AMI in patients who died or were readmitted with heart failure compared to those who did not

Median MPO(ng/ml)	
AMI admission (median[range])	44.7(5.3-105.6)
No AMI admission (median[range])	34.5(6.6-400.2)
P value	NS

Table 3.54. Plasma levels of median MPO post AMI in patients who reinfarcted compared to those who did not

Median MPO(ng/ml)	
Death or AMI (median[range])	50.6(15.3-124.1)
No Death or AMI (median[range])	34.5(6.6-400.2)
P value	0.001

Table 3.55. Plasma levels of median MPO post AMI in patients who died or reinfarcted compared to those who did not

Median MPO(ng/ml)	
MACE (median[range])	44.4(6.6-124.1)
No MACE (median[range])	33.3(6.6-400.2)
P value	0.001

Table 3.56. Plasma levels of median MPO post AMI in patients who experienced MACE compared to those who did not

3.41 MPO correlations

There was no correlation of MPO with age, troponin I, peak CK, eGFR, creatinine, presentation neutrophil count, biplanar ejection fraction or LVWMI.

There was a statistically significant correlation of MPO with NTproBNP days 2, 3, 4 and 5 ($r_{s<}$ 0.176, P<0.001).

3.42 Determinants of MPO secretion

Univariate general linear model analyses were not conducted due to the inadequate number of statistically significant factors.

3.43 Relationship between MPO and echocardiographic parameters

For the whole population, mean LVWMI was 1.53 (range 1.08-2.83) and EF was 36% (range 8-49%). The LVWMI score in those subjects with anterior AMI was higher than in those with inferior AMI (1.8 [1.08-2.75] vs. 1.4 [1.00-2.83], p<0.001) and LVEF was lower in anterior AMI than inferior AMI (37 [8-48] vs. 40.1 [14-49])

%, p=0.05). There was no correlation of MPO with LVWMI (r= 0.104, p> 0.147). However NTproBNP correlated with LVWMI (r=0.434, p<0.0001) at all time points.

3.44 MPO and NTproBNP as predictors of death

Binary logistic regression was performed to test for independent predictors of death in the form of one or more of the peptide factors. Demographic and clinical factors were entered into the model as standard predictors; these included age, sex, serum creatinine, Killip class, territory of AMI, whether the patient received thrombolysis or not and peptide markers (including troponin I). The only independent predictors of death were MPO (OR 14.72, 95% CI: 1.30-166.26, p=0.03) and age (OR 1.10, 95% CI: 1.01-1.21, p=0.032) (Table 3.57).

When clinical and demographic characteristics were entered into a Cox proportional hazards model, the independent predictors of death were MPO (HR 13.05, 95% CI: 1.68-101.29, p=0.014) and age (HR 1.11, 95% CI: 1.02-1.21, p=0.015) (Table 3.58).

Variable	Odds Ratio	95% CI	p value
NTproBNP	5.87	0.94-36.66	NS
Male gender	0.30	0.06-1.33	NS
MPO	14.72	1.30-166.26	0.03
Age	1.10	1.01-1.21	0.032
Serum creatinine	48.50	0.50-49068	NS
Killip class	1.00	0.25-3.99	NS
Peak CK	0.49	0.10-2.53	NS
Territory of AMI	0.61	0.15-2.45	NS
Thrombolysis	1.00	0.23-4.35	NS
Troponin I	2.13	0.55-8.25	NS

Table 3.57. Multivariate binary logistic regression model of predictors of death

Variable	Hazards Ratio	95% CI	p value
NTproBNP	4.88	0.93-25.69	NS
Male gender	0.33	0.09-1.24	NS
MPO	13.05	1.68-101.29	0.014
Age	1.11	1.02-1.21	0.015
Serum creatinine	67.20	0.23-19526	NS
Killip class	1.31	0.45-3.86	NS
Peak CK	1.20	0.32-4.52	NS
Territory of AMI	0.59	0.19-1.87	NS
Thrombolysis	1.48	0.47-4.62	NS
Troponin I	1.27	0.43-3.79	NS

Table 3.58. Multivariate Cox proportional hazards regression model of predictors of death

3.45 MPO and NTproBNP as predictors of death or heart failure

Binary logistic regression was performed to test for independent predictors of death or heart failure, in the form of one or more of the peptide factors. Demographic and clinical factors were entered into the model as standard predictors; these included age, sex, serum creatinine, Killip class, territory of AMI, whether the patient received thrombolysis or not and peptide markers (including troponin I). The only independent predictors of death or heart failure were age (OR 1.06, 95% CI: 1.00-1.13, p=0.035) and gender (OR 0.23, 95% CI: 0.08-0.70, p=0.01) (Table 3.59).

When clinical and demographic characteristics were entered into a Cox proportional hazards model, the only independent predictors of death or heart failure were age (HR 1.07, 95% CI: 1.01-1.12, p=0.023) and gender (HR 0.31, 95% CI: 0.11-0.82, p=0.019) (Table 3.60).

Variable	Odds Ratio	95% CI	p value
NTproBNP	2.74	0.89-8.40	NS
Male gender	0.23	0.08-0.70	0.01
MPO	4.09	0.64-26.18	NS
Age	1.06	1.00-1.13	0.035
Serum creatinine	6.46	0.03-1465.83	NS
Killip class	1.66	0.58-4.75	NS
Peak CK	0.52	0.15-1.83	NS
Territory of AMI	0.91	0.33-2.36	NS
Thrombolysis	0.61	0.20-1.82	NS
Troponin I	2.09	0.83-5.30	NS

Table 3.59. Multivariate binary logistic regression model of predictors of death or heart failure

Variable	Hazards Ratio	95% CI	p value
NTproBNP	2.21	0.79-6.20	NS
Male gender	0.31	0.11-0.82	0.019
MPO	5.02	0.99-24.40	NS
Age	1.07	1.01-1.12	0.023
Serum creatinine	8.16	0.10-683.48	NS
Killip class	1.68	0.72-3.94	NS
Peak CK	1.05	0.37-3.03	NS
Territory of AMI	0.82	0.36-1.89	NS
Thrombolysis	0.94	0.39-2.29	NS
Troponin I	1.38	0.63-3.01	NS

Table 3.60. Multivariate Cox proportional hazards regression model of predictors of death or heart failure

3.46 MPO and NTproBNP as predictors of death or non-fatal MI

Binary logistic regression was performed to test for independent predictors of death or non-fatal MI in the form of one or more of the peptide factors. Demographic and clinical factors were entered into the model as standard predictors; these included age, sex, serum creatinine, Killip class, territory of AMI, whether the patient received thrombolysis or not and peptide markers (including troponin I). The only independent predictor of death or non-fatal MI was MPO (OR 5.04, 95% CI: 1.14-22.25, p=0.033) (Table 3.61).

