Plasma matrix metalloproteinases as predictors of prognosis and left ventricular remodelling after acute myocardial infarction

Submitted for the degree of Doctorate of Medicine (MD)

Dr Dominic Kelly. BSC. MBChB. MRCP.

Correspondence:

Dr Dominic Kelly
White Gates

Waterworks Rd
Otterbourne
Hampshire
SO21 2DP

kelly.dominic@talk21.com

Word Count: 33, 398
No. References: 363

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Acknowledgements

I would like to acknowledge the following for their help and support in the completion of the work leading to this thesis:

Many thanks to **Dr Iain Squire** for acting as my supervisor throughout this project. I could not have asked for a more supportive mentor allowing me to form my own ideas and views and yet aiding in the development of skills required in this research field. Thanks to **Prof. Leong Ng** and **Pauline Quinn** for your support in the laboratory work leading to this thesis. Thanks to **Dr Joan Davies** for your support and mentoring in echocardiography. This project has allowed me to further develop my echo skills under your supervision and without your help I would not have achieved British Society of Echocardiography accreditation in transthoracic echo. Thanks to **Prof Nilesh Samani** for your frequent input and support. Thanks to **Gillian Cockerill** and **Prof. Matt Thompson** at St George's Hospital Medical School, London for their aid with MMP and TIMP analysis. Many thanks to my wife **Pauline** for your support without you none of this would be possible

Thanks to the British Heart Foundation for their supporting grant.

Most of all thanks to the patients for agreeing to take part in this study, offering your time for little in return.

Statement of Originality.

With the exception of that acknowledged, I confirm that this thesis is entirely my own work completed between July 2004 and April 2008 under the supervision of Dr Iain Squire, University Hospitals Leicester, UK.

Contents:

List of Figures:

Figure. 7.4 Kaplan-Meier survival curves for patients stratified by optimum MMP-9 and TIMP-1 levels. Both levels above optimum (solid line) v either above optimum (middle broken line) v both below optimum (top broken line)

Figure. 7.5 MMP-9 levels in subjects with increasing v decreasing End Diastolic Volumes (EDV) between admission and follow up.

Figure. 7.6 Receiver Operator Curves (ROC) for the Prediction of Death or Heart Failure (Top), Death (middle) and Heart failure (bottom) by TIMP-1/N-BNP.

Figure 7.7. Kaplan-Meier survival curves for patients stratified by: Top panel - NTproBNP <1472fmol/ml (broken line) or ≥1472fmol/ml (continuous line) and Bottom panel – TIMP-1 <135ng/ml (broken line) or ≥135ng/ml (continuous line)

Figure 7.8. Kaplan Meier survival curves for the prediction of Death or Heart Failure (above), death (middle) and heart failure (lower) in subjects with both TIMP-1 and N-BNP above optimum (blue), versus both below optimum (green) versus either above optimum (grey).

Figure 7.9. Kaplan-Meier survival curves for patients stratified first by NTproBNP, then by TIMP-1.

Top panel: Low risk BNP group (NTproBNP <1472fmol/ml) and TIMP-1 <135ngml (broken line) v ≥135ng/ml (solid line)

Bottom Panel: High risk BNP group (NTproBNP >1472fmol/ml) and TIMP-1<135ng/ml (broken line) v ≥135ngml (solid line)

Figure. 8.1. TIMP-1 concentration according to glucose quartile.

Figure. 8.2. LV function (LVEF above & WMIS below) according to glucose quartile

Figure. 8.3. LVEF according to MMP-9 (above) and TIMP-1 (below) Quartile

App. 11.13. Patient Information and consent form.

Printed on letter headed paper

List of Tables:

Table. 11.11 Difference in MMP-3 levels between categorical variables in patient demographics

Table. 11.12 Correlation between MMP-3 and echocardiographic markers during admission[1] (above) and follow up[2] (below) for each time period

Abstracts / Publications related to this thesis:

British Cardiovascular Society. Manchester 2008

- A prospective comparison of 2D echocardiography and N terminal pro-BNP in the prediction of adverse outcome post acute myocardial infarction. **Kelly D**, Thompson M, Samani N, Khan S, Ng L, Squire I
- MMP-3 is associated with heart failure and all cause mortality post acute myocardial infarction. **Kelly D**, Thompson M, Samani N, Khan S, Ng L, Squire I

BJCA Research Awards. Birmingham. 2007 - Awards Finalist. D Kelly

- TIMP-1 predicts death and heart failure but not re-infarction after acute myocardial infarction – A comparison with N-BNP.
- Stromelysin-1 is associated with all cause mortality and heart failure post acute myocardial infarction

European Society of Cardiology, Vienna 2007

- N-BNP and TIMP-1 Predict Death and Heart Failure but not Re-infarction after Acute Myocardial Infarction in Man. **Kelly D**, Thompson M, Samani N, Khan S, Ng L, Squire I.

- Stromelysin-1 is associated with all cause mortality and heart failure post acute myocardial infarction. **Kelly D**, Thompson M, Samani N, Khan S, Ng L, Squire I.

Both presented as oral presentations during, "State of the Art featured research session". Highest scoring abstracts in topic.

British Cardiovascular Society. Glasgow 2007

- Plasma MMP-9 and TIMP-1. Novel markers of LV dysfunction and adverse prognosis after acute myocardial infarction in man. **Kelly D,** Cockerill G, Thompson M, Ng L, Squire I.

American Heart Association. Scientific Sessions. Chicago 2006

- Matrix Metalloproteinase-9 in the Pathogenesis of Left Ventricular Remodelling and Heart Failure Post Acute Myocardial Infarction. **Kelly D**, Khan S, NJ Samani, M Thompson, Ng L, Squire I.

British Society of Echocardiography. Birmingham 2006

- Echocardiography v NT-BNP as markers of prognosis post acute myocardial infarction. **Kelly D.** Khan S. Hickey P. Squire I

World Congress Cardiology/European Society of Cardiology. Barcelona 2006

- **M**MP-2 and N-BNP in the Prediction of Left Ventricular Impairment Post Acute Myocardial Infarction.

  Kelly D, Khan S, NJ Samani, M Thompson, Ng L, Squire I.

- N terminal pro B type natriuetic peptide is better than TIMI risk score at predicting death following acute myocardial infarction. Khan S, Kelly D, Quinn P, Ng L.

British Cardiac Society. Glasgow 2006

- Matrix metalloproteinase -2 &-9 in the pathogenesis of left ventricular remodelling post acute myocardial infarction in humans. Oral presentation/abstract. **D Kelly**,S Khan, M Thompson, LL Ng, NJ Samani, IB Squire.

- N Terminal B Type Natriuretic peptide is better at predicting death following myocardial infarction than TIMI risk score**. Abstract.**,S Khan, **D Kelly** M Thompson, LL Ng, NJ Samani, IB Squire.

- Matrix metalloproteinase -2 & -9 in the pathogenesis of left ventricular dysfunction post acute myocardial infarction in humans. Oral presentation/abstract. **D Kelly,** S Khan, M Thompson, LL NG, NJ Samani, IB Squire.

Other Conferences

- Metalloproteinases 2 and 9 following acute myocardial infarction, potential for clinical use. **D Kelly,** I Squire

  Poster Presentation. "LNR Delivering the Best", regional research conference.

- Matrix metalloproteinase -2 & -9 post acute myocardial infarction in humans – LV function. Royal College of Physicians/Medical Research Society – Clinical Scientists in Training Conference. **D Kelly** RCP London Feb 2006

Publications:

- Plasma Matrix Metalloproteinase-9 and left ventricular remodelling after acute myocardial infarction in man: a prospective cohort study
  D Kelly, G Cockerill , LL Ng, M Thompson, S Khan, NJ Samani, IB Squire. Eur Heart J. 2007 Mar;28(6):711-8.

- Circulating Stromelysin-1 (MMP-3): a novel predictor of LV dysfunction, remodelling and all-cause mortality after acute myocardial infarction. Eur J Heart Failure. **D Kelly**, G Cockerill, LL Ng, M Thompson, NJ Samani IB Squire. Eur J Heart Failure. Jan 28 2008

In Press

- Plasma TIMP-1 and MMP-9 predict LV dysfunction and adverse prognosis after AMI in Man:Comparison with N-terminal proBNP. Eur Heart J. 2008
  D Kelly, G Cockerill, LL Ng, M Thompson, NJ Samani IB Squire

Submitted

- Hyperglycaemia is associated with higher plasma TIMP-1 and MMP concentration after acute myocardial infarction: A mechanistic link to LV dysfunction in diabetes. Diabetologia. **D Kelly**, G Cockerill, LL Ng, M Thompson, NJ Samani IB Squire
- A Prospective Comparison of 2-D Echocardiography and N-Terminal Pro-BNP in the Prediction of Adverse Outcome Post Myocardial Infarction. **D Kelly**, G Cockerill, LL Ng, IB Squire.

Chapter 1.

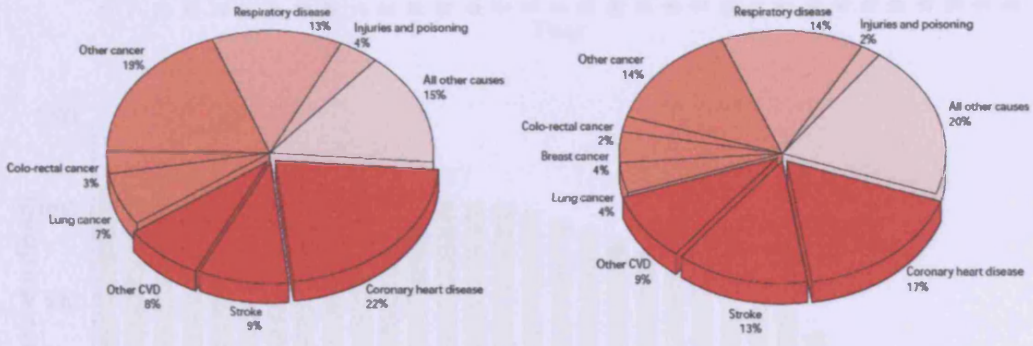
Coronary Heart Disease and Heart Failure: Incidence, Prevalence and Pathophysiology

1.1

Background:

Despite increased awareness and improvements in primary prevention, diseases of the circulatory system are the main cause of death in the UK with around 216,000 deaths in 2004 of which around 49% are due to coronary heart disease (Allender, Peto et al. 2006). Coronary heart disease alone is the most common cause of death in the UK and in addition is the most common cause of premature death (death <75yrs).

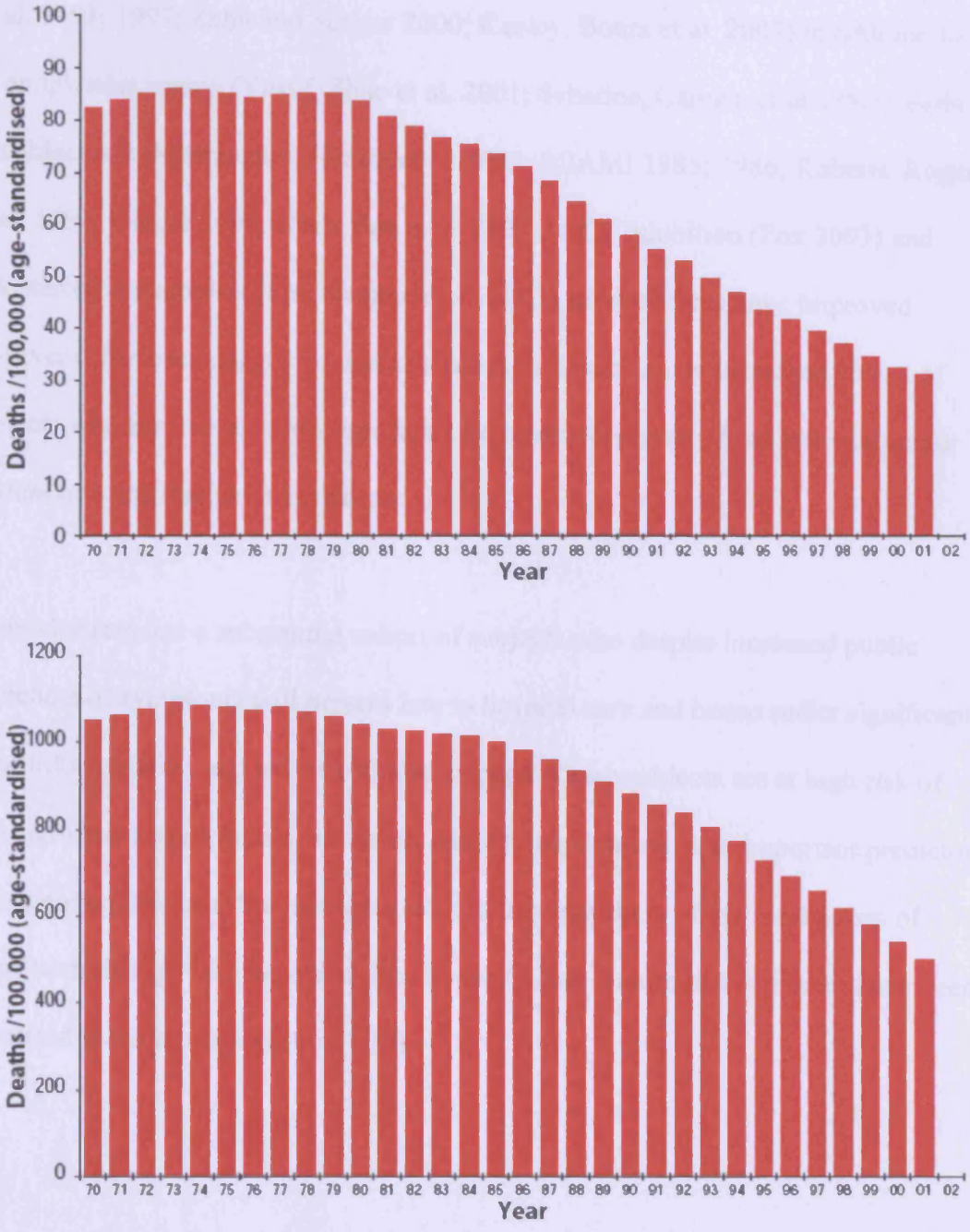
Figure. 1.1. Deaths by cause in 2002 in males (left) and females (right) – (BHF stats 2002)



In response to these figures, cardiovascular disease has been given priority in both primary and secondary prevention through governmental documents such as our healthier nation (Health 1999) and NSF targets for coronary heart disease (NHS 2000).

Recent trends have shown an overall decrease in death due to coronary heart disease. These changes are likely to be related to improved primary prevention via reduction in major risk factors, predominantly smoking in the general population (Unal, Critchley et al. 2004) in addition to improvement in treatment including those of secondary prevention.

Figure. 1.2. Death rates from coronary heart disease 1970-2001. Age<65years (above) and 65-74years (below) – (BHF stats 2002)



The incidence of acute myocardial infarction in the UK is estimated to be around 600 per 100,000 males and 200 per 100,000 females between the age of 30-69yrs with the incidence increasing with age (Volmink, Newton et al. 1998). With improvements in reperfusion therapy for acute myocardial infarction based on large randomized controlled trials such as thrombolysis (Group 1986; 1993; Aguirre, Younis et al. 1995; Califf, White et al. 1996; Van de Werf, Cannon et al. 1999) and recently more favourable results with primary percutaneous coronary intervention (Grines, Browne et al. 1993; 1997; Zahn and Senges 2000; Keeley, Boura et al. 2003) in addition to use of antiplatelet agents (Yusuf, Zhao et al. 2001; Sabatine, Cannon et al. 2005), early beta blockade (Hjalmarson, Herlitz et al. 1983; MIAMI 1985; 1986; Roberts, Rogers et al. 1991; Dargie 2001; Chen, Pan et al. 2005), ACE Inhibition (Fox 2003) and aldosterone antagonists (Pitt, Remme et al. 2003) survival rates have improved however it has been suggested that as a result there may be an increased cohort of subjects who survive but with significant morbidity especially from left ventricular dysfunction and clinical heart failure.

There also remains a substantial cohort of subjects who despite increased public awareness of symptoms will present late to hospital care and hence suffer significant myocardial necrosis and left ventricular damage. These subjects are at high risk of both significant heart failure and death. Time to reperfusion is an important predictor of prognosis (De Luca, Suryapranata et al. 2004) regardless of the mechanism of reperfusion therapy and targets of door to needle and door to balloon times have been prioritised in the management of AMI.

1.2

Heart Failure Incidence & Prevalence:

Heart failure is a clinical syndrome which results from structural or functional changes to the myocardium or cardiac tissue which impairs the ability to maintain cardiac output. Current estimates of heart failure incidence suggest around 66,000 new cases in the UK per year (Allender, Peto et al. 2006). The Hillingdon Heart Failure study found an incidence rate of 140 per 100,000 for males and 120 per 100,000 for females and the Heart of England screening study reported a prevalence of definite left ventricular systolic dysfunction (LVEF < 30%) of 2.9% in an urban population with only 1.5% being symptomatic indicating that there is a likely to be a significant percentage of the population with sub-clinical heart failure.

The predominant aetiology for heart failure is ischaemic heart disease. Clinical trials recruiting patients with heart failure report a history of previous MI in around 60% (Krum and Gilbert 2003). McDonagh et al demonstrated that in subjects with heart failure and a left ventricular ejection fraction <30%, a history of previous MI was seen in around 50% with a history of some degree of ischaemic heart disease in almost 95% (McDonagh, Morrison et al. 1997). The Heart of England screening study showed 53% of subjects with left ventricular dysfunction to have a history of ischaemic heart disease (Davies, Hobbs et al. 2001) with previous MI being the most powerful predictor of heart failure and Fox et al showed that over half of subjects with heart failure aged less than 75 years were attributable to coronary disease with the majority of these cases developing heart failure at the time of AMI (Fox, Cowie et al. 2001).

As stated above, improvements in reperfusion therapy for acute myocardial infarction has lead to improved survival rates, however it has been suggested that as a result there may be an increased cohort of subjects who survive, but with significant morbidity especially from left ventricular dysfunction and clinical heart failure. Data to support this theory are limited and others have suggested that early reperfusion therapy will result in not only increased survival but a lower incidence of heart failure due to preservation of myocardial tissue.

Hellerman et al (Hellermann, Goraya et al. 2003) observed a 28% reduction in the incidence of post MI heart failure between 1979 and 1994; in contrast the Framingham Heart Study showed no decline in post MI heart failure over time. It must be noted that this study excluded non Q wave Myocardial infarction (Guidry, Evans et al. 1999). In addition the Worcester Heart Attack Study reported a small decline in the incidence of heart failure during initial hospital stay for AMI however post discharge data were not presented (Spencer, Meyer et al. 1999). Factors influencing the amount of myocardial necrosis include a preconditioning stimulus (Murry, Jennings et al. 1986), collateral flow (Habib, Heibig et al. 1991), the volume of ischaemic tissue as well as time to reperfusion.

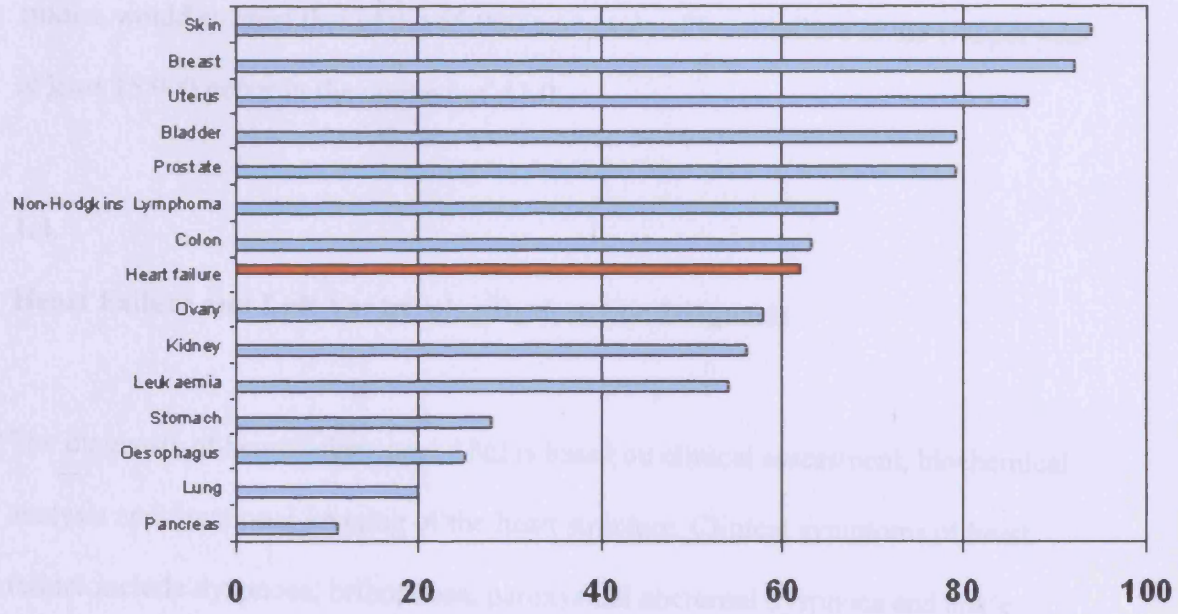
Further data to support the likely rise or fall of heart failure incidence are currently lacking in the UK and additional difficulties arise due to the discrepancy in diagnostic criteria used for heart failure and the lack of correlation between heart failure class, clinical symptoms and objective assessment of LV function. What is certain is that heart failure remains a significant cause of mortality and morbidity in the UK.

1.3.

Left Ventricular Dysfunction and Heart Failure after AMI

Heart failure mortality is comparable to that of most cancers and hence may be considered a "malignant", disease.

Figure. 1.3. 1 year survival rate of Heart Failure as compared to cancers (BHF stats 2002)



Data from the original Framingham studies suggest that the probability of someone with a diagnosis of heart failure dying within 5 years was between 60-75% in men and 38-42% in women (Ho, Anderson et al. 1993). Heart failure due to ischaemic heart disease is associated with a particularly adverse prognosis, the extent of infarcted tissue and hence the degree of remodelling of the left ventricle and changes in LV volume correlating closely with the likelihood of adverse prognosis (Group 1983; White, Norris et al. 1987; Pfeffer and Braunwald 1990).