When clinical and demographic characteristics were entered into a Cox proportional hazards model, the only independent predictor of death or AMI were log median

MPO (HR 5.07, 95% CI: 1.38-18.66, p=0.015) and age (HR 1.04, 95% CI: 1.00-1.08, p=0.045) (Table 3.62).

Variable	Odds Ratio	95% CI	p value
NTproBNP	1.93	0.89-4.19	NS
Male gender	0.72	0.29-1.77	NS
MPO	5.04	1.14-22.25	0.033
Age	1.03	0.99-1.08	NS
Serum creatinine	5.22	0.07-401.5	NS
Killip class	1.27	0.57-2.83	NS
Peak CK	0.76	0.27-2.11	NS
Territory of AMI	0.63	0.28-1.45	NS
Thrombolysis	2.13	0.90-5.09	NS
Troponin I	1.29	0.69-2.42	NS

Table 3.61. Multivariate binary logistic regression model of predictors of death or non-fatal MI

Variable	Hazards Ratio	95% CI	p value
NTproBNP	1.58	0.79-3.15	NS
Male gender	0.68	0.32-1.58	NS
MPO	5.07	1.38-18.66	0.015
Age	1.04	1.00-1.08	0.045
Serum creatinine	3.47	0.10-125.4	NS
Killip class	1.32	0.68-2.37	NS
Peak CK	1.26	0.50-3.16	NS
Territory of AMI	0.62	0.30-1.27	NS
Thrombolysis	2.04	0.97-4.27	NS
Troponin I	1.07	0.62-1.05	NS

Table 3.62. Multivariate Cox proportional hazards regression model of predictors of death or non-fatal MI

3.47 MPO and NTproBNP as predictors of MACE (death, MI, need for urgent revascularisation)

Binary logistic regression and Cox proportional hazards model was performed to test for independent predictors of MACE in the form of one or more of the peptide factors. Demographic and clinical factors were entered into the model as standard predictors; these included age, sex, serum creatinine, Killip class, territory of AMI, whether the patient received thrombolysis or not and peptide markers (including troponin I). No independent predictors of MACE were found.

3.48 Survival curve for MPO and NTproBNP (death)

The Kaplan-Meier survival curve revealed a significantly better clinical outcome in patients with median NTproBNP below the median compared with those with median NTproBNP above the median (log rank 11.47, p=0.0007, graph not shown). A positive MPO and NTproBNP was associated with a significantly higher rate of the death than having one raised peptide level or two low levels of peptide (log rank 22.63, p <0.00001).

3.49 Kaplan-Meier survival curve for MPO and NTproBNP (death or non-fatal MI)

The Kaplan-Meier survival curve revealed a significantly better clinical outcome in patients with median MPO below the median compared with those with median MPO above the median (log rank 12.62, p=0.0004, figure 3.20); this was also true for NTproBNP (log rank 20.24, p<0.0001, figure 3.21).

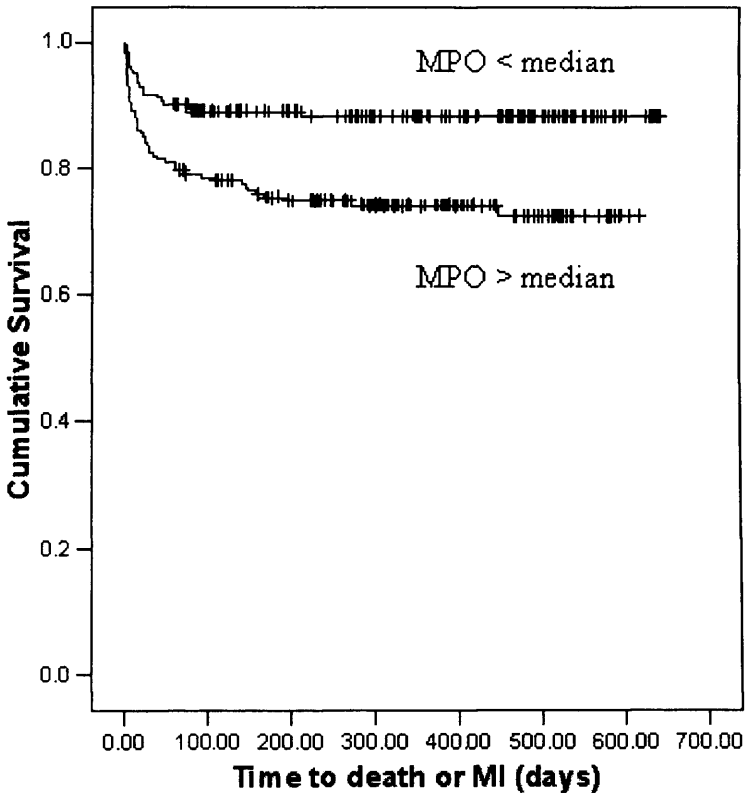

Figure 3.20. Kaplan-Meier Curve: Time to death or non-fatal MI related to median serum MPO

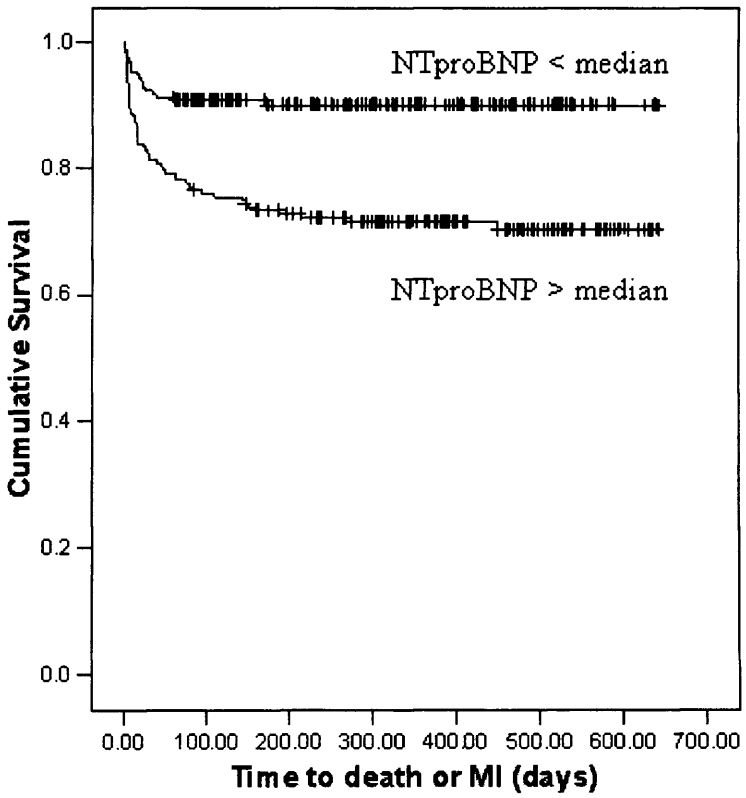

Figure 3.21. Kaplan-Meier Curve: Time to death or non-fatal MI related to median serum NTproBNP

3.50 Combined Kaplan-Meier survival curve for MPO and NTproBNP (death or non-fatal MI)

MPO had predictive power in patients with NTproBNP levels below or above the median (log rank 5.60, p=0.020, log rank 5.12, p=0.024 respectively). In addition there was a grading to death or non-fatal MI, which increased as the levels of MPO or NTproBNP increased. A positive MPO and NTproBNP (i.e. both above their respective median values) was associated with a significantly higher rate of death or non-fatal MI than having either peptide level above their medians, or both peptides below their medians (log rank 30.73, p <0.00001, figure 3.22).

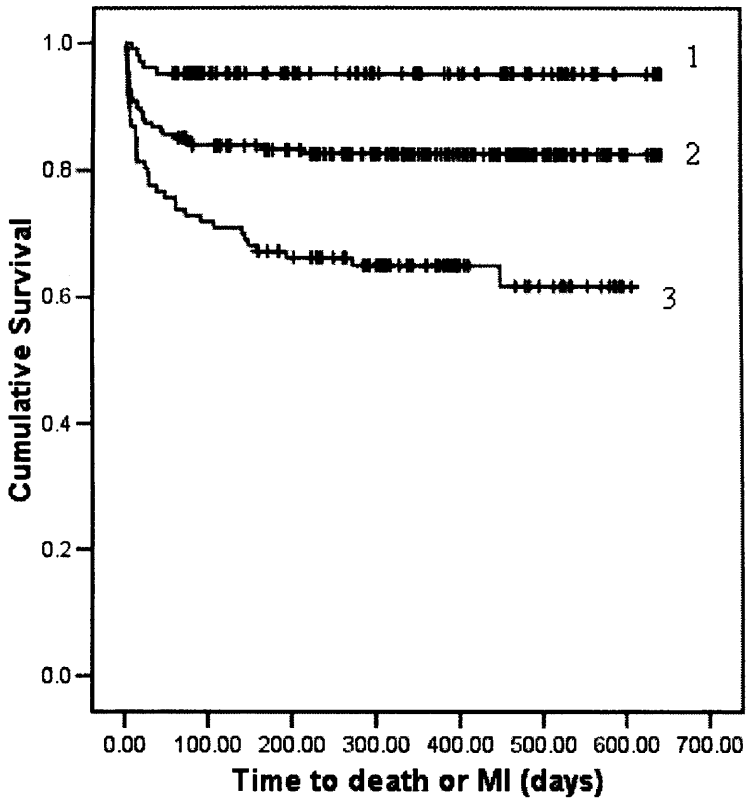

Figure 3.22. Kaplan-Meier Curve: Time to death or non-fatal MI related to low or high median serum MPO and NTproBNP levels 1) Low MPO and NTproBNP, 2) Low MPO or NTproBNP 3) High MPO and NTproBNP

3.51 Receiver operating characteristic curve for MPO and NTproBNP (death or non-fatal MI)

When patients were examined for one or more raised MPO or NTproBNP peptide levels the receiver operating characteristic curve for median NTproBNP yielded an area under the curve (AUC) of 0.72 (95% CI: 0.65-0.79, $p<0.001$); for median MPO the AUC was 0.62 (95% CI: 0.55-0.69, $p=0.001$). The logistic model combining the 2 markers yielded an AUC of 0.76 (95% CI: 0.69-0.82, $p<0.001$), which exceeded that of either peptide alone (figure 3.23). Discharge MPO and NTproBNP was better at predicting death or non-fatal MI than admission measurements (discharge AUC for

MPO 0.62, p=0.05, for NTproBNP 0.66, p=0.013 vs. admission AUC for MPO 0.56, p>0.05, for NTproBNP 0.69, p<0.001).

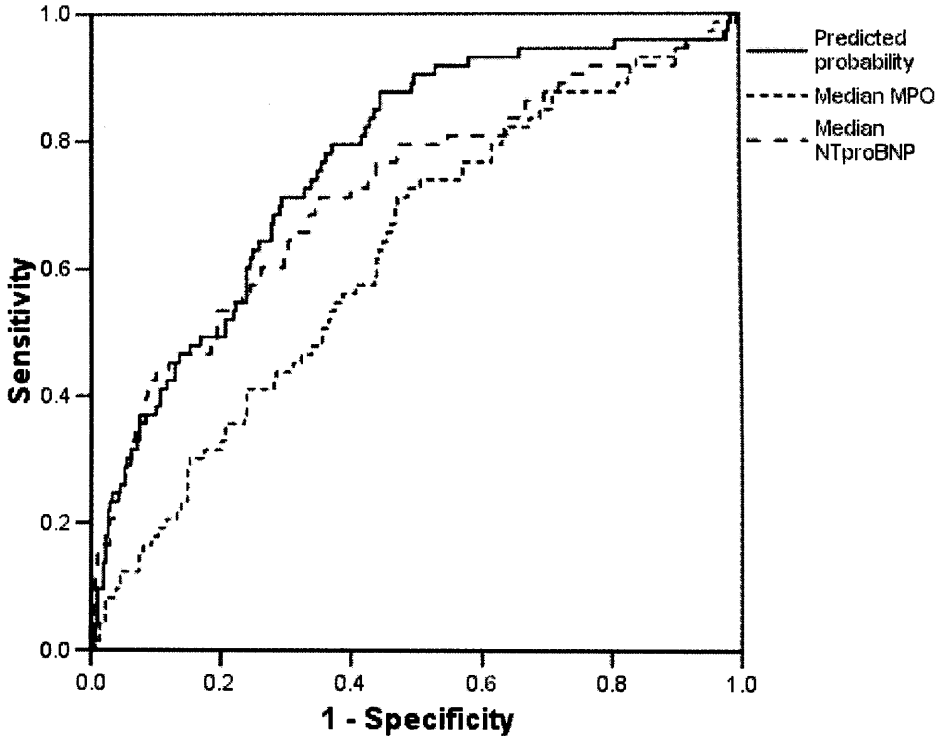

Figure 3.23. Combined receiver operating characteristic curve comparing NTproBNP, MPO and the combined predicted probabilities of death or non-fatal MI