Some degree of left ventricular dysfunction is common after AMI. The incidence of clinical heart failure is variable with poor correlation between the degree of left ventricular dysfunction and New York Heart Association status. Hellerman et al demonstrated an overall incidence of clinical heart failure following AMI of 36% between 1979 and 1994 with an annual fall in incidence of 2% per year over this time period (Hellermann, Goraya et al. 2003). The incidence of heart failure has been discussed above with a significant proportion of such being post AMI. Taken overall, studies would suggest that of the 65,000 new cases of heart failure in the UK per year, at least 15,000 occur in the context of AMI.

1.4.

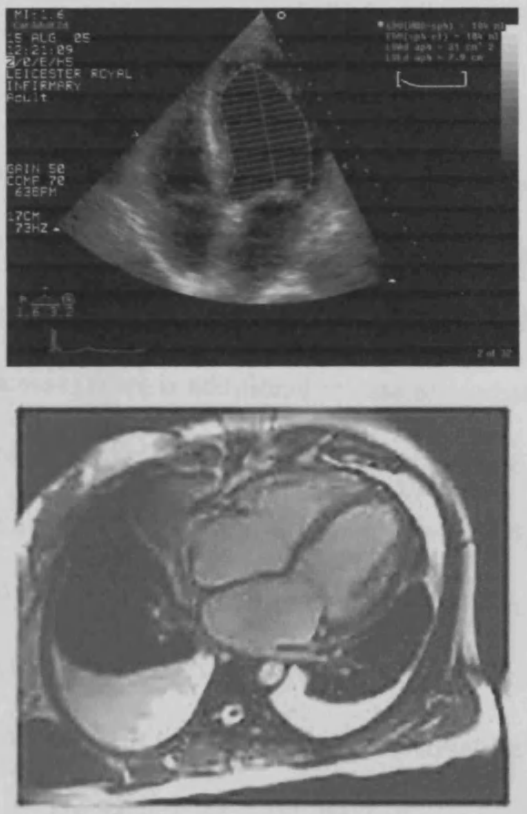
Heart Failure and Left Ventricular Dysfunction Diagnosis

The diagnosis of heart failure post AMI is based on clinical assessment, biochemical analysis and functional imaging of the heart structure. Clinical symptoms of heart failure include dyspnoea, orthopnoea, paroxysmal nocturnal dyspnoea and ankle oedema. Clinical signs are related to fluid overload with raised venous pressures, lung crepitations due to interstitial oedema and 3rd or 4th heart sounds. These findings however are not specific for congestive heart failure due to left ventricular dysfunction and additional information is often required to confirm diagnosis. Radiological imaging via chest x-ray may aid diagnosis and the recent use of natriuretic peptides especially Brain (or B-type) Natriuretic Peptide (BNP) has further added to clinical assessment tools (Chapter 3).

Current "Gold Standards", for the diagnosis of left ventricular dysfunction post MI involve direct imaging of cardiac structure and assessment of left ventricular systolic

function via echocardiography or more recently cardiac magnetic resonance imaging. Both of these modalities allow quantitative assessment of left ventricular ejection fraction in addition to assessment of contributory factors in reduced cardiac output such as valvular pathology, restrictive filling or intra-cardiac shunts.

Figure. 1.4. Cardiac imaging for assessment of LV dysfunction via echocardiography (above) or Cardiac MRI (below).



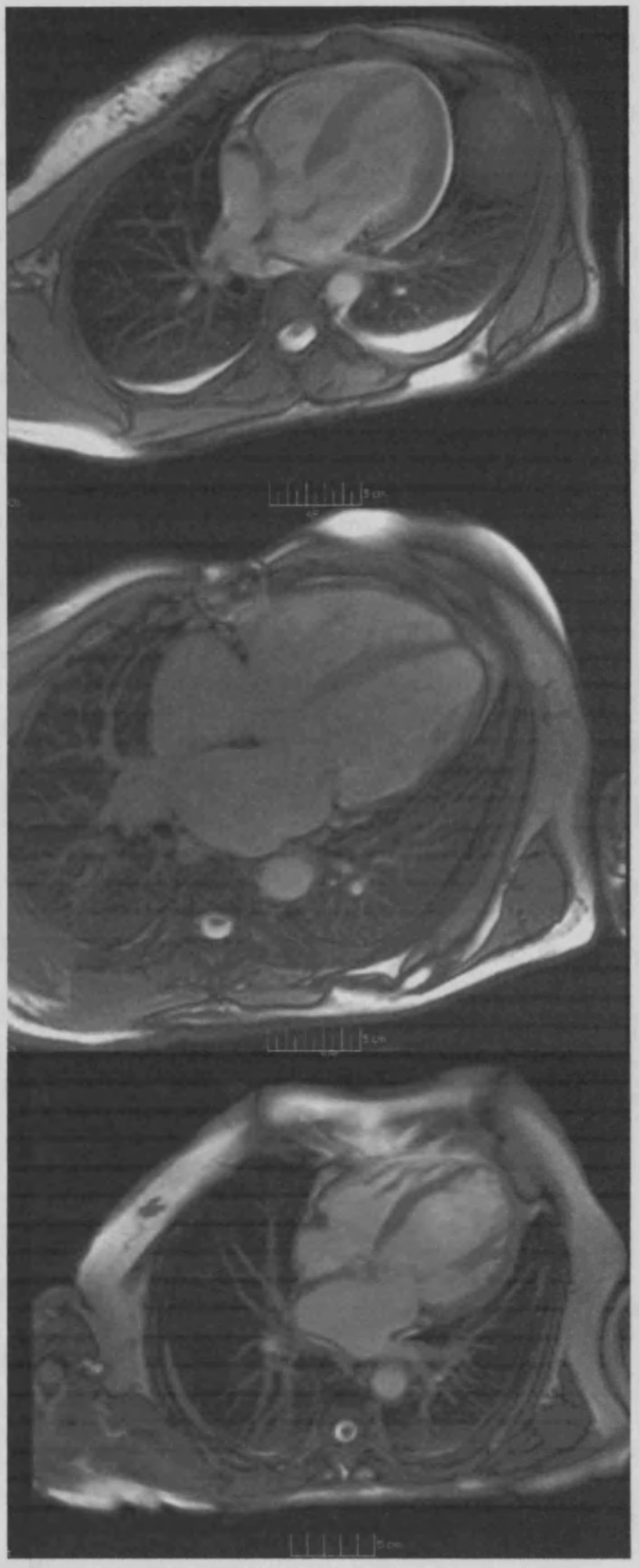
1.5.

Left Ventricular Remodelling - Pathophysiology:

Recent insights into the pathophysiology of heart failure have been drawn from studies of the mechanisms that lead to ventricular remodelling. Myocardial necrosis and loss of functional myocardial tissue leads to an abrupt increase in loading conditions and neurohormonal activation. This neurohormonal activation has been considered to be an adaptive response to the falling cardiac output in heart failure. The decline in stroke volume produces arterial under filling which in turn leads to a haemodynamic response driven by sympathetic activation, peripheral vaso-

constriction and salt and water retention (Schrier and Abraham 1999). These changes are mediated by an increase in circulating levels of hormones such as noradrenaline (Benedict, Shelton et al. 1996), angiotensin II (Liu, Gao et al. 2006), aldosterone (Hirsch, Talsness et al. 1991), arginine vasopressin (Chatterjee 2005) and endothelin (Wackenfors, Emilson et al. 2004). Although these adaptive processes are effective in the short term in order to increase cardiac output and hence organ perfusion, long term activation is detrimental leading to an increased cardiac workload, increased ventricular pressures and a spiralling decrease in LV function. With the activation of these maladaptive processes there is additional release of mediators with the opposite effect such as natriuretic peptide (Hall 2005), cytokines (Levine, Kalman et al. 1990; Torre-Amione, Kapadia et al. 1996; Aukrust, Ueland et al. 1999) and adrenomedullin (Oie, Vinge et al. 2000). Often a balance of these opposing effects is reached leading to a state of stable heart failure. However this balance is easily offset leading to further deterioration. Evidence for the neurohormonal activation state comes for numerous in vitro and in vivo studies. ACE inhibitor studies such as SOLVD (SOLVD 1991) and Concensus (CONSENSUS 1987) demonstrated a relationship between neurohormonal activation and mortality with patients who died during follow up having a higher plasma noradrenaline, angiotensin II and aldosterone.

Figure. 1.5. Increasing LV volumes as imaged via cardiac MRI Above – Normal LV size, Middle – Moderate LV dilatation, Below – Severe LV dilatation



The final common pathway in LV remodelling appears to involve structural changes within the myocardium. The remodelling process involves changes in ventricular geometry, structure and function via dilatation, hypertrophy and the formation of a discrete collagen scar affecting both infarcted and non-infarcted myocardium. The extracellular matrix of the myocardium forms the scaffold for myocyte function, the framework is comprised of predominantly type I and III collagens and couples adjacent myocytes by intercellular struts that align myofilaments to optimize force development, distribute force evenly to the ventricular walls and prevent sarcomeric deformation (Erlebacher, Weiss et al. 1984). Degradation and remodelling of this matrix will hence lead to loss of cardiac structure and decreasing function.
In the healthy state, structure and function of the extracellular matrix are maintained by the matrix metalloproteinase family of endopeptidases. Maintenance of extracellular matrix structure is an active process which requires equilibrium between degradation and new tissue formation. Instability in this balance will lead to quantitative and qualitative alterations in matrix composition with a tendency towards degradation with new tissue formation being non-structured. The extra-cellular matrix is composed predominantly of collagen although all four categories of proteinases (serine, cysteine, and aspartic proteinases, and metalloproteinases) have been implicated in its proteolytic degradation.

Acute MI leads to LV remodelling which has traditionally been divided into early (within 72hrs) and late (beyond 72hrs) phases. Myocardial infarction results in the migration of macrophages, monocytes and neutrophils into the infarcted zone and a local inflammatory response with release of matrix degrading enzymes such as MMPs. Early remodelling involves infarct expansion resulting from the degradation

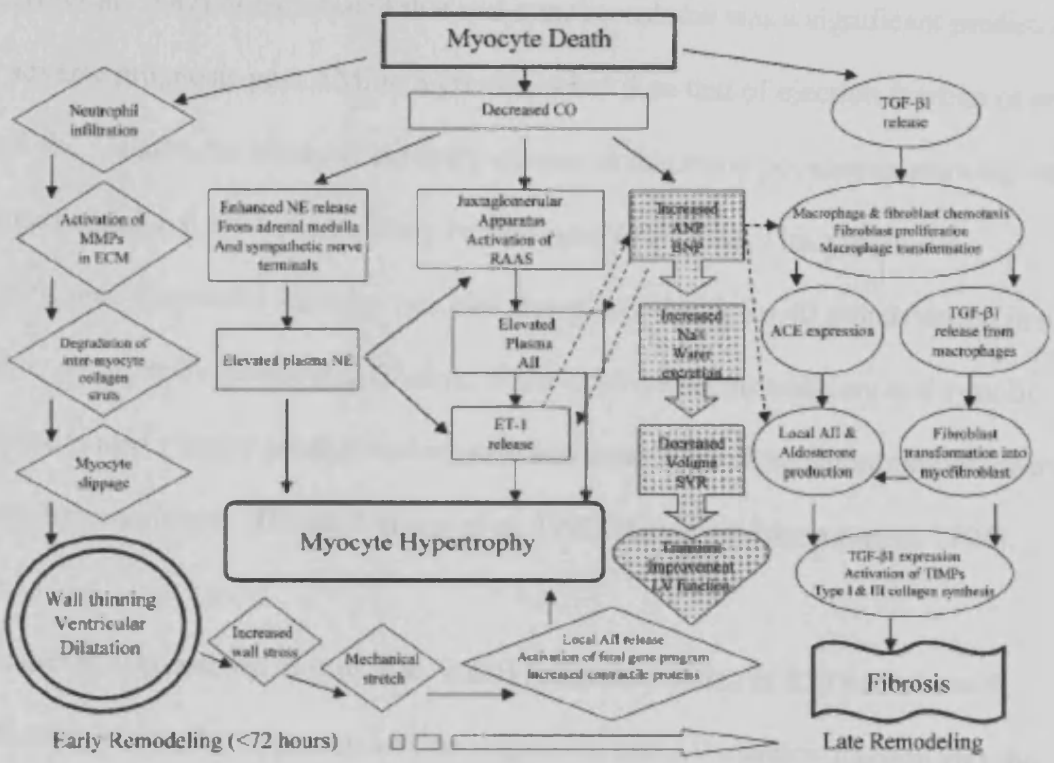
of the inter-myocyte collagen struts. This degradation is likely to be secondary to the activation of serine proteases and MMPs (Cleutjens, Kandala et al. 1995) with a shift in the tight balance between matrix formation and degradation in favour of the latter. Infarct expansion occurs rapidly following infarction (Warren, Royal et al. 1988) resulting in wall thinning, ventricular dilatation and increased wall stress. The extent to which this occurs ranges from clinically unapparent to ventricular rupture and is dependent on location of infarct, size of initial infarct, thickness of the myocardium and loading conditions of the ventricle.

The increase in wall stress is a strong stimulus for compensatory hypertrophy which subsequently occurs in the non infarcted regions (Lew, Chen et al. 1985; Sadoshima, Jahn et al. 1992). Simultaneously alterations in the haemodynamics trigger the sympathetic adrenergic system to stimulate catecholamine synthesis and activation of the renin-angiotensin-aldosterone system as discussed above.

Late remodelling involves myocyte hypertrophy and alterations in ventricular architecture to redistribute ventricular stress and formation of a collagen scar. Myocyte hypertrophy is seen microscopically (Anversa, Beghi et al. 1985) and is an adaptive response to offset the increased load conditions and reduce further deformation and dilatation. This myocyte hypertrophy is induced by neurohormonal activation, myocardial stretch and local activation of the renin angiotensin system in addition to catecholamine production and the secretion of natriuretic peptides. Catecholamine release has a direct effect on myocyte hypertrophy. Noradrenaline stimulates $\alpha 1$ receptors leading to hypertrophy via $G\alpha q$-dependent signalling pathways (Ju, Zhao et al. 1998) and $\beta 1$ stimulation in the juxtaglomerular apparatus

leads to enhanced renin release hence increased angiotensin II activity which in turn further stimulates catecholamine release and augments endothelin-1 release which is also a stimulant for hypertrophy (Levin, Gardner et al. 1998). In addition localised myocyte hypertrophy may occur secondary to cell stretch without the requirement of neurohormonal activation (Yamazaki, Komuro et al. 1995). The complex interactions occurring in the process of LV remodelling are summarized in the figure below reproduced from Sutton et al (Sutton and Sharpe 2000)

Figure. 1.6. Mechanisms associated with LV remodelling post AMI



1.6

Left Ventricular Remodelling – Prognostic Implications:

One of the most prognostically significant consequences of acute myocardial infarction is the development of adverse left ventricular (LV) remodelling. The degree of left ventricular remodelling, in particular the extent of changes in left ventricular volume, correlates closely to the severity of left ventricular dysfunction and the likelihood of the development of CHF and adverse prognosis. White et al (White, Norris et al. 1987) demonstrated that end systolic volume was a significant predictor of adverse prognosis post AMI to a greater extent than that of ejection fraction or end-diastolic volume, the extent of coronary disease in this study population showing only a small additional prediction of only borderline significance. This prognostic significance of systolic volumes was also shown to extend to a 40 month period in a follow up paper by Norris et al (Norris, White et al. 1992). In addition, end systolic volume is also a major predictor of adverse outcome in subjects undergoing coronary artery bypass surgery (Hirata, Nakano et al. 1992; Hamer, Takayama et al. 1994).

Castaner et al (Castaner, Betriu et al. 1984) followed a series of 259 patients with AMI over 34 months observing on Cox regression that LV ejection fraction was the only independent predictor of survival. These findings were consistent with those from the GISSI-2 study in which a marked increase in 6 month mortality was observed in patients with LV ejection fraction <40% (Volpi, De Vita et al. 1993). In another study of 834 subjects post AMI with LV ejection fraction <40%, Otterstad et al showed that initial baseline echocardiographic measures of LV volumes and

ejection fraction had no value in the prediction of adverse outcome (composite of death, infarction or heart failure) over subsequent follow up. In this study however an increase in end-systolic volume between index admission and 3 months was the strongest predictor of adverse events especially heart failure. This study concluded that early changes in left ventricular structure (LV remodelling) held the greatest prognostic significance post AMI (Otterstad, St John Sutton et al. 2002). Other studies have also demonstrated that progressive left ventricular dilatation predicts poor prognosis and ACE inhibitor therapy attenuates left ventricular dilatation and subsequent improvement in clinical outcomes in subjects with poor left ventricular function (St John Sutton, Pfeffer et al. 1994).

1.7

Chapter Summary.

Coronary artery disease remains a common cause of morbidity and mortality in the western world. Improved reperfusion and secondary preventative measures have improved survival following acute myocardial infarction however significant numbers of patients survive these events with resultant left ventricular dysfunction. Myocardial necrosis leads to complex structural and neuroendocrine changes in the infarcted and non-infarcted regions of the left ventricle with progressive dilatation and clinical heart failure, mortality being associated with the degree of left ventricular remodelling.

Chapter 2

An Overview of the Matrix Metalloproteinase System

2.1

Background

Matrix metalloproteinases (MMPs), also known as matrixins are a family of endogenous zinc dependant endopeptidases which selectively degrade components of the extracellular matrix (ECM) and are involved in physiological events such as wound healing (Lund, Romer et al. 1999; Calabro, Grappone et al. 2004; Krampert, Bloch et al. 2004; Salmela, Pender et al. 2004; Ahokas, Skoog et al. 2005), angiogenesis (Stetler-Stevenson 1999; Liekens, De Clercq et al. 2001; Rundhaug 2005) and ovulation (Smith, McIntush et al. 1999; Ogiwara, Takano et al. 2005). In addition they have been implicated in various pathological processes such as arthritis (Close 2001), peridontitis (Birkedal-Hansen 1993; Sorsa, Tjaderhane et al. 2004), multiple sclerosis (Leppert, Lindberg et al. 2001; Opdenakker, Nelissen et al. 2003), cancer invasion (Parsons, Watson et al. 1997; Kahari and Saarialho-Kere 1999; Kleiner and Stetler-Stevenson 1999) and cardiovascular disease (Table 2.1). First discovered in 1962 after experiments to investigate how the metamorphosing tadpole lost its tail in its transformation to adult frog (Gross and Lapiere 1962), since this time over 60 MMPs including over 20 human MMPs have been cloned and sequenced (Ye 2000).

Table 2.1 Normal and physiological processes in which MMPs have been implicated

Normal	Pathological
Development	**Tissue Destruction**
Blastocyst implantation Embryonic development Nerve Growth Growth plate cartilage removal Skeletal bone growth Nerve outgrowth Enamel maturation Primary tooth resorption	Rheumatoid arthritis Osteoarthritis Cancer Invasion Cancer metastasis Decubitus ulcer Gastric ulcer corneal ulceration periodontal disease
Reproduction	**Fibrotic disease**
Endometrial cycling Graafian Follicle rupture Luteolysis Cervical dilatation Postpartum uterine involution Mammary gland involution Rupture of fetal membranes	Liver cirrhosis Fibrotic lung disease otosclerosis atherosclerosis Multiple sclerosis
Maintenance	**Weakening of matrix**
Remodelling of bone Hair follicle cycle Wound healing Angiogenesis Apoptosis Nerve regeneration Macrophage function Neutrophil function	Dilated cardiomyopathy Epidermolysis bullosa Aortic aneurysm

2.2

MMP Sub-Classes

The MMP family are divided into classes based on in-vitro substrate specificity although there is considerable overlap between groups:

1) Collagenases (MMP, 1, 8, 13) cleave triple helical fibrillar collagens type I, II and III at Gly_{775}-Ile_{776} or Gly_{775}-Leu_{776} site (Billinghurst, Dahlberg et al. 1997).

2) Gelatinases (MMP 2, 9) degrade various substrates such as type IV, V, VII and X collagens, elastins and gelatins (Seltzer, Akers et al. 1990).

3) Stromelysins (MMP, 3, 7, 10, 11) these have a wide range of substrate specificities including laminins, proteoglycans and fibronectin (Okada, Nagase et al. 1986)

4) Elastases (MMP 12). Involved in degradation of elastins as well as proteoglycans, laminin and heparin sulphate (Shapiro, Kobayashi et al. 1993; Gronski, Martin et al. 1997)

5) Membrane type or MT-MMP (MMP 14, 15, 16, 17, 24, 25) are similar in structure to other MMPs however in addition they contain a 75-100 amino acid extension at their c-terminus which includes a hydrophobic region serving as a transmembrane domain (Pei and Weiss 1996), these enzymes unlike other MMPs are expressed as membrane associated enzymes rather than soluble proteins. The MT-MMPs have been shown to degrade gelatine, fibronectin, laminin, proteoglycan and can also activate other MMPs.