Chapter 4:

Discussion

Reperfusion therapy has reduced mortality post MI, however the outcome of patients despite this is still poor (142); for this reason risk stratification at an early stage after AMI remains important and may be useful in helping to select treatment regimes in the future. Previous risk stratification involved assessment for residual ischaemia and evaluation of left ventricular dysfunction. Risk stratification has moved on from this and is now in a stage where assessment is being undertaken to determine risk of future adverse events including further myocardial infarction, heart failure and death. Early risk stratification is necessary as it can identify groups of patients who are suitable for certain therapies. (143) This was borne out by the FRISC study group who were able to identify patients with unstable coronary artery disease who would benefit from treatment with antithrombotic therapy on the basis of troponin T.
A multimarker strategy for outcome post-AMI using independent biomarkers has benefits in that it integrates the different pathways involved in the hope that complementary information can be gained (144).

The aim of this thesis was to:
Investigate the prognostic utility of NTproBNP as a marker for risk stratification of AMI patients over and above clinical variables.
Establish the secretory patterns of CT-1, myotrophin and MPO in patients with AMI by frequent blood sampling within the first five days following the ischaemic event.
Compare the secretory patterns of CT-1, myotrophin and MPO to that of NTproBNP, which is known to peak within the first 24 hours and then fall. Investigate the prognostic utility of CT-1, myotrophin and MPO as markers for risk stratification of AMI patients especially in combination with NTproBNP.

4.1 TIMI scoring vs. NTproBNP

Although both the TIMI score and NTproBNP have been validated as good risk stratifying tools post AMI this is the first study to compare the two head to head.

The aim of this study was to compare the TIMI risk score for STEMI and compare it to NTproBNP in determining the prognosis of AMI patients. Our results confirm previous findings with regards to NTproBNP secretion patterns. We have shown a biphasic response to the secretion of this peptide. (145) This is similar to the secretion pattern of BNP (67)

We have also shown higher levels of NTproBNP in univariate analysis in patients who died or were re-admitted with heart failure and in those patients who were readmitted with a further non-fatal AMI. The findings of raised levels of NTproBNP and BNP in patients who later die or are re-admitted with heart failure is a consistent finding in several studies in univariate and multivariate analysis. (68,69,70)

Some studies have also found NTproBNP and BNP to be raised in those at increased risk of new ischaemic events. The mechanisms underlying the association of raised plasma natriuretic peptides in this setting are not fully known. (146)

There was strong positive correlation with NTproBNP levels and LVWMI and strong negative correlation with NTproBNP levels and ejection fraction re-iterating the fact that that the likely source of secretion is the myocardium. The correlation of NTproBNP levels and ejection fraction was weaker in our study than that of Richards and co-workers (146) but we must remember that the assessment of ejection fraction was done by echocardiography in our study and LV function was assessed by radionuclide ventriculography in Richards's study. The timing of echocardiography was also different. We performed echocardiography between days 2 and 5 whereas

Richards performed radionuclide ventriculography within 24 hours of blood sampling. We have also shown a significant increase in the NTproBNP levels as Killip grade increases; this can be thought of as a grading of LV systolic dysfunction following an AMI (68,70). In addition in patients with Killip class I, NTproBNP was able to give discriminatory information with regard to mortality and was significantly higher in those patients who died in this group compared to survivors. This result is in keeping with a report from Omland (71) which showed that NTproBNP had prognostic capacity in patients with Killip class I also.

NTproBNP was positively correlated with age and negatively with eGFR. We know that levels of NTproBNP increase with age. (54) The levels of NTproBNP also increase with declining renal function. We know that NTproBNP and creatinine clearance provide complementary information with respect to mortality (74) and the combination of NTproBNP and creatinine clearance provides the best combination of markers to predict 1-year mortality as borne out in a GUSTO-IV sub-study.

Our results also confirm the previous findings that TIMI risk score is of prognostic value in patients with STEMI. STEMI have historically been regarded as a high-risk AMI due to full occlusion of the coronary artery. Risk scoring to try and predict death in this group of patients is therefore thought to be very useful.

We were interested to see how NTproBNP (also a good marker of death after an AMI) would compare to the TIMI risk score which has been well validated.

Our results show that in multivariate testing NTproBNP is superior to TIMI risk scoring and is of independent prognostic value in determining death in patients who have an acute STEMI. The predictive value of NTproBNP provides risk prediction independent of the TIMI score, which includes known clinical predictors of death.

Kaplan-Meier analysis revealed that both raised NTproBNP and higher TIMI scores were predictive of poor outcome. However from ROC curve analysis the AUC for NTproBNP was greater than that for TIMI risk score showing that NTproBNP is more accurate than TIMI score at predicting death. This was also borne out in multivariate binary and Cox regression analyses with NTproBNP, but not TIMI score, independently predicting mortality. Post-AMI drug therapies with beta-blockers and ACE inhibitors or ARB were also significant with odds ratios less than 1 suggesting clinical benefit.

In Morrow et al's original paper (34) the c statistic obtained for the prognostic value of the TIMI risk score was 0.779. In our cohort of patients the c statistic for the TIMI risk score (equivalent to the receiver-operating characteristic curve AUC) is 0.67. The reasons for the difference are probably accounted for by the different population groups. In our cohort of STEMI patients only 68.5% of the patients received thrombolytic therapy. The TIMI risk score was derived from a population of patients who were all given thrombolytic therapy (lanoteplase or alteplase). When the TIMI risk score was used previously in a real world sample of patients the c statistic was 0.65 (33) which is similar to what we have found. We would argue that our patient population is more in keeping with the real life situation where not all patients are eligible for thrombolytic therapy and indeed these patients may in fact be at higher risk. (147) The utility of the TIMI risk score and NTproBNP have been investigated individually at predicting death in numerous studies (36,70,148,149) but the 2 have never been compared directly.

The Kaplan-Meier curves also show a clear delineation in terms of mortality with regard to above and below median values of NTproBNP in keeping with other studies. This is also the case when the TIMI variables are investigated as low,

intermediate and high-risk groups. The combination of NTproBNP with TIMI score did not significantly improve risk prediction for mortality; this tells us that NTproBNP is probably offering equivalent information rather than complementary information to the TIMI score.