Besides the degradation of ECM products, MMPs can also degrade non-matrix proteins such as cytokines, receptors and adhesion molecules which may explain their additional functions such as regulating growth, cell pathways and angiogenesis (Egeblad and Werb 2002)

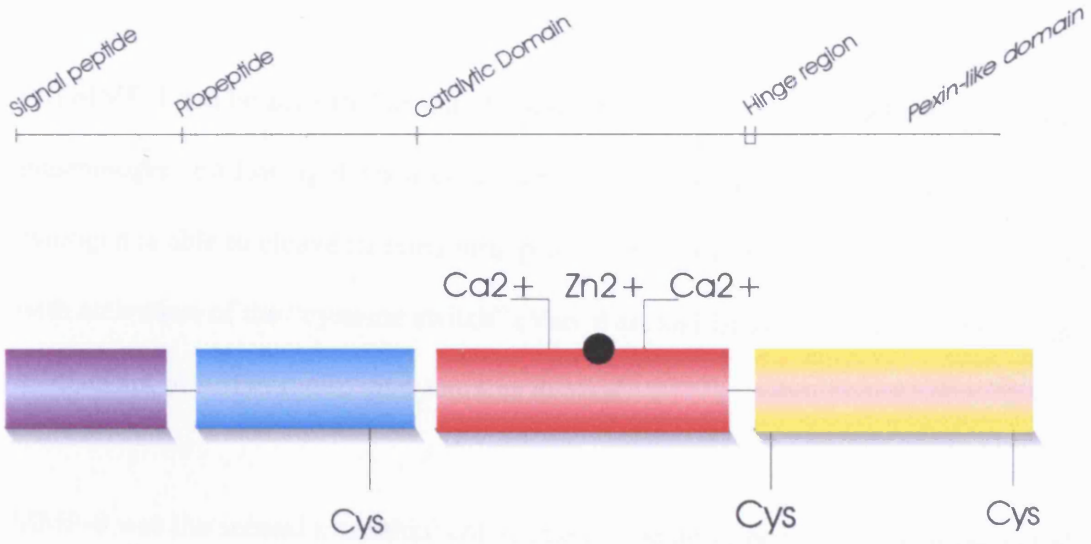
In addition, there exist natural inhibitors of metalloproteinases known as Tissue Inhibitors of Metalloproteinases (TIMPs). The TIMP family are low molecular weight proteins falling into 1 of 4 groups designated TIMP 1-4.

2.3

The Collagenases (MMP-1, -8, -13)

The first vertebrate collagenase identified originated from human fibroblasts (Stricklin, Welgus et al. 1982) and has been designated matrix metalloproteinase -1 (MMP-1). MMP-1 has been the most extensively investigated of the MMP family. MMP-1 and all members of the MMP family appear to have a very similar basic structure composed of a signal peptide, a proenzyme domain, a catalytic domain, hinge region and a hemopexin like c terminus which aids binding.

Figure. 2.1 The basic structure of the MMP family



All MMPs contain Zinc at the catalytic site and require calcium for stabilization and activity. Various studies have shown the importance of the hemopexin domain. MMP-1 lacking this region has no activity against collagens although it still displays activity against other low molecular weight peptides (Knauper, Osthues et al. 1993) and substituting this region with the hemopexin found in MMP-3 also results in loss of collagenase activity (Sanchez-Lopez, Alexander et al. 1993).

MMP-1 is known to degrade types I, II, III and X collagens, type I gelatins, α-1 anti-trypsin and is likely to have many other substrates in addition (Welgus, Jeffrey et al. 1981). This enzyme cleaves the triple helix of collagen at the Gly_{775}-$Leu/Ileu_{776}$ bond (Miller, Harris et al. 1976). MMP-1 as with most MMPs are synthesized and secreted as latent inactive pro-enzymes with a propeptide domain and a unique cysteine containing sequence known as a "cysteine switch", which binds zinc in the catalytic domain rendering the enzyme inactive. Disruption of this bond and removal of the

pro-peptide domain exposes the catalytic region hence leading to activation.(Van Wart and Birkedal-Hansen 1990).

Pro MMP-1 can be activated in vitro by a wide range of enzymes such as trypsin and plasminogen. Following the action of such a proteinase the resulting truncated zymogen is able to cleave its remaining pro regions resulting in mature active enzyme with activation of the "cysteine switch".(Van Wart and Birkedal-Hansen 1990). The mechanism of activation in vivo remains unclear.

MMP-8 was the second interstitial collagenase to be described (Lazarus, Brown et al. 1968). MMP-8 has similar structure to MMP-1 however is significantly glycosylated leading to a higher molecular mass. This enzyme has the unique property of being pre-synthesized and stored in cells (neutrophils) and released when required, other MMPs being synthesized as required. MMP-8 has action on different substrates to MMP-1 with preference for Type I collagen over type III as well as action on type II collagen which is highly prevalent in cartilage. Activation in vitro appears similar to that of MMP-1.

MMP-13 was originally described in rats and subsequently cloned from human breast carcinoma cells (Freije, Diez-Itza et al. 1994) and has predominant action on type II collagen in addition to type I and III. MMP-13 has been extensively investigated in the pathogenesis of rheumatoid arthritis (Burrage, Mix et al. 2006).

2.3

The Gelatinases - (MMP-2 & -9)

MMP-2, also known as Gelatinase A, was first described in 1979 (Liotta, Abe et al. 1979) as an enzyme secreted from metastatic murine tumour which was seen to degrade soluble type IV collagen but appeared to have little action on types I, II an III. MMP-2 has subsequently been shown to have action on gelatine, type V, VII (Seltzer, Eisen et al. 1989) and X collagen (Gadher, Schmid et al. 1989) as well as elastin (Senior, Griffin et al. 1991) via cleavage of several peptide bonds such as Gly-Val, Gly-Leu, Gly-Glu. As with other MMPs, MMP-2 is secreted as a proenzyme and is unique in that it is constitutively expressed by many cells and is usually isolated in complex with TIMP-2. MMP-2 has been implicated in various physiological and pathological processes such as cancer invasion (O'Grady, Dunne et al. 2007; Quaranta, Daniele et al. 2007), arthritis (Burrage, Mix et al. 2006) and peridontitis (Gurkan, Emingil et al. 2007).

MMP-9 or Gelatinase B is the second member of the gelatinase family. After discovery in 1962 of a collagenase which degraded native collagens into discrete fragments, it was observed that this enzyme had no activity against these denatured collagen fragments (gelatins). It was hypothesized that further enzymatic degradation was occurring due to additional agents. In 1974 a protease was identified in human neutrophils which could degrade such peptides (Sopata and Dancewicz 1974). Action against type IV and V collagens in addition to gelatins was subsequently identified (Murphy, Cawston et al. 1981; Murphy, Reynolds et al. 1982) and such activity has

also been shown in pig neutrophils (Murphy, Ward et al. 1989) and human macrophages (Hibbs, Hoidal et al. 1987).

MMP -2 & -9 have similar structure to that described above however in addition they possess a fibronectin-like domain inserted into the catalytic region.

In most cells MMP-9 is secreted complexed with TIMP-1 and requires activation which can be induced by various enzymes such as trypsin, elastases and other MMPs. Unlike MMP-2 which is constitutively expressed by many cells, MMP-9 is normally only expressed by trophoblasts, osteoclasts, neutrophils and macrophages. However expression can be induced in a variety of cells by growth factors, cytokines and ECM molecules.

2.4

The Stromelysins (MMP, 3, 7, 10, 11)

MMP-3 and MMP-10, also known as stromelysin 1 & 2, degrade various components of the extracellular matrix but not the triple helix regions of interstitial collagens. MMP-3, first purified by Galloway et al in 1983 (Galloway, Murphy et al. 1983) from rabbit bone was initially named proteoglycase due to its action on cartilage proteoglycans. MMP-3 and -10 have a similar structure to that described above and are secreted as pre-proenzymes which require activation via a number of proteases. Activated MMP-3 and -10 can also act on other proMMPs (MMP-8 (Knauper, Wilhelm et al. 1993), -9(Ogata, Enghild et al. 1992), and -13) to cause activation although auto-activation of their own pro-enzymes does not appear to occur. Stromelysins are inhibited by TIMPs which play an important part in their regulation (see below).

2.5

Elastases – (MMP-12)

Macrophage elastase (MMP-12) was identified by Werb et al in 1975 (Werb and Gordon 1975) in mouse peritoneal macrophages. The name elastase reflects its ability to degrade insoluble elastin. In addition macrophage elastase may cleave various other distinct substrates. While its structure is similar to other MMPs and it is inhibited by TIMPs, MMP-12 is unique in that it is predominantly expressed in macrophages, hence its terminology. MMP-12 activation may occur secondary to enzymes such as trypsin, plasmin and several MMPs, in particular MMP-3.

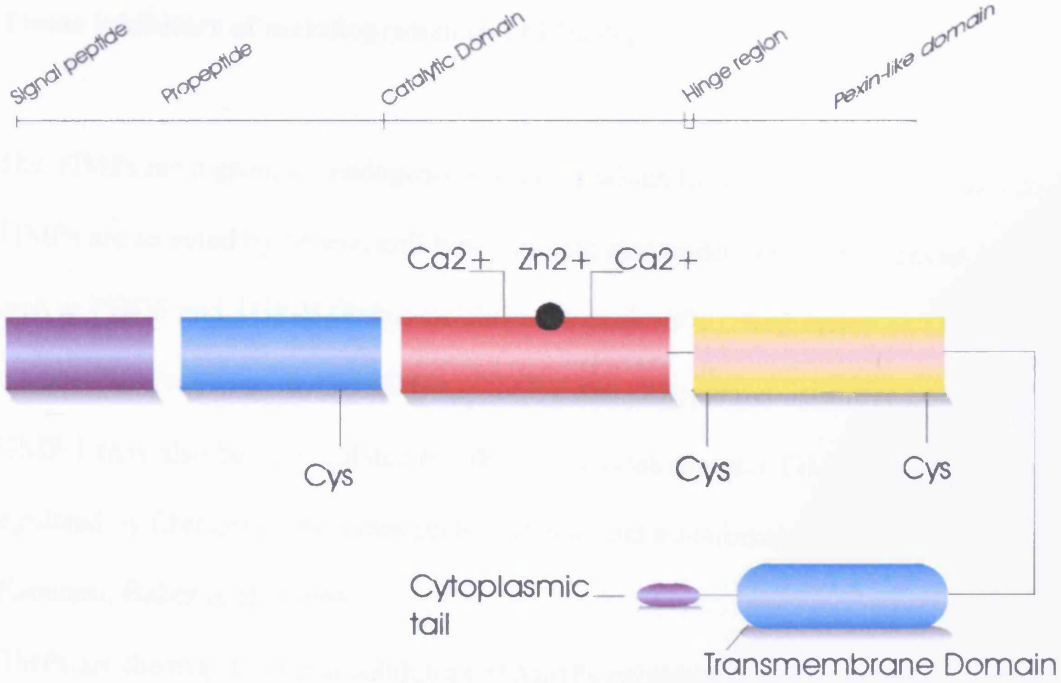
Human macrophage elastin has been associated with several macrophage mediated destructive diseases including atherosclerosis, cancer and COPD.

2.6

Membrane type or MT-MMP (MMP 14, 15, 16, 17, 24, 25)

In contrast to the above soluble MMPs which are secreted into the pericellular and extracellular environment, membrane type MMPs contain a transmembrane hydrophobic region followed by a short cytoplasmic tail.

Figure. 2.2 The structure of membrane type metalloproteinases



As with other MMPs, MT-MMPs require pro-enzyme activation which can occur in vitro due to a variety of enzymes such as plasmin (Okumura, Sato et al. 1997) suggesting that the pro-enzyme may be transported to the cell surface followed by activation by extracellular enzymes. However knowledge regarding in vivo activation is limited.

MT-MMPs are involved in activation of other enzymes such as pro-gelatinase A to gelatinase A (MMP-2) (Butler, Will et al. 1997). In addition MT-MMPs have action on other enzymes and peptides such as proteinases, collagens, cartilage aggrecans and laminin. These data suggest that MT-MMPs may regulate extracellular matrix integrity both through a direct action on its components and via activation of other enzymes known to influence ECM biology.

2.7

Tissue inhibitors of metalloproteinases (TIMPs)

The TIMPs are a group of endogenous proteins which firmly regulate MMP activity. TIMPs are secreted by several cell types and are also under control by various factors such as PDGF and TGF-B (Fabunmi, Sukhova et al. 1998). Regulation of TIMPs appears different from that of MMPs. TIMP-1 and -2 appear constitutive however TIMP-1 may also be up-regulated by fibrogenic cytokines and TIMP-3 may be up-regulated by fibrogenic cytokines such as PDGF and transforming growth factor-B (Fabunmi, Baker et al. 1996)

TIMPs are the major cellular inhibitors of MMPs exhibiting varying efficacy against different members as well as differing tissue expression and modes of regulation. Four mammalian TIMPs have been cloned and characterised designated TIMP 1 to 4. All TIMPs inhibit MMPs with different specificities (Gomez, Alonso et al. 1997) by binding to the active site of MMP in a 1:1 molar ratio and forming non-covalent bonds with very high Kd. TIMPs can also bind MMPs at the amino terminus to prevent activation. These secreted proteins are therefore thought to regulate the remodelling effects of MMPs.

The TIMPs share a common structure with 6 disulphide bonds and comprise a 3 loop N terminal domain and an interacting 3 loop C sub-domain and are variably glycosylated having molecular weights of 21 to 28 KDa. Although the TIMPs have similar basic structures, they exhibit distinctive biochemical properties and expression patterns. TIMP-1 is known to be soluble and has association with Pro-MMP-9 but lacks action on MMP-1, -2, -3, -5 and MMP-19 whereas TIMPs 2-4 all exhibit actions on these MMPs. TIMPs -2 and -4 are expressed as soluble proteins but may also have cell surface association and TIMP-3 is predominantly localised to the extracellular matrix bound to heparin sulphate proteoglycans (Yu, Yu et al. 2000).

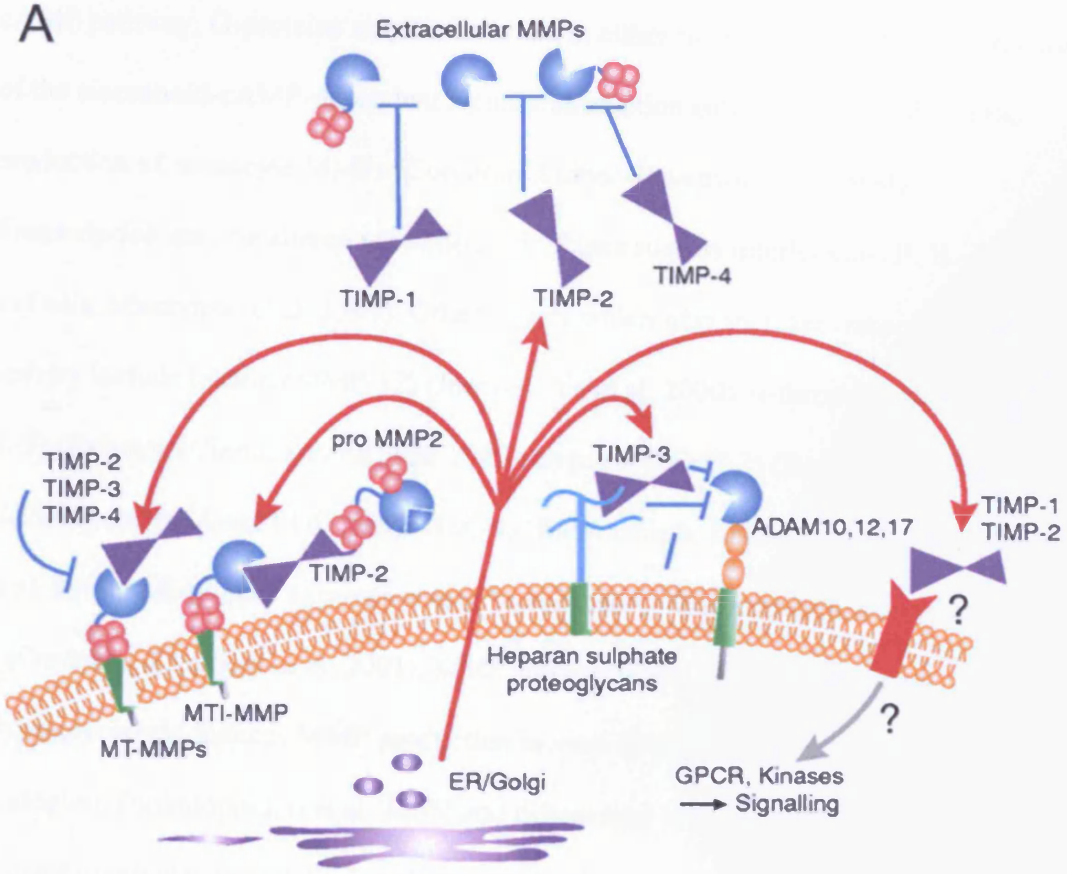
In addition to blocking the action of MMPs numerous studies have reported other activities such as cell growth promotion (Bertaux, Hornebeck et al. 1991), anti-apoptotic and anti-androgenic activity (Lambert, Dasse et al. 2004) which appear independent of their metalloproteinase inhibition activity. TIMP-1(Gasson, Golde et al. 1985) and TIMP-2 (Stetler-Stevenson, Bersch et al. 1992) have been shown to have erythroid potentiating activity and TIMP-2 stimulates the growth of lymphoma cells (Hayakawa, Yamashita et al. 1994). More recently both TIMP-1 and TIMP-2 have been shown to stimulate osteoclasts involved in bone resorption (Sobue, Hakeda et al. 2001). The mechanism by which TIMPs exert their effects independent of metalloproteinase inhibition is however to date poorly described.

TIMP knock-out models have addressed the biological roles of TIMPs. Mice deficient in TIMP-3 develop emphysematous changes to the lungs (Leco, Khokha et al. 1994). Of more relevance to the current studies, Fedak et al (Fedak, Smookler et al. 2004) observed that mice deficient in TIMP-3 developed LV dilatation, cardiomyocyte

hypertrophy and contractile dysfunction. Its absence also resulted in cardiac matrix disruption, increased MMP-9 activity and altered inflammatory cytokine expression.

TIMP activity may be altered in both experimental and human heart failure. Fedak et al (Fedak, Altamentova et al. 2003) observed reductions in TIMP-3 in human subjects with congestive cardiac failure and recovery of cardiac function in human hearts is associated with an upregulation of TIMP activity (Li, Feng et al. 2001; Walther, Schubert et al. 2001). More recently Ahmed et al (Ahmed, Clark et al. 2006) have shown that human subjects with hypertensive heart failure express increased TIMP-1 activity with a TIMP-1 level >1200ng/ml being predictive of CHF. Subjects with hypertension but no heart failure exhibited TIMP activity similar to controls suggesting that LV remodelling and not hypertension per se is associated with altered TIMP activity. In this study it was not possible to state whether the increased TIMP-1 levels seen in subjects with heart failure contributed to the LV remodelling process or was a secondary response.

Figure. 2.3 The mechanisms of action of TIMPs. TIMP's are largely MMP inhibitors modulating the activity of soluble, matrix bound and cell associated MMPs. TIMP-3 is an extracellular matrix protein which binds heparin sulphate and is an inhibitor of the function of some membrane bound ADAMs. TIMP-2 acts in conjunction with MT1-MMP as a receptor for the pro-form of MMP-2. TIMP-1 and TIMP-2 may also have receptors linked to intracellular signalling pathways regulating cell behaviour.



Taken from: Baker, A. H., D. R. Edwards, et al. (2002). "Metalloproteinase inhibitors: biological actions and therapeutic opportunities." J Cell Sci ***115****(Pt 19): 3719-27.*

2.8

MMP Regulation

MMP activity can be regulated at 3 levels i) altered gene transcription, ii) altered pro-enzyme activation iii) via interaction with inhibitors of MMP.

2.8.1: Gene transcription:

Altered gene transcription of MMPs is thought to be through a prostaglandin E_2 - cAMP pathway, G-proteins may be involved in either the enhancement or suppression of the eicosanoid-cAMP-dependent signal transduction pathway that results in the production of monocyte MMPs (Corcoran, Stetler-Stevenson et al. 1994). Transcription may be altered via various cytokines such as interleukin-1B, IL-6, TNF-α.(Galis, Muszynski et al. 1995). Other factors which may increase transcriptional activity include insulin (MMP-12) (Jormsjo, Ye et al. 2000), α-thrombin (MMP-1, -2 &-3) (Duhamel-Clerin, Orvain et al. 1997), hypoxia (MMP-2) (Ben-Yosef, Lahat et al. 2002) and oxidised LDL (MMP-1 &-9) (Rajavashisth, Liao et al. 1999; Xu, Meisel et al. 1999). MMP gene expression may be reduced by indomethacin, steroids and IL-4 (Creemers, Cleutjens et al. 2001). MMP activity is also altered in diabetes. Hyperglycaemia induces MMP production in vitro (Death, Fisher et al. 2003; Kadoglou, Daskalopoulou et al. 2005) and differential MMP activity may be causative in cerebrovascular remodelling and increased risk of stroke and cerebral ischaemia in diabetes(Harris, Hutchinson et al. 2005). Altered MMP activity occurs in children with type I diabetes mellitus(Derosa, Avanzini et al. 2005) and in adult subjects with type I (Shiau, Tsai et al. 2006) and type II diabetes mellitus (Sampson, Wall et al. 2006). In addition treatment with the oral hypoglycaemic agent rosiglitazone is

associated with reduction in MMP-9 (Goldstein, Weissman et al. 2006) and cardiovascular risk reduction in diabetic patients is associated with reduced MMP activity (Tayebjee, Lim et al. 2004). Altered MMP activity is seen in coronary artery sections in patients with, compared to those without, diabetes (Chung, Luo et al. 2007). Further, glucose may alter and augment MMP activity in endothelial cells (Kadoglou, Daskalopoulou et al. 2005) and monocyte-derived macrophages in vitro (Death, Fisher et al. 2003). In a rat model of diabetes altered MMP expression occurs and may be causative in cerebrovascular remodelling and increased risk of stroke (Harris, Hutchinson et al. 2005). The mechanism by which diabetes stimulates MMP production is also likely to be via increased gene expression. Pre-exposure of histiocytes to high glucose in-vitro markedly increases lipopolysaccharide-stimulated MMP-1 secretion and mRNA expression through the nuclear factor kappaB and mitogen-activated protein kinase cascades(Maldonado, He et al. 2004).

Altered gene transcription may also be due to promoter region polymorphisms such as the C to T polymorphism in the promoter region of MMP-9 which is associated with increased expression of this enzyme (Zhang, Ye et al. 1999). In addition the 5A variant polymorphism in the promoter region of MMP-3 has been shown to be associated with increased expression compared to the 6A variant (Ye, Eriksson et al. 1996). Both have been shown to have effects on clinical phenotype.

2.8.2: Pro-enzyme activation:

Pro-enzyme activation is the second regulatory step of metalloproteinase activity. This may occur both intra and extra-cellular. Activation of the cysteine switch can be achieved by proteases such as plasmin (Van Wart and Birkedal-Hansen 1990), trypsin

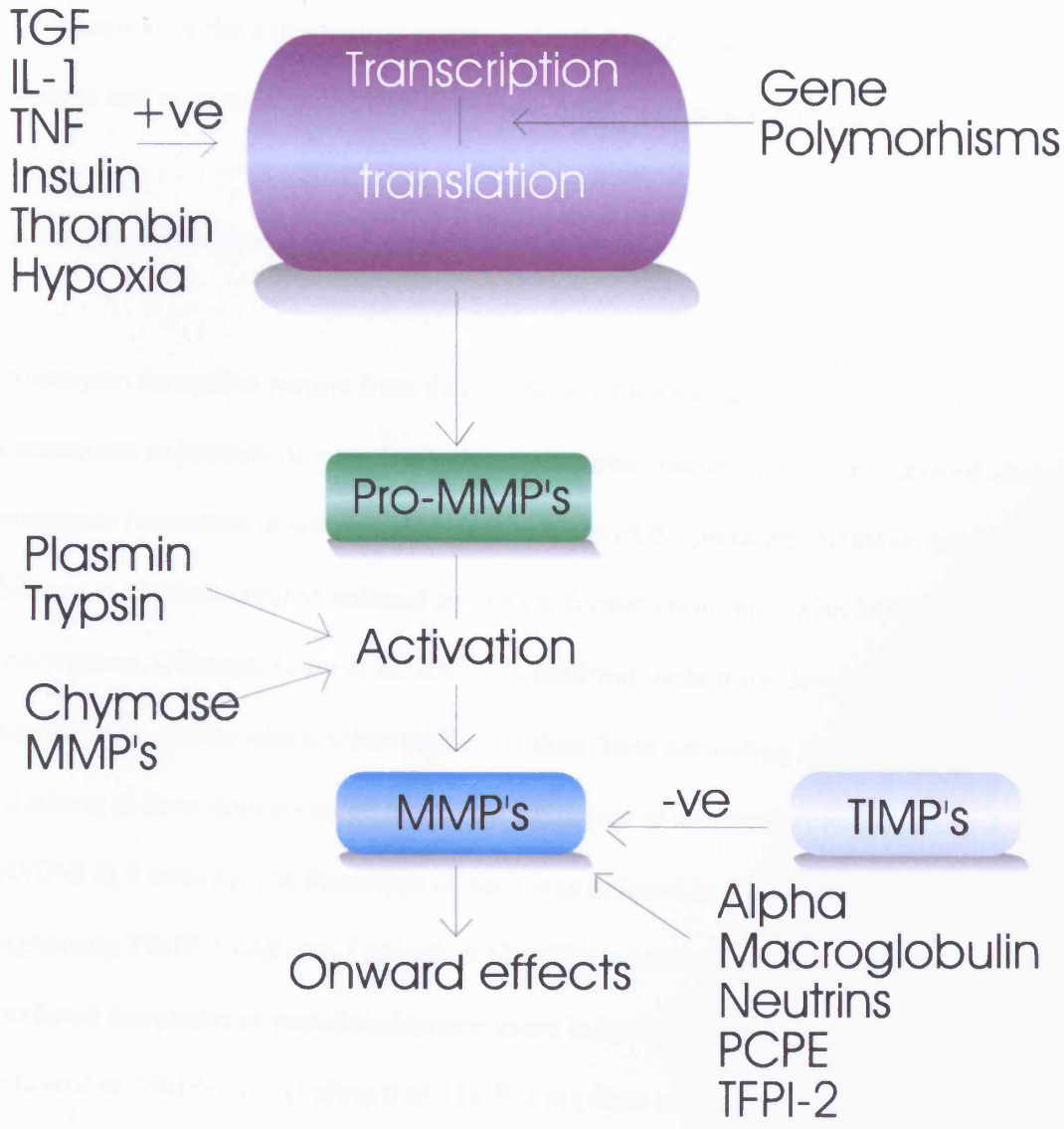
and mast cell derived chymase (Lees, Taylor et al. 1994). Other MMPs such as -3, -7 - 10 and Membrane type (MT)-MMPs (Sternlicht and Werb 2001) are also involved in activation, the most widely studied being activation of MMP-2 at the cell surface via membrane type MMP especially MT1-MMP (Strongin, Collier et al. 1995; Sternlicht and Werb 2001). TIMP-1 binds to the catalytic site of MT1-MMP acting as a receptor for MMP-2 via interaction with the non catalytic COOH terminus, a second MT1-MMP then cleaves the MMP-2 prodomain. It therefore appears that activation of MMPs can form a complex cascade with activation of other enzymes leading to varying outcomes. Products such as reactive oxygen species produced during cell death may be involved in the initiation of this cascade following vascular injury (Rajagopalan, Meng et al. 1996).

2.8.3 Inhibitors of MMP:

Interaction with inhibitors of MMPs also influences MMP activity. The structure and role of the natural inhibitors of MMPs - Tissue Inhibitors of Metalloproteinases (TIMPs) is discussed above. In addition other molecules may block the actions of MMPs. α2 macroglobulin is a 772 kDa proteinase inhibitor comprising of 4 nearly identical disulphide bonded domains. It is synthesized predominantly in the liver and may inhibit many proteases including MMPs. While α macroglobulin is likely to be the predominant inhibitor of MMPs in plasma, its role in the extracellular matrix is poorly understood. In addition, the actions of MMPs may be inhibited by other molecules such as the TIMP like proteins- netrins, secreted frizzled-related protein and type 1 collagen C-proteinase enhancer protein (PCPE) (Banyai and Patthy 1999).

The serine proteinase inhibitor - tissue factor pathway inhibitor 2 (TFPI-2) also inhibits MMP-1, -2, -9 and –13 (Herman, Sukhova et al. 2001).

Figure. 2.4 . The regulation pathways of MMP activity



2.9

Matrix Metalloproteinases in Cardiovascular Disease

Degradation of the extracellular matrix is central to several aspects of cardiovascular disease and consequently MMPs have been implicated in these processes.

2.9.1: Aneurysm formation

Aneurysm formation results from disregulation of the extracellular matrix with consequent expansion of vessel size. Several animal studies have demonstrated altered aneurysm formation in subjects deficient in areas of the metalloproteinase family. Silence et al demonstrated reduced aneurysm formation in mice with MMP-3 gene inactivation. (Silence, Lupu et al. 2001). In addition these mice developed significantly greater atherosclerotic lesions than those exhibiting MMP-3 production. Allaire et al have demonstrated aneurysm formation in association with increased MMP-2 & 9 activity, the formation of such was reduced in those animals over-expressing TIMP-1 (Allaire, Forough et al. 1998) whereas Lemaitre et al showed increased formation of pseudo-microaneursms in apolipoprotein E knockout mice deficient in TIMP-1 concluding that TIMP-1 prevents medial degradation through its ability to inhibit MMPs (Lemaitre, Soloway et al. 2003). Wilson et al (Wilson, Anderton et al. 2006) observed increased MMP-8 and -9 in subjects with ruptured versus un-ruptured abdominal aortic aneurysms whereas MMP-2 appears ubiquitous in subjects with stable aortic aneurysms (Goodall, Crowther et al. 2001) and the

MMP-2 – TIMP-1-MT-1 complex appears localised to the media of the arterial wall (Crowther, Goodall et al. 2000).

2.9.2: Arterial remodelling

Metalloproteinases may not only be involved with the enlargement of vessels leading to aneurysm formation. Evidence supports their involvement in arterial remodelling due to neointimal hyperplasia leading to reduction of the vessel lumen (constrictive remodelling) as occurs after direct trauma via angioplasty or atherectomy. The normal intima of large arteries is a highly complex structure consisting of a single layer of endothelial cells seated on basement membrane. The intima is also thickened by a hyaluronan rich matrix with sparse vascular smooth muscle cells (VSMC). The basement membrane contains type IV collagen, laminin and proteoglycans. The normal media contains contractile VSMC's, resident macrophages and fibroblasts surrounded by an interstitial matrix of type I and III collagen, a variety of glycoproteins and elastin arranged in distinct layers. The adventitia contains fibroblasts, small blood vessels and fat in a loose interstitial matrix.

In animal studies, intimal hyperplasia appears to be due to migration of pre-existing smooth muscle cells from the media and since these cells are surrounded by ECM, this migration and hence constrictive remodelling requires the breakdown of the ECM. Lijnen et al (Lijnen, Lupu et al. 1999) investigated the temporal and topographic expression of MMPs after perivascular electric injury in wild type and urokinase deficient mice. This group demonstrated increased MMP-2, -3, -9, 12 and -13, activity after such an insult and suggested varying origins to the individual

enzyme types. Likewise Webb et al (Webb, Henney et al. 1997) used an adenoviral vector containing the human TIMP-1 cDNA to induce increased expression of TIMP-1 in a rat model of vascular injury. Infection of rat SMCs with Av1.TIMP1 reduced migration of smooth muscle cells in vitro by 27% in injured rat carotid artery with the neointimal area 14 days after injury showing a 30% reduction in volume compared with controls.

The development of giant cell arteritis (GCA), a condition of medium sized and large arteries which involves not only breakdown of the internal elastic lamina of vessels but also intimal hyperplasia leading to luminal narrowing has also been shown to involve metalloproteinases. Rodriguez-Pla et al (Rodriguez-Pla, Bosch-Gil et al. 2005) demonstrated the presence of MMP-9 but not MMP-2 more frequently in temporal artery biopsy samples from subjects with confirmed GCA compared to controls. They detected MMP-2 and -9 in giant cells and macrophages within the media and intima particularly adjacent to the internal elastic lamina region and in myofibroblasts and smooth muscle cells of the media and intima. The expression of MMP-9 but not MMP-2 was seen in the smooth muscle layer of the vasa vasorum in all layers of the arterial wall. This group concluded that MMP-9 expression is associated with the histological diagnosis of GCA and is likely to contribute to the pathophysiology of its development. Interestingly GCA is associated with other clinical complications of large vessel arteritis such as aortic aneurysm, aortic valve incompetence and upper limb claudication in around 20% of cases suggesting a possible systemic effect of metalloproteinase activity (Brack, Martinez-Taboada et al. 1999).

MMP activity is also up-regulated in other forms of vasculitis. Matsuyama et al (Matsuyama, Sakai et al. 2003) investigated Takayasu Arteritis, a chronic vasculitis that predominantly affects large elastic arteries in women of reproductive age leading to disruption of vessel architecture. This group demonstrated elevated levels of MMP-2 in subjects over controls and also showed a correlation between MMP-3 and -9 levels and disease activity with higher levels being associated with active disease and suppression in those subjects classified as being in remission. Senzaki et al (Senzaki, Masutani et al. 2001) have also shown that patients with Kawasaki disease and coronary arterial lesions have higher MMP-9 levels compared to controls with other febrile illnesses.

2.10

Metalloproteinases and Coronary Heart Disease

A large amount of interest has arisen with regards to metalloproteinase activity and the pathogenesis of atherosclerosis and plaque rupture involved in coronary heart disease. In the 19th century Virchow first described the key histological features of thickening of the intima in atherosclerosis i.e. the presence of cholesterol in foam cells. Steinberg et al (Steinberg, Parthasarathy et al. 1989) based on work by Goldstein and Brown formulated the, "modified LDL hypothesis", to explain the uptake of cholesterol into cells, and Ross and Glomset (Ross and Glomset 1973) offered the "response to injury", hypothesis of atherosclerosis formation which explained the formation of fibrous caps and localisation of atherosclerotic lesions in areas of disturbed flow. According to this hypothesis the vessel wall responded by endothelial dysfunction and VSMC proliferation when injured mechanically by

abnormal flow or via noxious agents. Atheromatous plaques depend on recruitment of inflammatory cells into the vessel wall and production of pro-inflammatory mediators such as interleukin-1 and TNF-α which encourage smooth muscle cell accumulation, lipid deposition and alteration of the plaque composition. MMPs facilitate migration of both smooth muscle cells and inflammatory macrophages hence promoting the development of the atheromatous plaque.

2.10.1: Plaque Rupture

Myocardial infarction itself has been shown to not only involve the above process of progressive luminal narrowing but also requires an acute event superimposed on this chronic process involving plaque rupture or plaque erosion (Davies and Thomas 1984; Davies and Thomas 1985; Falk, Shah et al. 1995). Plaque rupture involves inflammation and disruption of the extracellular matrix of the fibrous cap, again indicating a potential role of MMPs. Although several animal models of intimal hyperplasia exist, models representing in vivo plaque rupture have been difficult to develop. In the absence of such models much work has used surrogate markers of atherosclerotic progression such as lesion size and extension; and plaque composition. Despite these difficulties, there exists evidence to implicate MMPs in the disruption of plaque fibrous cap leading to acute myocardial infarction. The fibrous cap of vulnerable atherosclerotic lesions is composed of types I and III collagen as well as elastin and proteoglycans. The accumulation of macrophage derived foam cells in atherosclerotic lesions correlates with local release of MMPs and fibrous cap thinning (Galis, Muszynski et al. 1995; Moreau, Brocheriou et al. 1999) Henney et al (Henney, Wakeley et al. 1991) demonstrated increased levels of MMP-2 mRNA in atherosclerotic plaques compared to normal arteries and localised this to both

macrophages and VSMC's and Noji et al have demonstrated MMP-2 activity to be closely correlated with the degree of plaque calcification (Noji, Kajinami et al. 2001). In a second study, Galis et al (Galis, Sukhova et al. 1994) demonstrated the presence of MMP-1, -3 and -9 in macrophages and VSMC's at the vulnerable shoulder regions of plaques, a site where rupture is more likely to occur. Since this work, several studies have demonstrated the presence of a variety of MMPs in atherosclerotic plaques and several polymorphisms of MMP genes have been shown to affect lesion development and clinical outcomes (Ye, Watts et al. 1995; Zhang, Ye et al. 1999). Other studies have related MMP levels to clinical and histological features of plaque instability. Schonbeck et al demonstrated MMP-11 confined to advanced atherosclerotic plaques as opposed to fatty streaks (Schonbeck, Mach et al. 1999) and Nikkari observed a correlation of MMP-1 with the percentage of lipid core occupied by haemorrhage (Nikkari, Geary et al. 1996). Loftus et al observed an increased level of MMP-9 expression in carotid endarterectomy tissue from patients with recent clinical evidence of instability (Loftus, Naylor et al. 2000), similar was also seen in patients with unstable versus stable coronary artery disease (Brown, Hibbs et al. 1995). Increased numbers of macrophages are seen in areas of plaques prone to rupture with isolated macrophages expressing increased MMPs over TIMPs (Chase, Bond et al. 2002). Also in a study by Galis et al (Galis, Sukhova et al. 1995), rabbit macrophages of atherosclerotic lesion origin expressed MMP-1 and -3 whereas alveolar macrophages did not and a reduction in MMP levels correlated with reduction in macrophages after discontinuation of high cholesterol diet. Infiltrating neutrophils have been shown to be an early source of MMP-9 after reperfusion of cardiac tissue and these may localise MMP activity (Lindsey, Wedin et al. 2001). MMP-7 and MMP-12 are also thought to play an important role in plaque rupture

secondary to their production in atherosclerotic plaques by foam cells lying along the perimeter of lipid rich cores and more recently MMP-8 which has a high affinity for collagen type I was reported to be expressed by human vascular endothelial cells, smooth muscle cells and macrophages (Herman, Sukhova et al. 2001) with its activity being increased in the shoulder regions which are most vulnerable to rupture. These studies however cannot establish whether the increased MMP expression is a cause or effect of the plaque instability.

Observation of altered metalloproteinase activity in subjects with acute coronary syndrome has also added weight to their involvement in this pathology. Kai et al (Kai, Ikeda et al. 1998) measured MMP-2 and MMP-9 levels in patients with acute myocardial infarction, unstable angina, stable angina and controls, observing increased MMP-2 levels in subjects with AMI or unstable angina. MMP-9 was initially increased in unstable angina and AMI however these levels decreased to the same as controls after 7 days follow up. Our own group has observed a temporal profile of MMP-2 and MMP-9 activity post acute myocardial infarction (Squire, Evans et al. 2004). In addition MMP-9 and TIMP-1 is increased in subjects with angiographically identified lesions in the left anterior descending artery versus normal controls in those subjects with AMI and unstable angina but not high grade lesions without symptoms (Inokubo, Hanada et al. 2001) and MMP-2 activity but not MMP-1 was elevated over controls in subjects with ST elevation AMI (Hojo, Ikeda et al. 2001).

MMP inhibition studies have also increased the evidence for the involvement of MMP in cardiovascular disease. Rouis et al (Rouis, Adamy et al. 1999) used an adenovirus that caused expression of TIMP-1 in ApoE knockout mice fed on high cholesterol diet. They observed a reduction in aortic plaque area compared to controls and observed features consistent with increased plaque stability. Other similar knockout studies have suggested roles for MMP-3 (Silence, Lupu et al. 2001) and MMP-9 (Luttun, Lutgens et al. 2004).

2.10.2: Platelet Aggregation

Metalloproteinase activity has also been implicated in the process of platelet aggregation occuring following plaque rupture. This process happens through the activation of von Willebrand factor (vWF) through its interaction with the platelet receptors glycoproteinase Ib (GPIb) and glycoproteins IIb/IIIa (GPIIb/IIIa). MMP-1 is located at the plasma membrane of platelets where its is involved in priming platelets for aggregation (Galt, Lindemann et al. 2002). MMP-2 has also been observed in the cytosol of human platelets from where it is translocated to the platelet surface and released during platelet aggregation (Sawicki, Sanders et al. 1998) and may also potentiate vWF-induced GPIb expression and platelet aggregation (Radomski, Stewart et al. 2001). MMP-2 also increases the pro-aggregation effects of collagen on platelets (Sawicki, Salas et al. 1997).

In contrast to the above other MMPs or altered concentrations of MMPs have been showed to have an inhibitory effect on platelet aggregation. Both excessive MMP-2 and MMP-9 have been shown to inhibit platelets (Sawicki, Salas et al. 1997; Fernandez-Patron, Martinez-Cuesta et al. 1999). Alternatively the association between

MMPs and platelets aggregation may be due to MMP accumulation secondary to platelet activity.

2.11

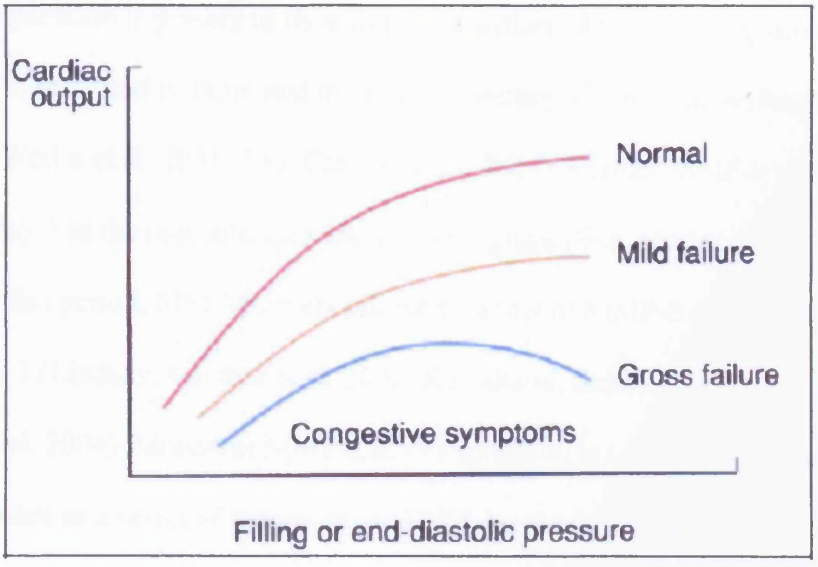
Metalloproteinases and LV Remodelling

Considering the known actions of MMPs in the extracellular space, it is perhaps unsurprising that these enzymes have been implicated in the remodelling process that occurs in the left ventricle in certain physiological and pathological processes. The cardiac ECM is composed of basic elements such as collagen and elastin and more specialized proteins such as fibrillin, fibronectin, proteoglycans and matricellular proteins (Scott-Burden 1994). The interstitial collagen fibres form a network providing supportive scaffolding for myocytes and blood vessels (Borg. T 1993). In the normal heart ECM synthesis and degradation are continuous and tightly regulated processes. Adverse ventricular remodelling is the process of changing shape, size and function of the ventricle due to disruption of these ECM components regulated by mechanical, neurohormonal and genetic factors (Pfeffer and Braunwald 1990; Rouleau, de Champlain et al. 1993). Remodelling may be physiological as occurs in normal growth or pathological in response to injury through myocardial infarction, cardiomyopathy, valvular heart disease or hypertension.

Acute myocardial infarction results in rapid loss of functional myocardial tissue. This triggers a complicated and unique pattern of ventricular changes involving the infarct border regions and non-infarcted tissue (Chapter 1). The loss of functional tissue leads to a marked increase in ventricular load triggering a cascade of reparative processes leading to ventricular enlargement, hypertrophy and the formation of a collagen scar.

The process of ventricular enlargement will continue until the loading forces of the ventricle are counterbalanced by the tensile strength of the scar in conjunction with the preserved myocardial tissue. The process of ventricular enlargement allows increased force of contraction as stated in Starlings law of contraction. However progressive changes eventually lead to the failure of the left ventricle and clinical heart failure. The time period over which this process occurs varies greatly between subjects and is dependant on many factors such as infarct size, revascularization, and other therapeutic intervention.