The variables included in the TIMI score for STEMI include predictors such as age, Killip class, heart rate, location of infarction, and weight. These are all important variables which we have shown to be directly related to NTproBNP or have been shown to be related in other studies. (56) Moreover, we have shown that there is no difference in risk prediction whether NTproBNP is measured early or late (OR for NTproBNP 72-96 hours, 6.25, 95% CI: 1.92-20.34, p=0.002) after an acute STEMI. There is now a bedside point of care assay for NTproBNP so results of such tests when taken should be readily available. This makes a simple NTproBNP blood test more easily applicable and as we have shown, the NTproBNP level has more predictive accuracy than a clinical risk score in a cohort of unselected STEMI patients.

The specific aim of this part of the study was to establish the fact that biochemical markers can provide superior information to prognostication than clinical markers. Although we have shown NTproBNP to be superior to TIMI scoring it would be interesting to see if these findings can be extrapolated to other clinical risk models such as the GRACE scoring and PURSUIT scoring both of which are validated scoring systems in their own right. (150,151)

There is some evidence to suggest that when the 3 scoring systems are compared the GRACE risk score is superior to TMII and PURSUIT in giving prognostic information. (40)

It must be remembered however that these head to head comparisons of the different scoring systems have not looked at TIMI scoring for STEMI, which includes different variables to those for TIMI score for NSTEMI.

The numbers of NSTEMI patients in our study were too small to be able to answer the question of whether NTproBNP is superior to TIMI scoring for NSTEMI; again it would be interesting to see if this is the case.

In conclusion, the present large single centre study reveals that in the first 24 hours following an acute ST-segment AMI, NTproBNP is superior to TIMI risk scoring for STEMI at predicting mortality. It gives complementary rather that additive information with regard to mortality. NTproBNP may integrate known prognostic factors such as age, heart rate, Killip class, location of infarction into one easily measured parameter. A simple blood test may be more easily applicable than a clinical risk score.

4.2 CT-1

Our study has compared levels of CT-1 in patients with an AMI with controls and we have shown increased levels of this peptide in patients following an AMI. Interestingly the peptide levels of CT-1 were found to be higher in patients who died or were re-admitted to hospital with heart failure.

Both CT-1 and NTproBNP are raised after an AMI and their secretion patterns differ over the 5 days following an AMI with significant differences noted for both peptides. CT-1 is raised early after an AMI with levels falling rapidly after the first 24 hours then rising again. This suggests that there may be a stored pool of CT-1, which is released after an AMI, with the second wave of release due to new synthesis of the peptide. The likely source of CT-1 is unknown but a possible source is the left ventricle. Northern blot and immunohistochemistry techniques have shown CT-1 to be expressed in a canine model of pacing induced experimental heart failure (152). We have shown that CT-1 levels are higher in STEMI patients compared with NSTEMI patients and that levels are higher in anterior AMI patients compared with other territory infarcts. This points towards the myocardium as a potential source of this peptide. We have also shown a weak but positive correlation between CT-1 and LVWMI and between CT-1 and NTproBNP. Other possible sources could well be the atria; CT-1 has been shown to be raised in the atrial tissue of WKY rats. (99) The release kinetics in our patient population is slightly different to those of Talwar et al (76) and this may be because of the greater number of patients that we recruited. (378 vs. 60) and the timing of the samples. but also, the assay which we utilised was different as it was a 1 site competitive assay for CT-1 hence more non-specific.

We found raised plasma concentrations of CT-1 in patients following AMI in comparison with control subjects. Our data is similar to the previous studies in which

CT-1 has been investigated showing raised levels after an AMI (76) and we know that there are raised levels of other cytokines such as IL-6 following unstable angina. (153,154)

The mechanism of production of CT-1 is not entirely clear but may in part be related to wall stress and stretch. This mechanism is known to activate the JAK/STAT pathway which may stimulate mRNA expression of CT-1 (155). There is also a known interplay between BNP and CT-1 which may explain the increase in this peptide following an AMI (156). CT-1 has also been shown to be raised in chronic heart failure and this may also be as a result of the activation of the JAK/STAT pathway (157)

The reason for increase in CT-1 may be cardioprotective we know that CT-1 plays a role in upregulation of protective heat shock proteins (91) and have anti-apoptotic effects. (158) This increased level of CT-1 particularly after the early phase of an AMI may have a role in cardiomyocyte protection. The ability of CT-1 also to cause the addition of sarcomeres in series may also augment cardiac function and cause a decrease in the local wall stress. (84,85)

CT-1 was also found to be more raised in patients who were readmitted with heart failure than in those who were not. CT-1 levels have previously been found to be raised in chronic heart failure (97) compared to controls and to correlate with LVWMI. CT-1 mRNA has also been found to be increased in patients with heart failure due to ischaemic cardiomyopathy or idiopathic dilated cardiomyopathy (159). This suggests that CT-1 may be contributing to the eccentric hypertrophy and also to remodeling.

The aim of this study was to assess the utility of CT-1 and NTproBNP in determining the prognosis of AMI patients. The results of this study confirm the independent

prognostic values of early CT-1 and NTproBNP levels in determining death or heart failure in patients who have an AMI. This has been borne out in multiple logistic regression modelling and the more superior Cox regression analysis. The information has been gained with a single blood test taken between 25-48 hours. Unlike NTproBNP there was no correlation of CT-1 with age or sex, which may make it a more discerning marker. The predictive value of CT-1 provides risk prediction independent of NTproBNP and other known clinical predictors of death or heart failure including Killip class and ejection fraction. The Kaplan-Meier analysis showed that levels of CT-1 above the median were associated with a worse outcome than levels of CT-1 below the median. This was also true for NTproBNP with regards to death and heart failure.

We have clearly shown the benefit of using each peptide alone at predicting death or heart failure; indeed NTproBNP is a well-established marker for predicting LV dysfunction and prognosis after an acute myocardial infarction. Both markers give good area under the ROC curves; however using a combination of CT-1 and NTproBNP in a multi-marker risk stratification approach in patients gives an increased area under the ROC curve and more predictive accuracy.

The relationships for prediction exists in quite a heterogeneous group of patients (NSTEMI, STEMI, previous history of cardiovascular diseases and age etc) it would be interesting to see if a separation could be made on the basis of presenting diagnosis. What also remains to be seen is the utility of CT-1 at being able to individually predict death or heart failure; a larger powered study would be necessary to answer such a question.

In conclusion, this is the first report of CT-1 as a prognostic marker of death or heart failure in patients with AMI. This study confirms previous findings that CT-1 is

involved during an AMI and it may be useful in a multimarker approach with NTproBNP for risk stratification in AMI patients.

4.3 Myotrophin

This is the first study to assess the usefulness of myotrophin in prognostication of AMI.