Figure. 2.5 Starling Curves to show the relationship between cardiac output and end diastolic pressure in normal versus heart failure subjects



Adverse left ventricular (LV) remodelling represents one of the most prognostically significant consequences of acute myocardial infarction (chapter 1). The degree of left ventricular remodelling, in particular the extent of changes in left ventricular volume, correlates closely to the extent of left ventricular dysfunction and the likelihood of the development of CHF. The consequences of remodelling are progressive ventricular enlargement and impairment of contractile function (Bolognese and Cerisano 1999). Changes in the extracellular collagen matrix of the myocardium are central to the

remodelling process after AMI and evidence increasingly implicates members of the MMP family in the process of left ventricular remodelling after AMI

2.11.1: Animal Studies

Several animal studies have lead authors to suggest that MMP production may be the final common pathway in LV remodelling.

Temporal Profiles:

A temporal profile of metalloproteinase release occurs following experimental AMI. MMP-9 expression is present in its active form within 24hrs following myocardial infarction in mice and is expressed in invading neutrophils and macrophages (Lindsey, Wedin et al. 2001; Tao, Cavasin et al. 2004). Further MMP-9 activity occurs at day 3 in the non-infarcted and border regions (Sun, Dawood et al. 2004). Following this period, MMP-9 levels fall while levels of MMP-2 increase to peak at around day 7 (Lindsey, Gannon et al. 2002; Kawakami, Saito et al. 2004; Tao, Cavasin et al. 2004). Moreover MMP-2 & -9 expression is temporally related to cardiac rupture as a result of inappropriate ECM degradation (Tao, Cavasin et al. 2004). MMP-3 activity is also up regulated following experimental AMI. In rabbits MMP-3 expression reaches its peak within 4 days and remains elevated to 14 days post event (Romanic, Burns-Kurtis et al. 2001). In rats MMP-1 mRNA increases at the infarct site and is reflected in an alteration of MMP-1 activity likely of fibroblast origin (Cleutjens, Kandala et al. 1995; Lu, Zhang et al. 2004).

MMP localisation:

In addition to the temporal pattern of MMP release post MI other data also implicate their involvement in left ventricular remodelling. Animal studies using a molecular probe to image expression of MMPs in the heart after AMI have shown that both MMP-2 and MMP-9 localise to the infarct region post event, maximal at 1 to 2 weeks and persisting to 4 weeks with early expression of MMP-9 (2-4days) and later expression of MMP-2 (1-2 weeks). In addition, high levels of MMP-9 are also seen in the non infarcted regions and dual labelled confocal microscopy also shows co-localization of MMP-9 activity with circulating leukocytes suggesting a potential source (Chen, Tung et al. 2005).

Knockout Models:

Targeted deletion of MMP-9 attenuates left ventricular enlargement and collagen accumulation in knockout models (Ducharme, Frantz et al. 2000). This study using mice deficient in MMP-9 showed that changes in LV end-diastolic and systolic volumes were decreased compared to wild type mice with differences persisting to 15 days post AMI. MMP-9 knockout mice also had less collagen accumulation in the infarcted region and showed enhanced expression of MMP-2, MMP-13 and TIMP-1 and reduced infiltrating macrophages. In addition MMP-9 deficiency has been shown to protect against cardiac rupture in a mouse model (Heymans, Luttun et al. 1999). In contrast, mice with targeted deletion of the TIMP-1 gene show spontaneous LV dilatation (Roten, Nemoto et al. 2000) and loss of TIMP-3 function in mice leads to spontaneous LV dilatation suggesting that loss of MMP control may exacerbate LV dilatation, myocyte hypertrophy and contractile dysfunction (Fedak, Smookler et al.

2004). These results however do not necessarily imply that MMP inhibition will directly lead to improvements in LV dilatation, indeed MMP inhibition may lead to a compensatory over-expression of alternative MMPs. MMP-9 deficient mice show increased expression of both MMP-3 and MMP-13 in ventricular tissue compared to wild type mice (Ducharme, Frantz et al. 2000).

MMP Inhibition:

Pharmacological MMP inhibition has been shown to reduce ventricular dilatation in several animal models. Both broad spectrum and selective MMP inhibitors have been investigated and have been shown to exert effects on LV remodelling. Rhode et al were the first to demonstrate that in vivo broad spectrum MMP inhibition attenuates left ventricular dilatation and dysfunction 4 days after AMI (Rohde, Ducharme et al. 1999) and in a pig model MMP inhibition at day 5 post AMI attenuated LV dilatation at 14 days to 8 weeks (Mukherjee, Brinsa et al. 2003). Broad spectrum MMP inhibition also attenuates LV enlargement in pacing induced congestive cardiac failure (Spinale, Coker et al. 1999), in rats with hypertensive heart failure (Li, Simon et al. 2000) and in a rat model of fluid overload induced heart failure (Chancey, Brower et al. 2002). Whether these effects are due to the broad spectrum MMP inhibition or effects on specific MMPs is difficult to conclude from these studies and further studies have used selective MMP inhibitors to attempt to improve understanding of this complex situation.

Selective MMP inhibition indeed has also been shown to have effects on LV remodelling. Lindsey et al (Lindsey, Gannon et al. 2002) using a selective MMP inhibitor with no effect on MMP-1 in a rabbit model of AMI demonstrated attenuation

in LV remodelling at 4 weeks post AMI with decreased infarct wall thinning. Interestingly the MMP inhibition group also had increased neovascularization in the subendocardial layer of the infarct with increases in capillaries and arterioles. In a pig model of AMI, MMP inhibition without action on MMP-1 or -7 had beneficial effects on LV remodelling (Yarbrough, Mukherjee et al. 2003). In view of the above MMP inhibition has been suggested as a therapeutic target in the failing heart (Woessner 1991). However the later above study (Yarbrough, Mukherjee et al. 2003) showed that the timing of MMP inhibition also exerts effects on outcome. Animals that had early (3 days before) versus late (3 days post) MMP inhibition had altered responses. All animals had a reduction in LV chamber size compared to controls however stroke volume, ejection fraction and fibrillar collagen content within the remote and border regions of the pre-MI MMP inhibition group differed from the post-MI inhibition group. Other data suggest that timing of MMP inhibition may have important effects on LV remodelling. In a mouse model of AMI, Spinale et al demonstrated that although short term MMP inhibition reduced ventricular dilatation as compared to controls, prolonged inhibition was associated with an increased mortality and increased ventricular dilatation (Spinale, Escobar et al. 2006). These results again suggest that with respect to pharmacological modulation of MMP activity there may be a temporal window in which benefits may be obtained.

2.11.2: Human Studies

MMP expression is altered in humans with heart failure. Reinhardt et al demonstrated up-regulation of MMP-9 activity in the failing myocardium regardless of aetiology whereas MMP-2 was only up-regulated in heart failure of idiopathic origin and was not elevated in coronary heart disease (Reinhardt, Sigusch et al. 2002). Orn et al

observed an inverse correlation between MMP-9 level and LV ejection fraction, functional myocardial mass and myocardial scar size as assessed via cardiac magnetic resonance imaging (CMR) in fifty two clinically stable, long-term survivors of AMI (Squire, Orn et al. 2005). In a community study of the Framingham population, detectable plasma MMP-9 levels were associated with increased LV diastolic dimensions and increased wall thickness in men but not women (Sundstrom, Evans et al. 2004) whereas TIMP-1 was associated with known major cardiovascular risk factors (Sundstrom, Evans et al. 2004). In addition Banfi et al showed MMP activity to be related to both echocardiographic markers of LV systolic function and neurohormonal activation. In this study of 42 patients with heart failure due to dilated cardiomyopathy of ischaemic and non ischaemic origin, MMP-2 and MMP-9 but not TIMP-2 were significantly higher in subjects compared to controls and across groups MMP-2 showed direct correlation with plasma noradrenaline levels (Banfi, Cavalca et al. 2005).

Differential expression of tissue inhibitors of MMPs is also seen in the failing heart with the cardiac expression of TIMP-1 and TIMP-3 transcripts and proteins being significantly reduced in patients with both idiopathic dilated cardiomyopathy and ischaemic cardiomyopathy (Li, Feldman et al. 1998). No difference was seen in TIMP-2 between subjects and controls. This data suggesting that MMP control in addition to the degree of metalloproteinase release is an important factor in its pathological effects. In addition TIMP-1 and TIMP-2 have been shown to be up-regulated and associated with the degree of collagen deposition, interstitial fibrosis, and LV hypertrophy and diastolic dysfunction (all being early features of ventricular remodelling) in subjects with aortic stenosis suggesting that an imbalance of the

TIMP/MMP is associated with chronic pressure overload and changes in the ECM (Heymans, Schroen et al. 2005)

Thomas et al (Thomas, Coker et al. 1998) observed increased MMP-3, MMP-9, TIMP-1 and TIMP-2 but suppressed MMP-1 activity in myocardial extracts of subjects undergoing orthotopic heart transplantation for idiopathic dilated cardiomyopathy and following insertion of LV assist devices myocardial MMP activity declines in line with functional LV recovery (Li, Feng et al. 2001).

Few studies have demonstrated the specific temporal profile of MMP expression in humans following MI. Squire et al observed a distinct temporal profile of MMP-2, & -9 post AMI (Squire, Evans et al. 2004) and correlated these levels with echocardiographic markers of LV dysfunction and volumes. Webb et al also observed specific temporal profiles of MMP/TIMP post acute myocardial infarction with an acute increase in MMP-9, MMP-8 and TIMP-1 post event. They demonstrated a link between this profile of enzyme release and left ventricular remodelling. Subjects with persistently elevated or increased MMP-9 levels at day 5 post MI suffering a greater degree of LV remodelling with greater increases in LV end diastolic volumes at 28 days (Webb, Bonnema et al. 2006). In this study no significant relationship was seen between LV dilatation and early changes in MMP-2, MMP-7, MMP-8 or TIMP-1 or TIMP-2. These results suggest that dynamic changes in metalloproteinase activity occur in patients following AMI and the profiling of this proteolytic system may hold clinical utility with respect to adverse LV remodelling. Similar results were seen in the recent study by Wagner et al in which high levels of MMP-9 were associated with late onset CHF and left ventricular remodelling following acute myocardial infarction

treated with primary mechanical reperfusion (Wagner, Delagardelle et al. 2006).In addition elevated levels of the gelatinases were associated with changes in LV geometry between 2 weeks and 6 months post AMI in a study from Japan (Matsunaga, Abe et al. 2005).

To date human studies of metalloproteinase inhibition have been lacking. The recent PREMIER study (Hudson, Armstrong et al. 2006) - (Effects of selective matrix metalloproteinase inhibitor [PG-116800] to prevent ventricular remodelling after acute myocardial infarction,) was a randomized double blinded, placebo controlled study investigating 253 patients with a first diagnosis of ST segment AMI and ejection fraction between 15 and 40%. PG-116800 is a selective MMP inhibitor known to have action on MMP-2, -3, -8, -9, -13 and -14 as well as minor action on MMP-1 and -7. PG-116800 had previously shown promising results in preclinical studies with significant reductions in LV volume along with infarct zone collagen content in a porcine model (Yarbrough, Mukherjee et al. 2003) and reducing LV volumes plus improving ejection fraction in a canine heart failure model. In this human study, remodelling data were obtained through serial echocardiography. All subjects were randomized to receive active drug or placebo twice daily for 90 days. This clinical study failed to show any significant reduction in LV remodelling or improve clinical outcomes after MI with a non significant higher rate of death and re-infarction in the active drug arm. The explanation for the failure of this study is likely to be multifactorial. Firstly, therapy was not commenced until greater than 48hours post MI hence missing the stage at which early remodelling is likely to be occurring. Later, therapy was continued for 90 days, a time during which scar formation and positive remodelling may have beneficial effects. The timing of therapy may therefore

have specific implications. In addition PG-116800 is not a truly, "selective", inhibitor and may indeed block actions of some enzymes with beneficial effects hence negating any benefit obtained by inhibition of detrimental enzymes.

2.12

Metalloproteinases and Prognosis

MMPs appear to have several roles in the pathogenesis of coronary disease. Firstly they may be responsible for atherosclerotic lesion development and rupture leading to acute myocardial infarction. Following this acute event, MMPs may subsequently be involved in the LV remodelling process and development of clinical heart failure. As both of these events are intimately related to survival it may be hypothesized that measurement of the MMP system may be useful in the prediction of prognosis in certain groups of subjects. In an extensive study using the "atherogene", population of 1979 patients with angiographically proven coronary artery disease, TIMP-1 was found to be an independent predictor of future cardiovascular deaths, the authors suggesting that plasma TIMP-1 level may be a marker of cardiovascular extracellular matrix remodelling, a process that may be associated with increasing cardiovascular risk (Lubos, Schnabel et al. 2006). Similar results were seen in 389 male patients undergoing coronary angiography at a Veterans Administration medical centre. In this study a single baseline measurement of plasma TIMP-1 but not MMP-9 was independently predictive of the subsequent risk of death or MI at 24 months and the ratio of TIMP-1 to MMP-9 was also an independent predictor. When patients were divided into those with or without acute coronary syndrome, TIMP-1 remained an independent predictor of all-cause mortality in both subgroups signifying that TIMP-1

may be a useful biomarker in both groups (Cavusoglu, Ruwende et al. 2006). In contrast to this study Blankenberg et al in a cohort of 1127 patients with documented coronary artery disease observed higher MMP-9 levels in those subjects who subsequently suffered a fatal cardiovascular event and demonstrated that the T allele of the C-1562T polymorphism in its encoding gene was associated with increased levels (Blankenberg, Rupprecht et al. 2003).

Wu et al have also shown that plasma MMP-3 levels are associated with future cardiovascular events in subjects with documented coronary artery disease (Wu, Leu et al. 2005) however both MMP-2 and MMP-9 did not appear to have any association with adverse outcomes.

2.13

Genetic Factors and MMP expression

Genetic factors influence MMP activity and in turn this has effects on physiological and pathological behaviour. Jormsjo et al (Jormsjo, Ye et al. 2000) demonstrated that within the common polymorphism in the promoter region of MMP-12, the A allele was associated with both increased MMP-12 expression and smaller luminal diameters in coronary lesions compared to the G allele. Morgan et al (Morgan, Zhang et al. 2003) observed several polymorphisms in the gene encoding MMP-9 and demonstrated that the incidence of the C\T and T/T genotypes of the -156C>T polymorphism were significantly higher in patients with coronary stenosis then those free of coronary disease. Haplotype analysis also showed the C-G-C haplotype (-1562C, +279Q, and +6C) to be protective against atherosclerosis. However these results are far from clear with Wang et al (Wang, Warzecha et al. 2001) observing no

relationship between this polymorphism and the severity of coronary atherosclerosis. In a study of MMP-9 plasma concentrations of 1127 patients with documented evidence of coronary artery disease, the T allele of the C-1562T polymorphism was associated with increased MMP-9 levels. There was also a significant association between the R279Q polymorphism and cardiovascular events in patients with stable angina over a mean follow-up period of 4.1 years (Blankenberg, Rupprecht et al. 2003). The C-1562 polymorphism has also been shown to be associated with increased area of complicated coronary plaques in post mortem studies again implicating this polymorphism in the development of significant coronary disease (Pollanen, Karhunen et al. 2001). The C-1562 polymorphism is also associated with restenosis following percutaneous coronary intervention (Cho, Chae et al. 2002)

The 5A polymorphism in the promoter region of the MMP-3 gene, which has increased promoter activity (Ye, Eriksson et al. 1996), is associated with coronary lesions in subjects with stable angina compared to healthy controls (Kim, Park et al. 2002). In the same study, polymorphisms of the MMP-9 gene were not associated with stable angina. In a similar study Terashima et al reported the frequency of the 5A allele of MMP-3 to be significantly higher in sufferers of AMI (Terashima, Akita et al. 1999) and Nojiri et al also showed the MMP-3 5A and MMP-1 1G polymorphisms to be associated with a risk of AMI (Nojiri, Morita et al. 2003). These data were replicated more recently showing the 5A genotypes of MMP-3 to be associated with young smokers with AMI compared to controls (Liu, Chen et al. 2003) and with the development of unstable plaques (Beyzade, Zhang et al. 2003). The 5A polymorphism has also been shown to be associated with the development of coronary aneurysms as assessed via angiography (Lamblin, Bauters et al. 2002)

Ye et al (Ye, Gale et al. 2003) in a UK population demonstrated a link between MMP-1 polymorphisms and coronary heart disease as assessed by Rose/WHO cardiovascular questionnaire regarding risk factors and current medications. The homozygous 2G allele of the MMP-1 gene had reduced risk of coronary heart disease as compared to homozygous 1G allele, with heterozygotes displaying an intermediate risk.

Promoter polymorphisms in the MMP-2 gene (-790T/G and –735C/T) have been shown to be more frequent amongst patients with chronic heart failure (NYHA class II-IV) as compared to matched control subjects (Vasku, Goldbergova et al. 2003). Mizon-Gerard et al (Mizon-Gerard, de Groote et al. 2004) investigated polymorphisms of the MMP-2 (-1306C>T), MMP-3 (-1171 5A > 6A), and MMP-9 (-1562 C > T) genes. They demonstrated that MMP-3 polymorphism had a different impact on survival in heart failure patients with ischaemic and non ischaemic aetiology, the MMP-3 5A/5A genotype being a predictor of cardiac mortality in patients with non-ischaemic but not ischaemic heart failure. The MMP-9 polymorphism was associated with cardiac survival independent of heart failure aetiology. There was no evidence for an association between MMP-2 polymorphism and cardiac survival.

2.14

Chapter Summary

In summary I have described the structure, function and mechanisms of control of the MMP system. I have presented compelling background data to link MMPs and their control with various aspects of cardiovascular disease including the process of LV remodelling leading to heart failure under various pathological conditions. These data form the background for the original hypotheses of this thesis.

The basis of this project is to investigate the role of matrix metalloproteinases and their natural inhibitors - tissue inhibitors of metalloproteinases (TIMPs) in the process of left ventricular remodelling and clinical heart failure. In addition we investigate the relationship of these enzymes with prognosis post acute myocardial infarction in humans.

Chapter 3

Natriuretic Peptides

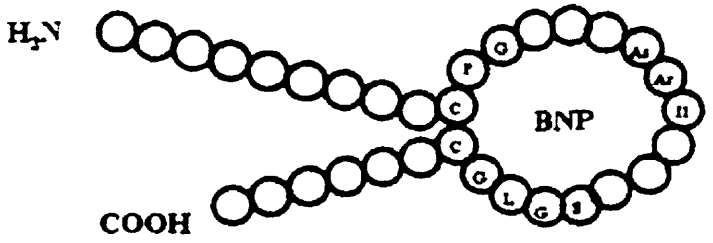
A complete review of the background and previous studies of natriuretic peptides is beyond the scope of this thesis. In our work we have compared the prognostic utility of MMPs and TIMPs with that of N terminal pro BNP (N-BNP) in the prediction of prognosis post AMI. In the following chapter we therefore review the previous data for the use of BNP and N-BNP in prognostication post AMI.

3.1

B Type Natriuretic Peptide (BNP) – Structure and Function

BNP is a cardiac hormone belonging to the natriuretic peptide family. In common with other members of the natriuretic peptide family BNP consists of a 17 amino acid ring and a disulfide bridge between 2 cysteine molecules.

Figure. 3.1 Structure of Brain Natriuretic Peptide



The functions of BNP are diverse including diuresis, natriuresis, vasodilatation and inhibition of the renin angiotensin system. BNP was first isolated from porcine brain (Sudoh, Kangawa et al. 1988) and subsequently identified in humans (Mukoyama, Nakao et al. 1991). It is secreted and synthesized predominantly by the left ventricle in response to myocardial stretch as a 108 amino acid pro-peptide and is subsequently cleaved into its biologically active 32 amino acid BNP and the biologically inactive 76 amino acid N terminal pro hormone fragment (N-BNP) (Hama, Itoh et al. 1995). The action of BNP is via interaction with the natriuretic peptide receptor type-A (NPR-A) causing intracellular cGMP production. BNP is cleared by binding to the NPR-C receptor followed by proteolysis whereas N-BNP is cleared via renal excretion and therefore has a longer biological half life making its measurement easier. Close correlation is seen in measurements of BNP or N-BNP in clinical practice (Hunt, Richards et al. 1997).

3.2

BNP and LV dysfunction

Over the last 10 years BNP and its N terminus have become well established as markers of the severity of LV systolic dysfunction (LVSD) and of prognosis in subjects with a variety of cardiovascular conditions. BNP is now widely used as a screening tool for the identification of LVSD (McDonagh, Morrison et al. 1997), as a marker of severity of LV impairment (Tsutamoto, Wada et al. 1997; Gardner, Ozalp et al. 2003), and of prognosis in subjects with left ventricular dysfunction.(Koglin, Pehlivanli et al. 2001; Richards, Doughty et al. 2001; Hulsmann, Berger et al. 2002).

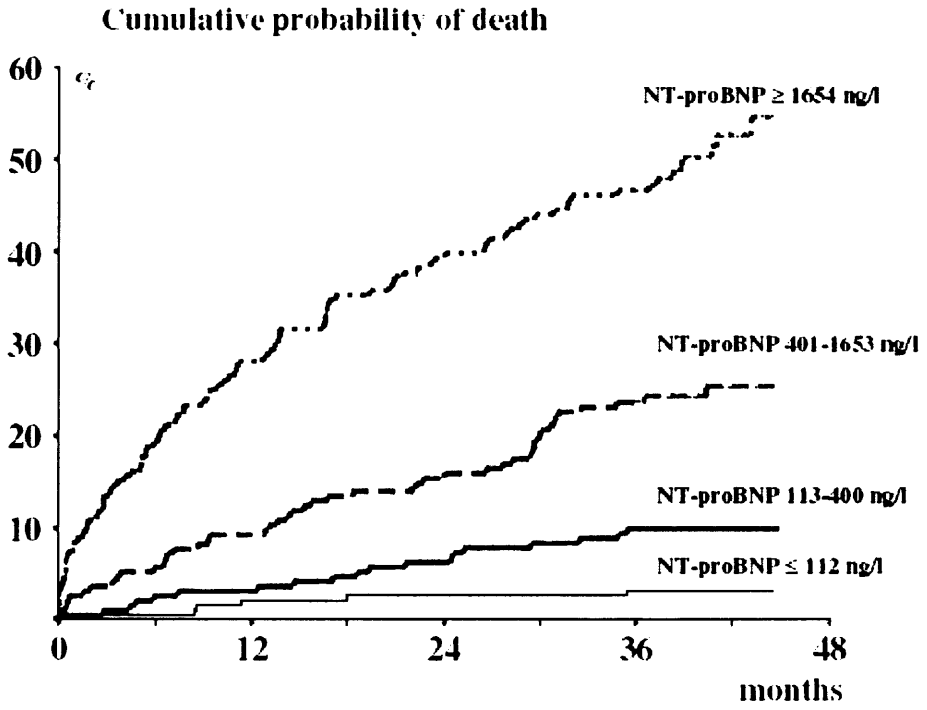
3.3

BNP post AMI

More recently increased BNP and N-BNP levels have been demonstrated post AMI (Morita, Yasue et al. 1993) and increased levels early after AMI appear to be a strong marker of long term mortality. This has now been validated in a large number of studies (Arakawa, Nakamura et al. 1996; Darbar, Davidson et al. 1996; Omland, Aakvaag et al. 1996; Richards, Nicholls et al. 1999; Omland, Persson et al. 2002; Richards, Nicholls et al. 2003; Mega, Morrow et al. 2004; Squire, O'Brien et al. 2004; Grabowski, Filipiak et al. 2005; Sun, Wang et al. 2006; Zhang and Wang 2006). Heeschen et al using the PRISM study population (Heeschen, Hamm et al. 2004) demonstrated that N-BNP was an independent determinant of short term cardiac risk in patients with non-ST segment elevation AMI and in addition observed that patients with early high levels of N-BNP and lack of a rapid decline or low early levels with subsequent rise had a very poor short term mortality. This adverse prognosis was evident regardless of stratification as to troponin positive or negative ACS and unrelated to levels of CRP. In the Orbofiban in Patients with Unstable Coronary Syndromes (OPUS)-TIMI 16 Trial, BNP was measured at 40 hours post onset of symptoms, during a 10 month follow up elevated BNP was associated with an increased risk of death or heart failure regardless of the type of ACS (de Lemos, Morrow et al. 2001). Later the same research group validated these findings in a second study including 1676 patients with unstable CAD. In this study a prospectively defined cut of 80ng/L was able to identify a group of patients with a 30 day and 6 month mortality of 5% and 8.4% respectively compared to 1.2% and 1.8% in the

group with lower levels of BNP (Morrow, de Lemos et al. 2003). Analysis of the GUSTO-IV study also demonstrated an adverse prognosis of subjects with elevated N-BNP regardless of diabetes status in subjects with non ST segment elevation MI (James, Lindahl et al. 2006). In addition measurement of N-BNP at the time of admission to the emergency department in subjects with chest pain and no ST segment elevation is associated with long term mortality (Jernberg, Stridsberg et al. 2002). This study included 775 non-selected patients admitted with symptoms suggestive of ACS. Patients were followed for a median of 40 months for the primary endpoint of death. Compared to the lowest quartile, patients in the 2nd, 3rd of 4th quartile had a relative risk of subsequent death of 4.2, 10.7 and 26.6 respectively.

Figure. 3.2. Comparison of cumulative death rates between subjects according to presentation N-BNP levels. (Jernberg, Stridsberg et al. 2002).



The same group also reported the N-BNP sub study of the GUSTO-IV trial including 6809 patients with CAD. In this study the magnitude of BNP release was related to

the degree of myocardial damage (troponin) and inflammation (CRP). Again increased mortality was observed with higher levels of N-BNP with survival curves beginning to separate at 48 hours however curves continued to diverge up to 12 months. Morrow et al have also presented data from the A to Z study. In a subgroup of this multi-national study which included patients with ST or Non ST elevation myocardial infarction, elevated BNP prior to discharge and at 4 and 12 months post discharge was associated with adverse outcome (death or heart failure). Patients with newly elevated BNP at 4 months also had an increased risk of adverse outcome (Morrow, de Lemos et al. 2005). A sub-study of the OPTIMAAL study investigated plasma natriuretic peptides in 236 patients with AMI complicated by clinical or radiological evidence of heart failure for up to 2 years after acute myocardial infarction. This data suggested that change in N-BNP from baseline to 30 days was associated with adverse outcome (Squire, Orn et al. 2005)

3.4

BNP and Adverse Prognosis – Mechanisms

The association between BNP and adverse prognosis are likely to be due to a number of factors. Certainly there is some association of BNP with other co-morbidities such as age, hypertension, diabetes and renal dysfunction which are all associated with an adverse prognosis in coronary artery disease. Secondly BNP levels may be related to the degree of left ventricular damage established prior to or as a consequence of the initial infarct which is an important predictor of outcome in ischaemic heart disease (St John Sutton, Pfeffer et al. 1994). Thirdly elevated BNP is related to the degree of adverse ventricular remodelling which itself is associated with adverse prognosis

(Chapter 1) (White, Norris et al. 1987; Crilley and Farrer 2001). In addition elevated BNP has also been shown to be associated with inducible ischaemia in subjects with stable coronary disease (Bibbins-Domingo, Ansari et al. 2003) and BNP levels may well be a marker of the size or severity of myocardial ischaemia rather than purely due to infarction and left ventricular stretch in subjects with AMI. Indeed animal studies have demonstrated that BNP synthesis is detectable in both infarcted and non infarcted myocardium (Hama, Itoh et al. 1995) and BNP elevations are seen post percutaneous coronary angioplasty possibly secondary to transient ischaemia during balloon inflation or stent deployment (Tateishi, Masutani et al. 2000) and are elevated after exercise in patients with stable coronary disease (Marumoto, Hamada et al. 1995).In a subgroup of the FRISC II study, coronary intervention was shown to reduce mortality in those subjects with elevated BNP but not in patients without BNP increase (Jernberg, Lindahl et al. 2003). This data supports the theory that BNP is associated with ischaemia and hence adverse prognosis, however BNP is also elevated and associated with prognosis in other cardiac conditions which cannot be explained through an ischaemic nature such as pulmonary embolism (ten Wolde, Tulevski et al. 2003; Tulevski, Wolde et al. 2006) and congenital heart disease (Hopkins, Chen et al. 2004)

3.5

Chapter Summary

In summary BNP is a cardiac hormone which is released in subjects with left ventricular dysfunction and is a well established marker of such. More recently BNP has also been shown to be elevated post AMI and to be a marker of adverse prognosis

in this situation. Our studies will compare our new MMP and TIMP biomarkers with.

N terminal pro BNP as markers of prognosis post AMI.

Chapter 4.

Aims and Methods

4.1

Original Hypothesis

MMPs have the ability to degrade the components of the cardiac extracellular matrix and are elevated post AMI. Our original hypothesis is MMP and TIMP levels are altered following acute myocardial infarction and alterations in these levels will be associated with LV dysfunction, LV remodelling and adverse prognosis post AMI.

4.2

Aims

The aims of this project were 3 fold:

1) To identify the specific temporal profiles of MMPs post acute myocardial infarction (AMI) in human subjects.
2) To identify relationships between MMPs and echocardiographic markers of LV size, function and remodelling.
3) To identify relationships between MMPs and prognosis post AMI over a predetermined follow up period.

The project was performed in 2 stages as described below:

Stage 1.

Stage 1 aimed to examine the temporal profile of several MMPs: MMP-1 (Collagenase-1), MMP-2 (Gelatinase A), MMP-3 (Stromelysin 1), MMP-9 (gelatinase B), and their relationship with markers of LV function, volumes and remodelling in a limited population size. This stage would provide essential information on a variety of MMPs and would allow us to identify the most appropriate MMPs to assay in our extended population (stage 2).

Stage 2

Stage 2 involved a more extensive population, concentrating on a limited number of MMPs and again investigating the relationship with markers of LV function, volumes and remodelling and to further the study by investigating the association of MMPs with prognosis post AMI.

4.3

Methods – Stage 1

4.4

Study population.

We recruited 91 patients admitted to our Coronary Care Unit (CCU) with AMI for stage 1 of this investigation . We excluded patients with known malignancy or with inflammatory or connective tissue disease which may be associated with altered metalloproteinase activity. In addition we excluded from analysis recruited patients with poor echocardiographic images making valid measurements difficult (N=15). No patients approached refused consent to inclusion, and no patient were lost to follow-up. The diagnosis of AMI was based on symptoms consistent with AMI in conjunction with appropriate ECG changes (dynamic ST segment elevation (STEMI, n=77, 84.6%) or ST segment / T wave changes (NSTEMI, n=14, 15.4%) and elevation in markers of myocardial necrosis (Creatine Kinase or Troponin I). All patients donated venous blood samples at 0-12hrs, 12-24hrs and at 24hr intervals post symptoms until discharge and underwent echocardiographic studies during their index admission and at follow up (median 176 days, range 138-262 days after AMI for stage 1 subjects). The local research ethics review committee approved the study and all patients gave written consent to participation. The conduct of the study was in keeping with the Declaration of Helsinki.

Control Population.

Our control population consisted of 196 age (median 62, range 31-86 years) and sex (76.5% male) matched subjects with stable coronary artery disease (AMI>90 days previous) with preserved LV function (LVEF>40%) and no change in cardiovascular therapy for a minimum of the previous 30 days.

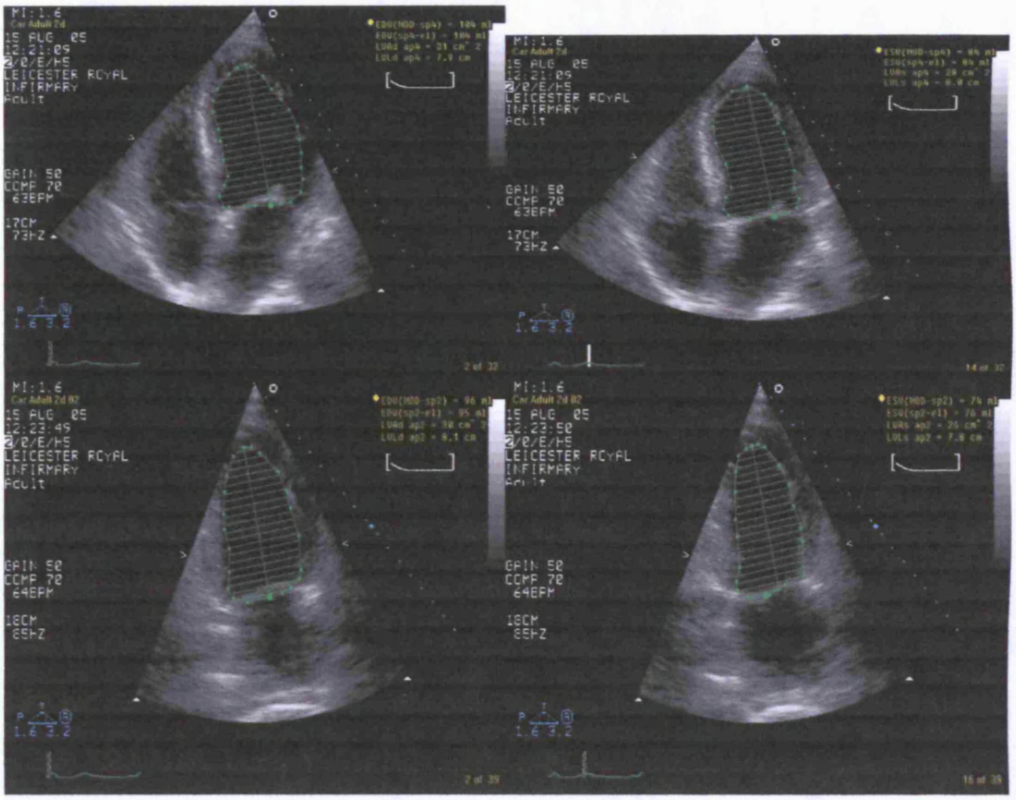
4.5

Echocardiographic assessment

Echocardiography is a readily accessible, non invasive imaging technique and hence frequently used in the coronary care setting. Echocardiographic assessment was carried out during the index admission (immediately prior to discharge) and at follow-up, by a single operator (DK) using either Sonos 5500 or IE33 scanner (Philips Medical Systems, Reigate, UK). Prior to commencement of this project, DK had performed over 200 independent echocardiography scans and had completed the British Society of Echocardiography Examination. Left ventricular end systolic volume (LVESV), end diastolic volume (LVEDV) and ejection fraction (LVEF) were estimated using the bi-planar modified Simpson's rule from apical 2 and 4 chamber views. This technique involves tracking endocardial borders on both apical 2 and 4 chamber views, the ventricular cavity is separated into 20 equally spaced perpendicular segments (assuming uniformity between the 2 cuts) to give 20 discs, overall volumes can therefore be calculated by summing the volume of each of these discs. Previous studies have shown echo to be a valid tool in the assessment of LV systolic function post AMI with close correlations seen between echocardiographic measurements of ejection fraction via and bi-planar model and radionuclide ventriculography (Galasko, Basu et al. 2004). The bi-planar method has fewer

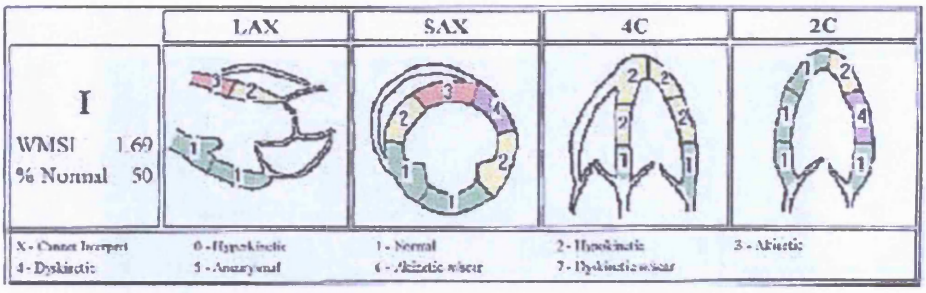
geometrical assumptions and hence is the more accurate of tools for measurement of LV volumes and ejection fraction and has been recommended for quantification of 2 dimensional echo by the American society of echocardiography (Schiller, Shah et al. 1989)

Figure. 4.1. Assessment of LV volumes and ejection fraction via bi-planar modified Simpson's' method. Upper images - apical 4 chamber, lower – apical 2 chamber. Diastolic frames to left, systolic to right.



Left ventricular function was also assessed using the left ventricular wall motion index score (WMIS), using a standard 16 segment model from para-sternal long and short axis and apical 2 and 4 chamber views. Each LV segment is scored as 0-hyperkinetic, 1-normal, 2-hypokineic, 3-akinetic, 4-dyskinetic, 5-aneurysmal, 6-akinetic with scar or 7-dyskinetic with scar. The total is divided by the number of segments analysed to give an overall score with higher values indicating more impaired LV function.

Figure. 4.2 Assessment of wall motion index scores



In addition, left ventricular internal dimensions in both systole (LVIDs) and diastole (LVIDd) were measured from para-sternal long axis views according to currently accepted techniques using either 2D images or M-mode where appropriate. LV internal dimensions are measured at the level of the mitral valve tips from the endocardial surface of the interventricular septum to the endocardial surface of the LV posterior wall. M-mode was used where a perpendicular cut could be obtained though the LV, otherwise measurements were made on a frozen 2D image at end systole or end diastole.

Figure. 4.3 Assessment of LV dimensions via 2D imaging - upper LVIDd, lower LVIDs

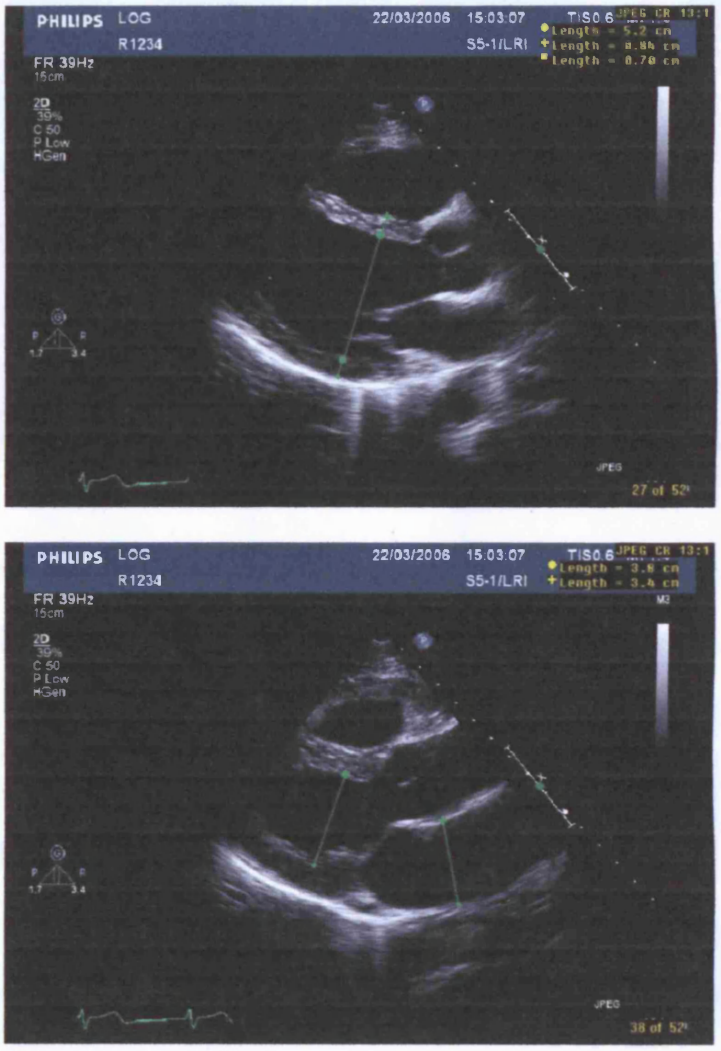
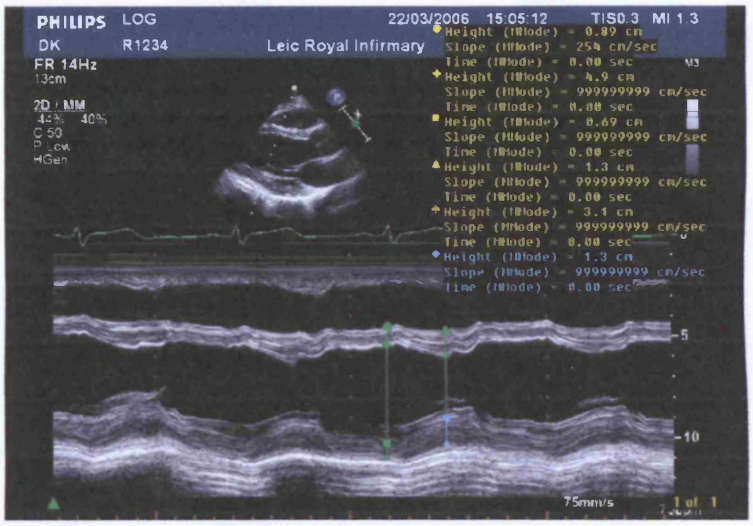


Figure. 4.4 Assessment of LVIDd and LVIDs via m-mode imaging.



For patients attending for echocardiography at follow-up, the change (Δ) in left ventricular end systolic (Δ LVESV) and end diastolic volume (Δ LVEDV) were calculated as percentage of the pre-discharge volume (bi-planar). Interpretation and measurements were made using off-line software (enconcert- Phillips medical). Every effort was made to obtain similar cuts between pre-discharge and follow up examinations. In order to ensure similar cuts, large numbers of images were stored on our digital echo reporting system, these were later reviewed together (admission and follow up) to match image slices.

The reviewer was blinded to the results of MMP analysis at the time of echo assessment.

Ethical approval did not include repeated scans on subjects and therefore intra observer variation was assessed via repeated measurements on digitally stored echo images. As all echo measurements were completed via a single operator (DK) we did not assess inter-observer variation. Intra-observer variation, assessed in a subset of the

cohort (N=45; Mean ± SD) was 0.36% ± 1.75 for WMIS, 5.2% ± 3.9 for EDV, 6.0% ± 6.6 for ESV and 6.7% ± 7.6 for LVEF. Pearsons correlation between analyses all >0.9, $p<0.001$.

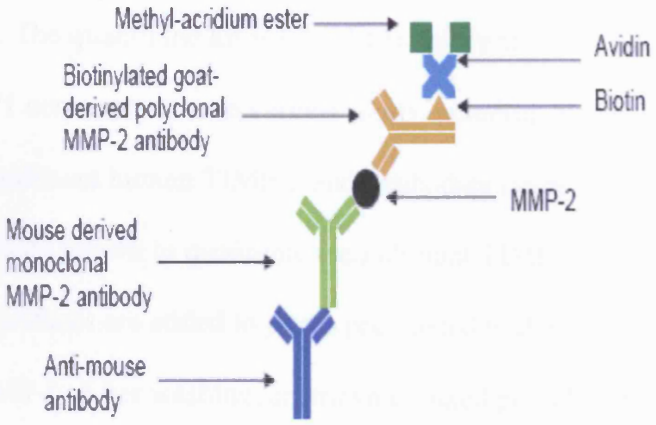
4.6

Laboratory Methods

4.6.1: MMP-2 & 9

Plasma MMP-2 &-9 were measured using an in-house non-competitive immunoluminometric assays. ELISA plates were coated with 100ng/well of anti-mouse IgG (Sigma Chemical Co., Poole, UK). Following washes, wells were blocked with 5g/L bovine serum albumin. For the assay, 100 μL of assay buffer containing 20ng of MMP9 or 50ng of MMP2 mouse monoclonal antibodies (Research Diagnostics, NJ, USA) were pipetted into the wells, followed by 10 μL of plasma samples or standards of recombinant MMP2 or MMP9 (Research Diagnostics, NJ, USA). ELISA plates were incubated for 24h, and after washes, the second antibody (biotinylated goat anti-MMP2 or anti-MMP9, 10ng/100 μL assay buffer in each well) was introduced and incubated for another 4 h. Plates were washed and streptavidin-MAE used to build up the final component of the sandwich assay. Chemiluminescence was elicited using sequential injections of H_2O_2 in 0.1 mol/L HNO_3, and 0.25 mol/L NaOH with cetylammonium bromide, integrating the light emitted for 1 sec after the final injection on a MLX luminometer (Dynex Technologies, Worthing, West Sussex, UK)(1). The lower limit of detection was 0.2ng/ml for MMP-2 and 0.4ng/ml for MMP-9. Interassay coefficient of variation was <8%.