Our study showed only a weak correlation between myotrophin and LVWMI and no correlation between myotrophin and peak troponin I. Myotrophin may be initially released from the myocardium but may not necessarily be a marker of myocardial necrosis. Both myotrophin and NTproBNP are raised after an AMI and their secretion patterns differ over the 5 days following an AMI with significant differences noted for both peptides. Myotrophin is raised very early after an AMI with levels staying fairly constant suggesting a possible extra cardiac source of secretion as well. Myotrophin has been shown to have growth promoting activities in rabbit skeletal muscle cells. (119). Indeed human myotrophin RNA has been shown to be widely distributed, being found in a variety of tissues including heart, skeletal muscle, liver, pancreas, and other cardiovascular tissue such as endothelial and vascular smooth muscle cells (114). It is not however clear from our work what the source of myotrophin is and it could potentially be coming from any of the above-mentioned sources. The independent determinants of myotrophin secretion are Killip class and the site of infarction which suggests a likely cardiac rather than non-cardiac source. Our results for the secretion of myotrophin differ from those of O'Brien (116) who showed that males have a higher level of myotrophin than females. It must however be remembered that this is in a different patient population. O'Brien was investigating myotrophin in heart failure patients and the release kinetics in this disease state may be different to those in an AMI. Also of interest was the fact that in heart failure myotrophin levels actually decrease as the New York Heart Association classification of heart failure increases. We did not find this inverse relationship between

myotrophin in AMI and Killip grade, in fact we found the exact opposite, myotrophin levels were raised in patients as Killip grade increased. Myotrophin is known to known to bind and activate NFkB (105,106) and our work suggests that this pathway may be important in the early stages of an AMI. Univariate analysis has shown myotrophin to be significantly raised in patients who suffered MACE compared to survivors.

A further aim of this study was to assess the utility of myotrophin and NTproBNP in determining the prognosis of AMI patients. The results of this study confirm the independent prognostic value of myotrophin in determining death, death or heart failure and MACE in patients who have an acute coronary syndrome. The predictive value of myotrophin provides risk prediction independent of NTproBNP and other known clinical predictors of death, death or heart failure and MACE.

We have shown that myotrophin is superior at predicting MACE than NTproBNP in a multivariate binary logistic regression model which was independent of established common clinical variables. The independent predictors were myotrophin levels, and serum creatinine. This finding was also confirmed on the more robust Cox proportional hazards model which showed myotrophin levels to be the only independent predictors of MACE. NTproBNP was excluded in either the binary logistic regression or Cox proportional hazards models. A raised myotrophin level was associated with a worse outcome than a below median myotrophin level. The same was true for NTproBNP with an above median NTproBNP being associated with a significantly worse outcome. When the two markers were combined myotrophin had predictive power even in the patients with NTproBNP levels above the median, suggesting that further risk stratification of this already high-risk group is possible. Furthermore using a combination of myotrophin and NTproBNP, elevation

of both above their respective medians was associated with a significantly higher MACE rate than having either peptide level above the median, or both peptides below the median. On the ROC analysis both markers give good areas under the ROC curves; however using a combination of myotrophin and NTproBNP in a multi-marker risk stratification approach in patients gives an increased area under the ROC curve and more predictive accuracy.

Myotrophin levels also gave independent prognostic information in patients who died or were readmitted with death or heart failure but not in patients who died or were re-admitted with a further AMI.

Myotrophin is raised in patients readmitted with heart failure but on multivariate analysis myotrophin does not give independent prognostic information.

It is possible that activation of myotrophin may lead to the development of heart failure and myotrophin is known to be increased in human heart failure. (116) Myotrophin is known to interact with NFkB and this may be the initiating step which drives cardiac hypertrophy. (107) This adaptive process after an AMI may prove to be beneficial in the early stages but ultimately leads to heart failure. Myotrophin is known to increase genes such as beta-myosin heavy chain (MHC) and atrial natriuretic factor (ANF), which are markers for hypertrophy (114). Our data would suggest that raised myotrophin is detrimental in the long term.

This is the first study showing the benefits of myotrophin as a prognostic marker in patients with acute coronary syndromes. Over 80% of the population consisted of STEMI. It would be interesting to see if the data can be replicated in both STEMI and NSTEMI groups. Currently the numbers are too small to give us meaningful information about this. Also admission or discharge bloods do not however offer prognostic capabilities.

One of the limitations of this study may be the number of patients recruited. A larger study may be appropriate to detect the utility of myotrophin in predicting death and heart failure individually.

However this is the first study reporting the utility of myotrophin in combination with NTproBNP in patients with ACS.

In conclusion, the present study reveals that the myotrophin system is activated during an AMI and that myotrophin is an independent predictor of death, death or heart failure and MACE in patients with AMI. Myotrophin may be useful for risk stratification in AMI patients.

4.4 MPO

This is the first study to assess the usefulness of MPO in prognostication of AMI. The aim of this part of the study was to assess the utility of MPO and NTproBNP in determining the prognosis of AMI patients. The results of this study confirm the independent prognostic value of MPO and NTproBNP in determining death or non-fatal MI in patients who have an acute ST-segment elevation MI. The predictive value of MPO provides risk prediction independent of NTproBNP and other known clinical predictors of death or non-fatal MI.

Consideration of both markers gave added prognostic information above existing clinical characteristics, enabling patients to be stratified into low, intermediate or high-risk groups. Neither marker however was predictive of recurrent myocardial infarction.

The complementary information provided by MPO to NTproBNP may suggest that the stimuli to the secretion of both markers are different, and plasma levels likely to reflect different aspects of cardiovascular homeostasis. In support of this is the clear difference in secretion profile post-AMI of both markers. Both MPO and NTproBNP are raised after an AMI and their secretion patterns differ over the 5 days following an AMI with significant differences noted for both peptides. It is clear that MPO is raised very early after an AMI with levels falling rapidly after the first 24 hours suggesting that neutrophil activation plays a role very early in AMI and may even precede the onset of AMI.

Our study showed only weak correlation between MPO and peak troponin I and no correlation between MPO and LVWMI, reiterating the fact that MPO is not a marker of myocardial necrosis. Recruitment and degranulation of the neutrophil leading to the release of MPO is seen as a key step in AMI. (160)

We have clearly shown the benefit of using each peptide alone at predicting death or death or MI. In addition MPO had predictive power even in the patients with NTproBNP levels above the median, suggesting that further risk stratification of this high-risk group is possible. Furthermore a positive MPO and NTproBNP was associated with a significantly higher rate of the primary endpoint than having one raised peptide level or two low levels of peptides.