Figure. 4.5. Diagram to demonstrate the MMP-2 assay



4.6.2: N-BNP.

N-BNP was also measured using our in house assay. Sheep antibodies were raised to the N-terminal of human N-BNP and monoclonal mouse antibodies were raised to the C- terminal. The N-terminal IgG was affinity-purified and biotinylated. Samples or N-BNP standards were incubated in C-terminal IgG-coated wells (100ng/well) with the biotinylated antibody for 24 hours at 4°C. Following washes, detection was with methyl-acridinium ester (MAE)–labelled streptavidin ($2x10^7$ relative light units/ml, incubated for 90 min at room temperature). Chemiluminescence was elicited using sequential injections of H_2O_2 in 0.1 mol/L HNO_3, and 0.25 mol/L NaOH with cetylammonium bromide, integrating the light emitted for 1 sec after the final injection on a MLX luminometer (Dynex Technologies, Worthing, West Sussex, UK)(1). The lower limit of detection was 0.3 fmol/ml. Interassay coefficient of variation was <8%.

4.6.3: TIMP-1(stage2)

TIMP-1 was measured using a commercially available ELISA kit (Quantikine-Cat. No. DTM100). The quantikine kit is a 3.5 hour solid phase ELISA designed to measure TIMP1 concentrations in various fluids including plasma. It contains NS0-expressed recombinant human TIMP-1 and antibodies raised against the recombinant factor and has been shown to quantitate recombinant TIMP-1 accurately.
Samples and standards are added to plates pre-coated with monoclonal antibody specific for TIMP-1. After washing, an enzyme linked polyclonal antibody specific for TIMP-1 is added to the wells. Following a further wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of TIMP-1. Colour intensity was measured using Dynex Revelation 4.24 ELISA reader. The lower limit of detection was 0.08ng/ml. Intra- and inter-assay coefficient of variation was <8%.

4.6.4: MMP-1 & MMP-3

MMP-1 & -3 were measured using a commercially available kit (R&D systems-Fluorokine multianalyte profiling, human MMP base kit. Cat No.LMP000). Analyte-specific antibodies are pre-coated onto colour coded microparticles. Microparticles, standards and samples are pipetted into wells and the immobilized antibody binds the analytes of interest. After was washing away any unbound substances, biotinylated antibodies specific to the analytes of interest are added to each well. Following a wash to remove any unbound biotinylted antibody, streptavidin-phycoerythin conjugate

(streptavidin-PE), which binds the biotinylated detection antibodies, is added to each well. A final wash removes unbound streptavidin-PE and the microparticles are resuspended in buffer and read using a luminex 100 analyser. One laser is microparticle-specific and determines which analyte is being detected. The other laser determines the magnitude of the phycoerythin-derived signal, which is in direct proportion to the amount of analyte bound. The lower limit of detection was 0.04ng/ml for both MMP-1 and -3. Intra- and inter-assay coefficient of variation was <8%.

4.7

Patient follow up.

Subjects were first invited for follow up echocardiography at 3 months post discharge. All patients were sent an appointment via mail including a reply slip to confirm attendance. Subjects also had the option to request alternative appointment times including out of hours and weekends in order to reduce the numbers lost to follow up. Reasonable travel costs were covered through the BHF research grant. Any subjects with significant abnormalities on follow up echocardiography were referred on for further investigation.

4.8

Statistical Analysis

4.8.1: Sample size

We based our sample size on the estimated change in LVEDV between discharge and follow-up echocardiographic examinations. We calculated that a sample size of 18

would give 80% power to detect an estimated 10% (SD 15%) change in LVEDV. We aimed to estimate ΔLVEDV in 50 patients. Allowing for 10% mortality between discharge and follow-up, and a further 15% non attendance, we estimated a sample size of 75, leading to 58 serial assessments, would be adequate. In reality we recruited 91 patients, in 65 of whom ΔLVEDV could be calculated from echocardiographic examinations. The observed mean ΔLVEDV between discharge and follow-up in previous studies in our population was 22% (SD 25%), giving our study over 90% power to detect a mean change of 20% in LVEDV.

4.8.2: Analysis

The main points of interest were the plasma profiles of MMP, and the relationship of plasma concentrations with echocardiographic parameters. Normality of distribution was assessed for continuous variables via Kolmogorov-Smirnov test. Non-Gaussian distributed variables were log-transformed prior to all analysis. Data are presented as mean ± SD or median (range) for non-Gaussian distributed data.

Differences among MMP concentrations at individual time periods were analysed by repeated-measures analysis of variance (ANOVA). We assessed the univariable association between log-transformed MMP and biologically plausible individual variables. Gender, anterior/inferior AMI, ST elevation/non-ST elevation AMI, prior AMI, history of angina, of hypertension, or of diabetes, individual pre-admission drug therapy, thrombolysis, and current smoking/not smoking were entered as categorical variables and levels of MMP between these groups were assessed using Mann-Whitney U tests. Age, Creatinine, CK and white cell/neutrophils count were entered as continuous variables and correlation between these and MMP levels were assessed using Pearson correlation co-efficients. We performed similar analyses for the relationship between log-transformed MMP concentration and echocardiographic

parameters (LVEF, WMIS, LVEDV, LVESV, and Δ LVEDV). For multi-variable analysis of the determinants of the variable of interest (MMP concentration or echocardiographic parameter), factors with univariable association at 2-sided significance level of $p<0.10$ were entered in to linear regression models using a forced entry method. Models including log-transformed MMP concentrations satisfied assumptions of normality of residuals and independence. For multivariable analyses, two-sided $p<0.05$ was regarded as significant. Analyses were carried out using SPSS for Windows version 12 (SPSS Inc. Chicago, USA).

4.9

Stage 2.

Stage 2 of this project involved MMP analysis in a larger cohort of subjects. Following analysis of stage 1 results (see results section) we identified appropriate MMPs/TIMPs to further investigate. The remainder of the methods are similar to stage 1. All subjects underwent echocardiographic assessment prior to discharge and at follow up and all subjects were followed for clinical endpoints.

Stage 2 involved recruitment of a further 310 subjects (total 400). This study size was calculated from prior power calculations. Planned assessment of relationships between: (1) LV function (EF and LVWMI) and serum levels of individual MMPs; (2) N-BNP and MMP; (3) LV dimensions and both N-BNP and MMPs; (4) LV function (EF and LVWMI) and N-BNP; (5) changes in LV dimensions (from 0-6 months) and both baseline and 6 month MMP/N-BNP values and clinical outcomes

Previous data in our population indicates an event rate (combined end-point of death or clinical heart failure) of approximately 19% per annum (Squire, O'Brien et al.

2004). Thus, in a study sample of 400 patients recruited over 12 months, with follow up of 6 months we will expect to see approximately 75 events, of which approximately 25 will be deaths and 50 will be individuals with heart failure episodes. Heart failure episode was defined as in-patient treatment for a primary diagnosis of heart failure requiring high dose IV diuretic (frusemide >40mg), intravenous GTN , ionotropic support or intra-aortic balloon pumping.

We have shown that this sample size is sufficient to allow robust determination of relationships between N-BNP and clinical outcomes (Squire, O'Brien et al. 2004), between plasma MMP and N-BNP (Squire, Evans et al. 2004) and between plasma MMP and echocardiographic parameters of remodelling (Squire, Evans et al. 2004). We have powered this study to allow determination of the relationship of plasma MMP levels with clinical outcome, the most difficult relationship to establish. The sample size of 400 patients is expected to be sufficiently large to demonstrate the relationship of MMP levels with all parameters of LV dysfunction listed above.

Statistics were performed as per stage 1. In addition we assessed the association between MMPs and survival. Our primary outcome was a composite of death or heart failure with secondary outcomes of the individual components of the primary plus re-infarction. We assessed end-points at 5 months (150 days) post event. End-points were identified through our hospital tracking system with review of medical records for each event. Checks were also made by telephone contact with all surviving patients at the end of the study to ensure complete capture of events.

Differences between groups experiencing or not experiencing each clinical endpoint were assessed using χ^2 analysis for categorical variables and Mann-Whitney U-test for continuous variables. Factors with univariable association with each endpoint at significance level of $p<0.10$ were entered into a binary logistic regression model

using a forced entry method. The strength of association with end-points is expressed as odds ratio (OR) per unit increase in log transformed plasma concentration of enzyme. For this study we considered both death and heart failure to be equally significant events as our hypothesis states that MMPs may have effects on prognosis through their influence on LV remodelling and heart failure. Therefore when considering the primary end-point of death or heart failure we assessed time to first event, patients being removed from further analysis thereafter.

Cox proportional hazards modelling was used to explore the relationship between underlying variables and the time to first clinical endpoint. Optimal prognostic thresholds for the prediction of death or heart failure were derived from Receiver Operator Characteristic (ROC) curves and Kaplan-Meier survival curves were constructed comparing survival in subjects with MMP levels above/below these optimum cut-offs. For all analyses, $p<0.05$ was regarded as significant. All statistical analysis was performed using SPSS V12.

Chapter 5

Patient Demographics and Temporal Profiles of MMPs after AMI.

5.1

Patient Demographics.

Patient demographics for our stage 1 population are shown below. Median age was 63 years (range 31-88) with a male:female ratio of 66:25. 84.6% were ST elevation myocardial infarction of which 65 (84%) received thrombolytic therapy which is the standard therapy in our unit. No patient received primary percutaneous revascularisation. Median CK was 1045 (range 75-5372). A limited number of subjects were receiving cardiovascular medications prior to admission (<25%). At discharge a high percentage of subjects were receiving secondary preventative medications in keeping with current guidelines (Aspirin 89%, Beta-blocker 90%, ACE-Inhibitor 90% Statin 96%).

Other factors are listed:

Table 5.1. Patients demographics – stage 1 population

	Median	Range
Age (yrs)	63	31-88
CK (I.U., NR 0-200)	1045	75-5372
Troponin I (NR <0.06)	7.02	0.08-120

	Number (%)
Male/Female	66/25 (73/27)
STEMI	77 (84.6)
Anterior / Inferior	36 (40)/55 (60)
Thrombolysis	65 (59)
Current Smoker	37 (40.6)
Ex Smoker	20 (22)
Never Smoked	34 (37.4)
History of:	
Diabetes	19 (21)
Previous Angina	15 (16.5)
Hypertension	36 (40)
Previous MI	9 (11)
Drug treatment at admission	
Antiplatelet agent	23 (25)
Beta Blocker	20 (22)
ACE-I	16 (17.6)
Statin	21 (23)
Drug treatment at discharge	
Aspirin	81 (89)
Clopidogrel	12 (13.2)
Beta Blocker	82 (90)
Ace-Inhibitor	82 (90)
ARB	8 (8.8)
Statin	87 (96)
Spironolactone	2 (2.2)
Ca Antagonist	11 (12.1)

NR = Normal Range; ACE-I=Angiotensin Converting Enzyme Inhibitor

5.2 – Temporal profiles

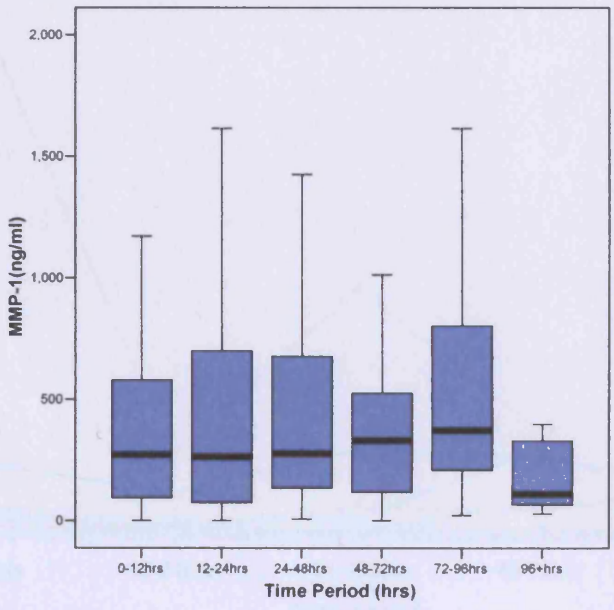
The following section presents data on the temporal profiles of each MMP subtype. Data is presented for the whole cohort as median, quartiles ± range. Data is also presented on a subgroup (n=10) to demonstrate the individual variation of release of each MMP subtype.

The Collagenases

5.2.1: MMP-1 (Collagenase 1)

The temporal profile of MMP-1 over the in patient stay is shown below.

Figure. 5.1 Box and whisker graph to show the temporal profile of MMP-1

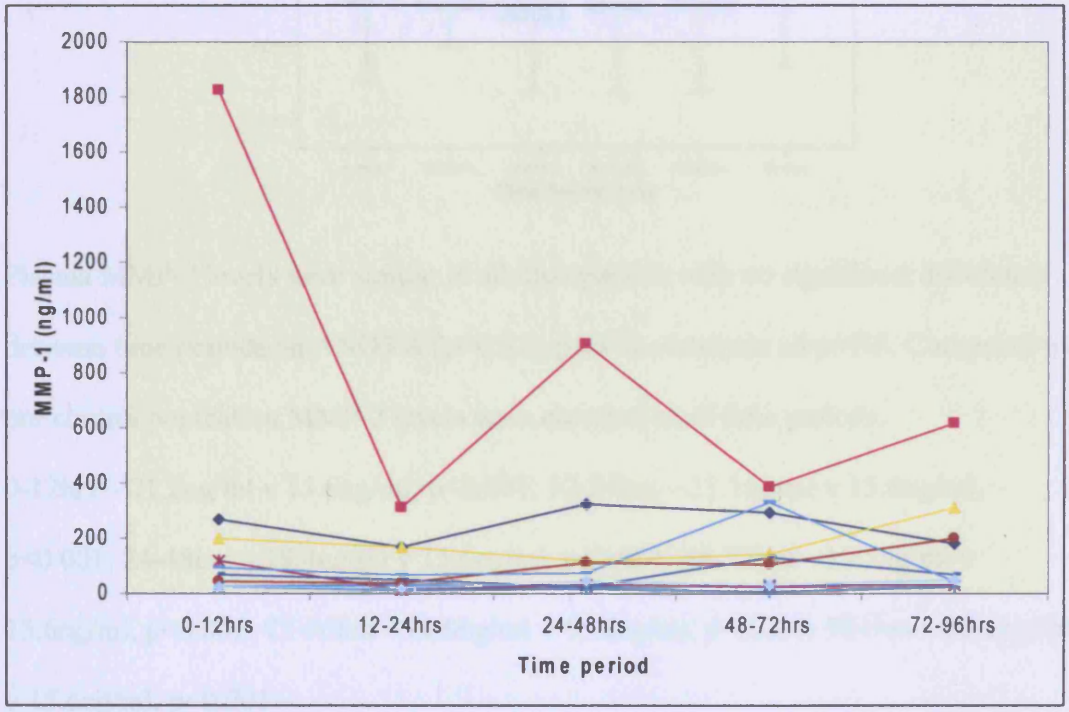


MMP-1 levels were stable from admission to 96 hours followed by a statistically non-significant fall beyond this period (72-96hrs (median 242.12ng/ml) v 96+ hours (median 116.60ng/ml), p=0.926). ANOVA revealed no significant variation between time periods (p=0.516).

Compared to our control population MMP-1 levels were elevated at all time periods until 96hrs however statistical significance was only reached at 72-96hrs.

0-12hrs – 279.1ng/ml v 217.3ng/ml, p=0.892; 12-24hrs – 253.3ng/ml v 217.3ng/ml, p=0.748: 24-48hrs – 282.1ng/ml v 217.3ng/ml, p=0.374: 48-72hrs – 315.5ng/ml v 217.3ng/ml, p=0.604: 72-96hrs – 328.4ng/ml v 217.3ng/ml, p=0.03: 96+hrs – 100.8ng/ml v 217.3ng/ml, p=0.168.

Figure 5.2. Temporal profile of individual patients (n=10). Each line represents 1 patient



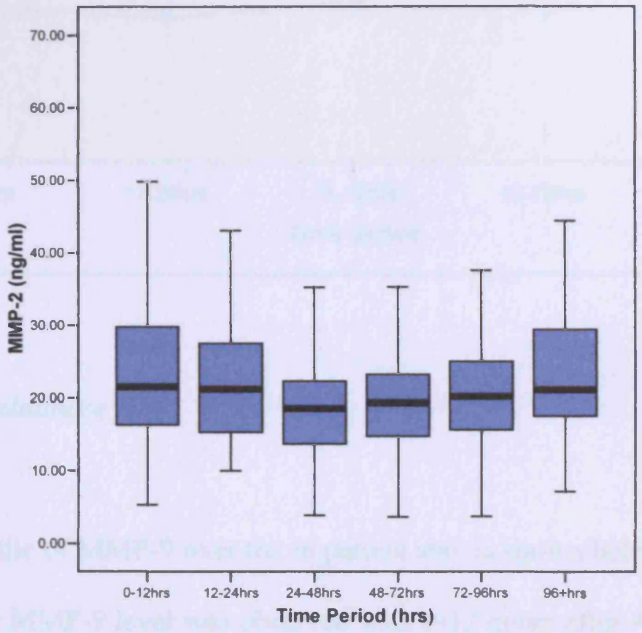
5.3

The Gelatinases

5.3.1: MMP-2 (Gelatinase-A)

The temporal profile of MMP-2 over the in patient stay is shown below. Data are median, quartiles ± range (with outliers excluded).

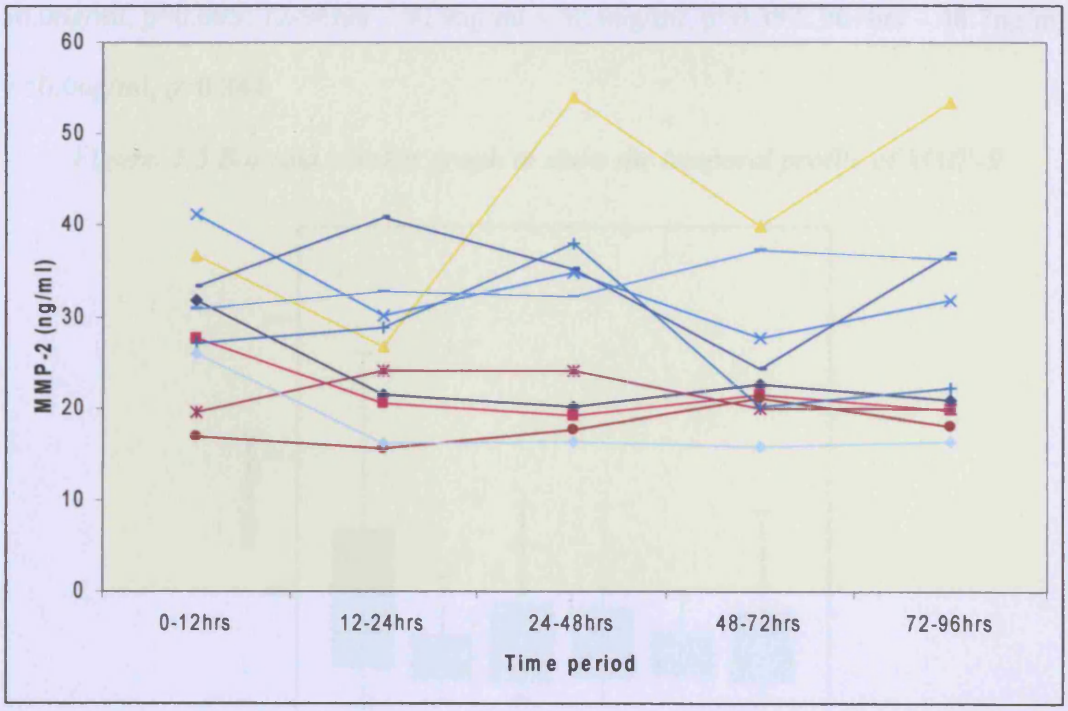
Figure. 5.3. Box and whisker graph to show the temporal profile of MMP-2



Plasma MMP-2 levels were similar at all time periods with no significant difference between time periods on ANOVA (p=0.31), post hoc analysis all p=NS. Compared to our control population MMP-2 levels were elevated at all time periods.

0-12hrs – 21.2ng/ml v 15.6ng/ml, p<0.001; 12-24hrs – 21.1ng/ml v 15.6ng/ml, p<0.001: 24-48hrs – 18.4ng/ml v 15.6ng/ml, p<0.001: 48-72hrs – 19.5ng/ml v 15.6ng/ml, p<0.001: 72-96hrs – 20.0ng/ml v 15.6ng/ml, p<0.001: 96+hrs – 20.5ng/ml v 15.6ng/ml, p<0.001

Figure 5.4. Temporal profile of individual patients (n=10). Each line represents 1 patient



5.3.2: MMP-9 (Gelatinase B)

The temporal profile of MMP-9 over the in patient stay is shown below. In contrast to MMP-2, maximal MMP-9 level was observed with 0-12 hours after AMI (median 71.0ng/ml [range 15.0-376.0]) with a fall to a plateau thereafter (ANOVA, p=0.001). Post hoc analysis revealed differences between 0-12hrs and all other time periods until 96hrs (12-24hrs 45.8 [20.5-293.2]ng/ml, p=0.003; 24-48hrs 49.1 [22.9-361.4] ng/ml, p=0.016; 48-72hrs 50.6 [18.75-296.5] ng/ml, p=0.02; 72-96hrs 50.1 [18.3-395.2] ng/ml, p=0.003; >96hrs 49.0 [23.0-398.1] ng/ml, p=0.105. Compared to our control population MMP-9 levels were only elevated at 0-12hrs. Following this period there was no significant difference between cases and controls.