Using a combination of MPO and NTproBNP in a multi-marker risk stratification approach in STEMI patients gives an increased area under the ROC curve and more predictive power. The utility of MPO as a prognostic marker has been borne out previously in patients with acute coronary syndromes, (136) where it was found to be an independent predictor of death or non-fatal MI in this population group. In another study the usefulness of MPO in patients presenting to the emergency room with chest pain was examined. (134) Here it was found to be useful as an independent predictor of early MI and major adverse cardiac events in the ensuing 30 days and at 6 months. Brennan et al study recruited all patients presenting with chest pain and the study included 23.5% of patients with a final diagnosis of AMI; in comparison our study has examined only patients with a diagnosis of STEMI, a relatively high-risk group. In univariate analysis both MPO and NTproBNP were significantly raised in patients who subsequently died compared to survivors. On multivariate analysis MPO retained independent prognostic information but not NTproBNP. This concurs with previous studies on the utility of NTproBNP in predicting death. (71,149) However neither peptide marker had utility in predicting non-fatal MI in univariate or multivariate analysis. One of the limitations of this study and the reason why MI did not achieve statistical significance may well have been due to the number of patients recruited. A larger study may be appropriate to detect the utility of this combination

of markers in predicting death and MI individually. The re-infarction rate is also high and this may be due in part to the fact that reperfusion was obtained with thrombolysis. Care must be taken to extrapolate these findings in patients undergoing mechanical reperfusion.

We saw no influence on plasma levels of MPO by prior treatment with statins. Acute treatment with statins has been reported to downregulate MPO expression in macrophages (159).

Our results are similar to those recently published by Mocatta and colleagues (137). They also have shown raised levels of MPO after an AMI compared to controls. Unlike our study however Mocatta has found increased levels of MPO in females. This study also concurs with ours regarding the weak correlation with NTproBNP and the finding that levels are no different in patients who are thrombolysed compared to those who are not.

Mocatta et al's study has also found that MPO levels are predictive of death, similar to our study. However the event rate for death in that study was 15% compared with 10% in our study. MPO appeared to give prognostic information for follow-up upto 5 years compared with 1 year in our study. Furthermore, MPO in combination with NTproBNP provided more discrimination than either marker measured alone. There are however important differences between these studies. Mocatta et al's study consisted of NSTEMI and STEMI patients whereas our cohort were STEMI patients. Unlike our study Mocatta did not investigate the outcome of AMI or heart failure.

One potential confounder may be heparin administration which has been shown to increase serum levels of MPO (162,163).

Previous multimarker strategies have used combinations of markers including, inflammatory markers, myocardial necrosis markers and markers of left ventricular

systolic dysfunction (144) in formulating a risk assessment profile in non-STEMI patients. However this is the first study reporting the utility of MPO in combination with NTproBNP in patients with STEMI.

In conclusion, the present study reveals that MPO is a predictor of death and death or non-fatal MI in patients with STEMI. This study confirms previous findings that MPO is involved during an AMI and it may be useful in a multimarker approach with NTproBNP for risk stratification in STEMI patients. MPO and NTproBNP may be useful tools for risk stratification of all acute coronary syndromes, including higher risk STEMI patients.

4.5 Conclusion

Several markers have now appeared and form part of the clinical armamentarium of the admitting physician to help gage risk in patients with acute coronary syndrome, the common ones include troponin (19,21), BNP (68,69,72), NTproBNP (70,71,73,74) and hsCRP (164).

Each in their own right can give information about adverse events in patients; however the combination of these markers provided independent and incremental prognostic value. (143) The reason for this is that the acute coronary syndrome is a complex syndrome consisting of plaque rupture which is the defining point with acute thrombosis; we need to remember that there is progressive mechanical obstruction of the underlying coronary vessel with dynamic obstruction and ongoing inflammation. It is rare for any of these events to occur in isolation. (165)

For this reason a multi-marker strategy at trying to define risk makes physiological sense. As our understanding of acute coronary syndrome grows so does our knowledge about how to quantify the participation of the various steps non-invasively (some or all). Sensitive information from biomarkers can provide us with this useful information as we can find out so far about haemodynamic stress, myocardial necrosis, inflammation, and vascular damage. The ongoing challenge will be to try and find the right marker or combination of markers. Our research has helped in this sense as we have driven forward the multi-marker based risk stratification hypothesis. In the future we will not only determine the type of treatments we give to patients but risk stratification will hopefully be able to identify patients who will benefit most from costly invasive procedures without the need for unnecessary risk. This has been shown to be the case using a multi-marker strategy using a combination of

NTproBNP and IL-6 at identifying patients who benefited the most from percutaneous coronary intervention. (166)

An invasive treatment strategy was found to only be beneficial if the patients had a raised NTproBNP and IL-6 level, where mortality was reduced by 7.6%; this was not the case for patients with lower levels of NTproBNP and IL-6. The same is true for the combination of NTproBNP and troponin T. (167)

These studies suggest that patient selection is certainly a possibility for the future, particularly if a multi-marker strategy includes the different facets of an acute coronary syndrome so that different pathways which are involved can be identified to gain maximum complementary information. The downside of both these studies however was the fact that they were retrospective analyses and hence all the potential criticisms and drawbacks associated with this. However whether this holds out in prospective studies remains to be seen as the data from a sub-study of TACTICS-TIMI 18 study has shown that there was no difference in the benefit of early invasive strategy between patients with and without BNP elevation. (168)

In the future proteomics may help unravel which markers are beneficial in contributing additional independent prognostic information; however that technology is some way off. (169)

A benchmark for biomarker use has been proposed by Morrow et al. (170) It has been suggested that a biomarker is only useful if it can be easily measured, give further information to the clinician and thirdly help in decision making. I think currently available markers are easily measured with robust assays which are now available and as this work has shown they can give clear prognostic information especially if used in a multimarker approach. Unfortunately currently the final benchmark is not quite achieved. It is difficult to assign therapies currently on the basis of biomarker results

as all trials looking at biomarkers, pharmacological or invasive have been retrospective and no clear treatment strategies are available which can alter risk in those deemed to be high risk. This however is not a drawback but merely shows that further work is needed in this area.

4.6 Limitations and strengths

This was a single centre study and the results need to be replicated in larger multicentre studies. There was a preponderance of ST elevation AMI, as such cut-points for non-ST elevation AMI may need to be independently established.
A possible limitation of this study is the fact that the circulating levels of the natriuretic peptides and other peptides before the AMI are unknown. Pre-existing ventricular dysfunction, renal impairment may potentially be the cause for the elevated levels of NTproBNP; however we did not demonstrate a correlation of renal function with the other peptides so it may be that they are not influenced as much. Pre treatment with drugs again could have an impact on the levels of NTproBNP which are reduced by certain drug therapies. The reperfusion strategy that was used for STEMI patients was predominantly thrombolysis; however this is still the major reperfusion strategy in many parts of the world. One of the overwhelming strengths of the study is the inclusion of consecutive patients with no restrictions on age. Our cohort of patients is similar to that which is encountered by clinicians around the world.

4.7 Future perspective

The role of a multimarker strategy has only recently been coming to light in the risk stratification of AMI. Our findings suggest that a multimarker strategy is certainly

feasible and serve to generate a number of hypotheses for the potential roles of CT-1, myotrophin and MPO in AMI. In the future risk stratification will take place on the measurement of an array of markers and this may well also be used for the assignment of drug therapies and invasive cardiological procedures. MPO is now available as a point of care assay along with NTproBNP, with results available on patients within a few minutes; it will be interesting to see if it will take off as a potential combined marker for risk stratification.

4.8 Concluding remarks

Our study is the first to establish that the natriuretic peptide, NTproBNP is better at predicting death than a well-established clinical risk score. Our study has also shown the secretory patterns of CT-1, myotrophin and MPO in the acute phase of a myocardial infarction. Patients with AMI have significantly higher levels of these peptides than control subjects. CT-1 levels were significantly higher in those patients who incurred the composite endpoint of death and heart failure, than in those patients spared of these events. Myotrophin levels were significantly higher in patients who died, died or were re-admitted with heart failure or had a composite endpoint of MACE (including death, recurrent AMI and need for urgent revascularisation) compared to those patients spared of these events. MPO levels were significantly higher in those patients who died or had the composite endpoint of death and recurrent AMI, than in those patients spared of these events.

Furthermore, we compared the utility of the natriuretic peptide NTproBNP with CT-1, myotrophin and MPO for the prediction of our endpoints. We showed that the predictive value of NTproBNP was markedly enhanced with the combination of plasma CT-1, myotrophin and MPO. Thus these novel peptides have complementary

predictive power with the natriuretic peptides in identifying patients at high risk of adverse events following AMI, independent of established conventional risk factors.

We can conclude that there is early activation of CT-1, myotrophin and MPO systems following an AMI. The CT-1 system may represent a compensatory measure and be beneficial following an AMI whereas MPO may be detrimental.

In conclusion a multimarker approach with NTproBNP is more informative and may be useful for risk stratification in AMI patients.

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Appendices

Appendix 1 Patient information Sheet

Study title: Study to look at proteins released during a heart attack to see if these will help predict future heart problems.

Principal Investigator: Professor Leong Ng, Professor of Medicine & Therapeutics, Leicester Royal Infirmary

Contact details: (0116) 252 3125 or 252 5839

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part in the study, as there is no obligation.

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

Myocardial infarction or "heart attack" is a common problem affecting many individuals. We wish to investigate certain proteins, which are released during a suspected heart attack to see if we can predict which patients are at risk of further heart damage in the future so we can address the needs of these patients. The study will run for 2 years during which time we will follow your progress to see how you are getting on.

2. **Why have I been chosen?**

You have been chosen to participate in this study as you have suffered a suspected "heart attack".

3. **Do I have to take part?**

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive in any way.

4. **What will happen to me if I take part?**

The study will involve a blood test, which may be performed on 2 days during your hospital stay or daily for 6 days. You will be informed how many samples will be taken before the blood tests are performed. On each occasion, we will collect approximately 10ml of blood (approx. 2 teaspoons) and the plasma will be stored for a period of 5 years. However, most of the blood tests will be performed in the first 18-24 months. You will also have an echo scan of the heart to look at the function of

your heart. It is not painful and is part of the normal investigation after a heart attack. This will be done during your initial hospital stay. The study will run for a total of 2 years; during this time we will follow you up and see how you are getting on. This will usually involve a brief phone call every 6 months. If you have a hospital admission during this period we would like to know about this. You will not be expected to attend for extra clinic visits. The Cardiovascular Unit is also registered with the Office of National Statistics, which periodically supplies us with information on a patient's progress.

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

Although there are unlikely to be any direct benefits to you, the information we get from this study may help us to assess future patients with heart attacks better and assess their risk of further heart damage.

6. **What are the possible disadvantages and risks of taking part?**

As this project does not involve you changing the drugs you are taking, the risks of taking part are minimal. However, you may experience some bruising and/or discomfort at the site of the blood test in your arm.

7. **What if new information becomes available?**

If you decide to withdraw from this study, your research doctor will make arrangements for your care to continue.

8. **What happens when the research study stops?**

At the conclusion of the study, we will be able to assess whether any of the blood proteins that we have measured are of value in estimating risk following a heart attack. These tests may then be used in future risk assessments in patients admitted with a heart attack.

9. **What if something goes wrong?**

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you.

10. **Will my taking part in this study be kept confidential?**

All information, which is collected, about you during the course of the research will be kept strictly confidential. Any information about you, which leaves the hospital, will have your name and address removed so that you cannot be recognised from it. Also your own GP will be notified of your participation in the trial.

11. **What will happen to the results of the research study?**

The results of this research are likely to be published in 2006 in a medical journal. All participants in the study will remain anonymous.

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

The British Heart Foundation is funding the research. Your doctor will not be paid for including you in this study.

13. **Who has reviewed the study?**

All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by an NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

14. **Contact for Further Information**

For further information about the study you may wish to contact:

Professor L Ng
University of Leicester
Clinical Sciences Building
Leicester Royal Infirmary
Leicester
LE2 7LX
Tel: 0116 252 3125 or 252 5839

Thank you for reading the above information

Appendix 2

PATIENT CONSENT FORM

Title of Project: Study to look at proteins released during a heart attack to see if these will help predict future heart problems.

Name of Researcher: Dr SQ Khan please initial boxes

1. I confirm that I have read and understand the information sheet dated 29.07.2003 (version 1) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that sections of any of my medical notes may be looked at by persons involved in the study. I give permission for these individuals to have access to my records. ☐

4. I agree to take part in the above study.

Name of Patient	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

Appendix 3

Letter to Patient's General Practitioner

Dear Dr

Your patient _____ has kindly agreed to take part in our study entitled "Risk stratification of myocardial infarction using cardiac peptides." This is a prospective study that hopes to enrol 1200 patients to look at the effect of novel cardiac peptides to try and formulate risk stratification of patients with acute myocardial infarction. This study will run for a period of two years.

If you have any further queries about the project then do not hesitate to contact us on the above number.

Yours sincerely

Dr SQ Khan