0-12hrs – 67.6ng/ml v 50.0ng/ml, p=0.002; 12-24hrs – 44.3ng/ml v 50.0ng/ml, p=0.192: 24-48hrs – 49.8ng/ml v 50.0ng/ml, p=0.998: 48-72hrs – 49.4ng/ml v 50.0ng/ml, p=0.695: 72-96hrs – 50.9ng/ml v 50.0ng/ml, p=0.397: 96+hrs – 48.7ng/ml v 50.0ng/ml, p=0.844

Figure. 5.5 Box and whisker graph to show the temporal profile of MMP-9

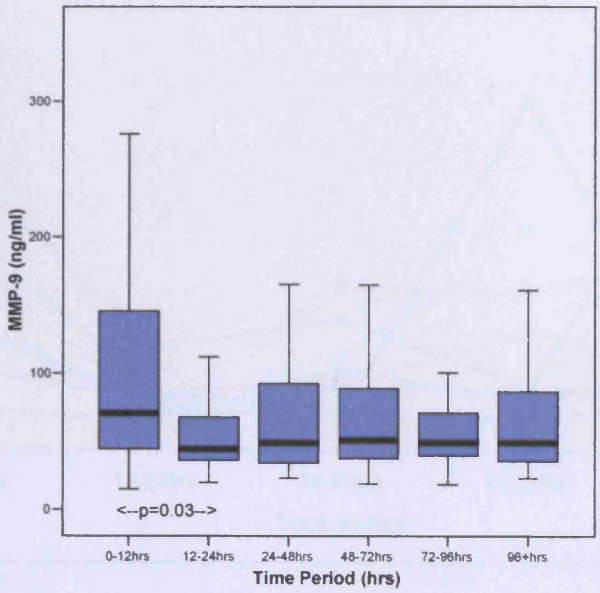
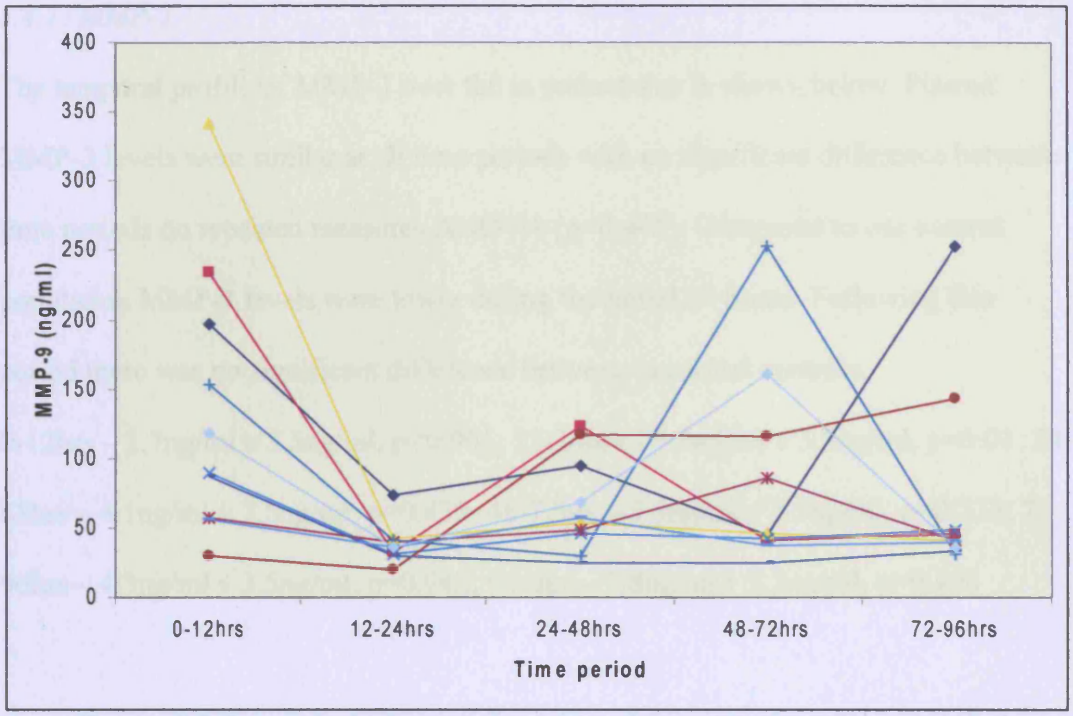


Figure 5.6. Temporal profile of individual patients (n=10). Each line represents 1 patient



5.4

The Stromelysins

5.4.1: MMP-3

The temporal profile of MMP-3 over the in patient stay is shown below. Plasma MMP-3 levels were similar at all time periods with no significant difference between time periods on repeated measures ANOVA (p=0.445). Compared to our control population MMP-3 levels were lower during the initial 24 hours. Following this period there was no significant difference between cases and controls.

0-12hrs – 2.7ng/ml v 3.5ng/ml, p<0.001; 12-24hrs – 3.0ng/ml v 3.5ng/ml, p=0.01: 24-48hrs – 4.1ng/ml v 3.5ng/ml, p=0.435: 48-72hrs – 3.5ng/ml v 3.5ng/ml, p=0.238: 72-96hrs – 4.3ng/ml v 3.5ng/ml, p=0.942: 96+hrs – 2.8ng/ml v 3.5ng/ml, p=0.498

Figure. 5.7 Box and whisker graph to show the temporal profile of MMP-3

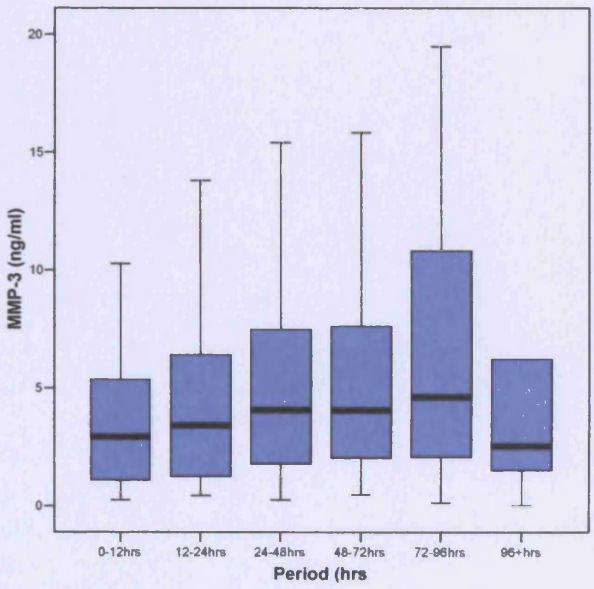
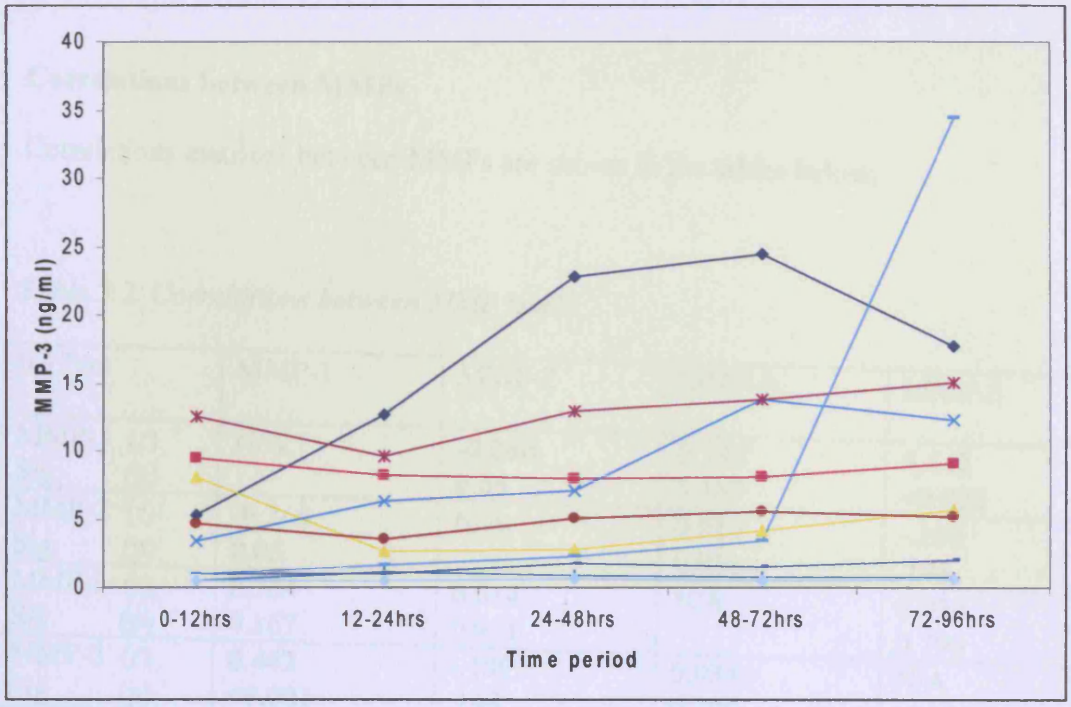


Figure 5.8. Temporal profile of individual patients (n=10). Each line represents 1 patient



5.5

Correlations between MMPs

Correlations matrices between MMPs are shown in the tables below.

Table 5.2. Correlations between MMP levels

0-12hrs	MMP-1	MMP-2	MMP-9	MMP-3
MMP-1 (r) Sig. (p)	N/A	**-0.260** **0.05**	0.184 0.167	**0.442** **<0.001**
MMP-2 (r) Sig. (p)	**-0.260** **0.05**	N/A	0.014 0.911	-.189 .148
MMP-9 (r) Sig. (p)	0.184 0.167	0.014 0.911	N/A	0.034 0.796
MMP-3 (r) Sig. (p)	**0.442** **<0.001**	-.189 .148	0.034 0.796	N/A

12-24hrs	MMP-1	MMP-2	MMP-9	MMP-3
MMP-1 (r) Sig. (p)	N/A	-0.145 .301	**0.267** **<0.001**	**0.177** **0.001**
MMP-2 (r) Sig. (p)	-0.145 .301	N/A	-0.045 0.736	0.158 0.254
MMP-9 (r) Sig. (p)	**0.267** **<0.001**	-0.045 0.736	N/A	0.110 0.06
MMP-3 (r) Sig. (p)	**0.177** **0.001**	0.158 0.254	0.110 0.06	N/A

24-48hrs	MMP-1	MMP-2	MMP-9	MMP-3
MMP-1 (r) Sig. (p)	N/A	-0.091 0.337	**0.146** **0.011**	**0.188** **0.001**
MMP-2 (r) Sig. (p)	-0.091 0.337	N/A	-0.053 0.550	-0.045 0.633
MMP-9 (r) Sig. (p)	**0.146** **0.011**	-0.053 0.550	N/A	-0.018 0.761
MMP-3 (r) Sig. (p)	**0.188** **0.001**	-0.045 0.633	-0.018 0.761	N/A

48-72hrs	MMP-1	MMP-2	MMP-9	MMP-3
MMP-1 (r) Sig. (p)	N/A	-0.016 0.883	-0.016 0.883	**0.341** **<0.001**
MMP-2 (r) Sig. (p)	-0.016 0.883	N/A	-0.075 0.472	0.011 0.922
MMP-9 (r) Sig. (p)	0.099 0.093	-0.075 0.472	N/A	**0.155** **0.008**
MMP-3 (r) Sig. (p)	**0.341** **<0.001**	0.011 0.922	**0.155** **0.008**	N/A

72-96hrs	MMP-1	MMP-2	MMP-9	MMP-3
MMP-1 (r) Sig. (p)	N/A	-0.142 0.302	**0.274** **<0.001**	**0.312** **<0.001**
MMP-2 (r) Sig. (p)	-0.142 0.302	N/A	-0.079 0.472	0.032 0.815
MMP-9 (r) Sig. (p)	**0.274** **<0.001**	-0.079 0.472	N/A	0.024 0.751
MMP-3 (r) Sig. (p)	**0.312** **<0.001**	0.032 0.815	0.024 0.751	N/A

Chapter 6

Determinants of Plasma MMP concentrations. Association of Plasma MMP with LV Structure, Function and Remodelling.

Before investigating the determinants of plasma MMP concentrations and association with LV structure and remodelling we considered the temporal profile of each MMP. Taking into account the varying temporal profiles as discussed in chapter 5, we considered MMP levels at each individual time period. In addition, for those MMPs in which we observed a flat/steady temporal response (MMP-1, -2, -3) we considered mean levels and for MMP-9 we considered peak and trough levels.

6.1

Determinants of Plasma MMP concentration.

6.1.1: The Collagenases (MMP-1)

Pearsons correlations co-efficients between MMP-1 and continuous patient demographics are presented in appendix section, table 11.1. MMP-1 shows a consistent but weak inverse correlation with troponin, reaching statistical significance only at 12-24hrs ($r=-.341$, $p=0.008$). In contrast, there does not appear to be any consistent relationship with CK. No significant correlation is seen with other markers. Mean MMP-1 showed weak but statistically significant correlation with white cell count ($r=0.214$, $p=0.048$).

Difference in MMP-1 levels between categorical patient demographics were compared using Mann-Whitney U tests (appendix table 11.2). No difference is seen between males v females. There appears a consistent relationship with both type of infarct and territory, with MMP-1 being consistently higher (but not reaching significance) in NSTEMI v STEMI and Inferior v Anterior infarcts. No consistent relationship is seen in those receiving thrombolysis or smoking, or in patients with previous history of angina, myocardial infarction or hypertension MMP-1 is elevated in diabetics over non diabetics, this relationship being consistent across all time periods and significant at 0-12hrs.(534.5 v 220.6 ng/ml , p=0.05)

In addition MMP-1 appears consistently elevated although again not reaching statistical significance in those subjects who at admission were receiving anti-platelet medication or ACE-Inhibitors. No difference was consistently seen in those receiving either Beta-blockers or Statins.

6.1.2: The Gelatinases

MMP-2 (Gelatinase-A)

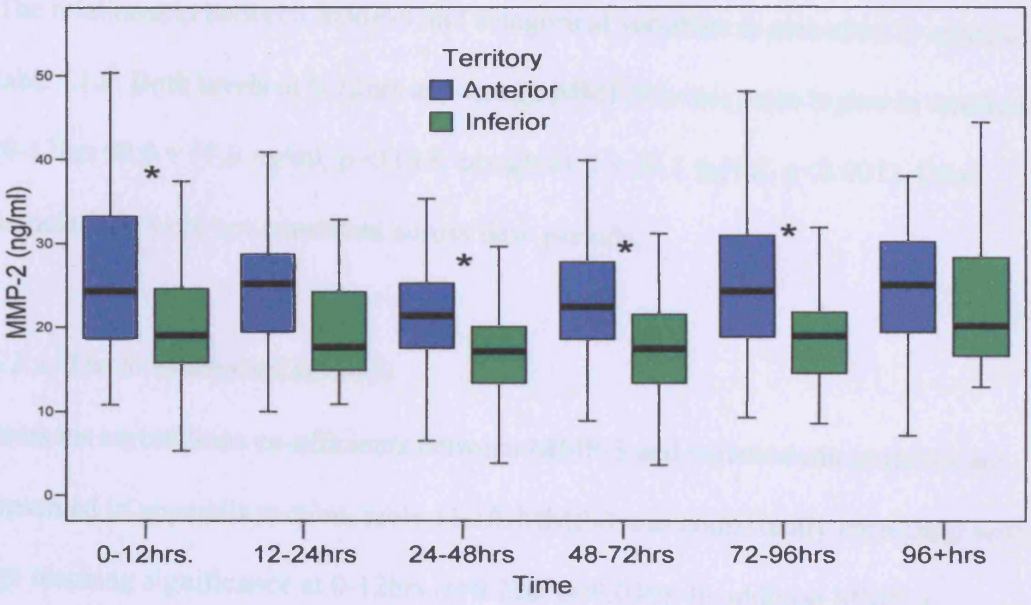
Pearsons correlations co-efficients between MMP-2 and continuous patient demographics are presented in appendix section, table 11.4.

MMP-2 was associated with patient age with direct correlation at 4 of 6 time periods and with mean value (0-12hrs r=0.335, p=0.006, 24-48hrs r=0.309, p=0.003, 48-72hrs, r=0.271, p=0.009, 72-96hrs r=0.332, p=0.002, mean r=0.211, p=0.044). Inverse correlation was seen with CK at all time periods until 96hrs (statistically significant at 0-12 r=-0.318, p=0.012, 24-48 r=-0.325, p=0.002 & 48-72hrs r=-0.245, p0.023). Mean MMP-2 also showed inverse correlation with CK (r=-0.280, p=0.009). In

addition there was an apparent inverse correlation between MMP-2 and WCC at all times and with mean levels (statistically significant at 0-12 r=-0.245, p=0.048, 24-48 r=-0.340, p=0.001 & 48-72hrs, -0.261, p=0.013)

Difference in MMP-2 levels between categorical variables were compared using Mann-Whitney U tests (appendix table 11.5). MMP-2 was higher in females v males, reaching statistical significance at 0-12hrs (28.7 v 19.6 ng/ml, p=0.032). Both mean levels and levels at all time periods between 24hrs – 96hrs were higher in NSTEMI v STEMI (24-48hrs 20.1 v 17.7 ng/ml, p=0.034; 48-72hrs 23.5 v 18.1 ng/ml, p=0.001; 72-96hrs 25.3 v 19.3 ng/ml, p=0.002, mean 23.3 v 19.8 ng/ml, p=0.04). MMP-2 was consistently higher in anterior v inferior infarcts (Figure 6.1) reaching statistical significance at 0-12hrs (p=0.02), 24-48hrs (p=0.007), 48-72hrs (p=0.008) and 72-96hrs (p=0.07). In addition mean levels were also higher in anterior v inferior infarcts (24.1 v 18.5 ng/ml, p=0.006). MMP-2 was also consistently higher in those subjects who were receiving beta-blocker medication prior to admission (0-12hrs 25.3 v 19.6 ng/ml, p=0.044; 24-48hrs 20.9 v 17.6 ng/ml, p=0.023; 48-72hrs 20.5 v 18.1 ng/ml, p=0.047; 72-96hrs 25.0v 18.8 ng/ml, p=0.002; 96+hrs 29.4 v 19.8 ng/ml, p=0.034; mean 24.4 v 19.4 ng/ml, p=0.01). No other significant differences were noted between MMP-2 and patient demographics.

*Figure. 6.1 Box and whisker graph to show the temporal profile of MMP-2 according to infarct territory (*p<0.05)*



MMP-9 (Gelatinase B)

Pearsons correlation coefficients between continuous variables and MMP-9 at the various time periods are presented in appendix table 11.7. MMP-9 at 0-12hrs (r=-0.294, p=0.014) and trough MMP-9 (r=-0.398, p<0.001) showed inverse correlation with patient age. MMP-9 at 0-12hrs was also inversely related to creatinine (r=-0.342, p=0.004).

MMP-9 at all time periods as well as peak and trough levels were related to both WCC (peak r=0.362, p=0.001; 0-12hrs r=0.368, p=0.002; 12-24hrs r=0.434, p=0.001; 24-48hrs r=0.147, p=0.165; 48-72hrs r=0.212, p=0.044; 72-96hrs r=0.284, p=0.008; 96+hrs r=0.350, p=0.031; trough r=0.332, p=0.001) and neutrophil count (peak r=0.362, p=0.001; 0-12hrs r=0.298, p=0.013; 12-24hrs r=0.403, p=0.003; 24-48hrs r=0.145, p=0.170; 48-72hrs r=0.215, p=0.041; 72-96hrs r=0.269, p=0.012; 96+hrs

r=0.298, p=0.069; trough r=0.285, p=0.006). No other significant correlations were noted.

The relationship between MMP-9 and categorical variables is presented in appendix table 11.8. Both levels at 0-12hrs and trough MMP-9 levels were higher in smokers (0-12hrs 99.0 v 57.0 ng/ml, p=0.018; trough 41.3 v 30.1 ng/ml, p<0.001). Other associations were not consistent across time periods.

6.1.3: The Stromelysins(MMP-3)

Pearsons correlations co-efficients between MMP-3 and patient demographics are presented in appendix section, table 11.10. MMP-3 was consistently correlated with age reaching significance at 0-12hrs (r=0.220, p=0.049). In addition MMP-3 correlated with serum creatinine across all time periods until 96hrs (0-12hrs r=0.441, p<0.001; 12-24hrs r=0.401; p<0.001; 24-48hrs r=0.422, p<0.001;48-72hrs r=0.498, p<0.001; 72-96hrs r=0.373, p=0.003). In addition mean levels correlated with age (r=0.220, p=0.043) and creatinine (r=0.498, p<0.001)

Difference in MMP-3 levels between categorical patient demographics were compared using Mann-Whitney U tests and are presented in appendix section 11.11. MMP-3 levels were consistently (but not statistically significantly) elevated in those subjects with NSTEMI over STEMI and in non smokers over current smokers. In addition levels appeared higher in those subjects with a history of hypertension reaching significance at time periods between 0-48 hrs (0-12hrs 4641.0 v 2412.6 pg/ml , p=0.05, 12-24hrs 4457.9 v 2451.4 pg/ml, p=0.015, 24-48hrs 4946.8 v 3020.7

pg/ml, p=0.049). Mean levels were also elevated in hypertensives (6054.9 v 2703.9 pg/ml, p=0.017). No other consistent differences were noted.

6.2

Association of plasma MMP concentration with LV structure, function and remodelling

6.2.1: *The Collagenases (MMP-1)*

Pearsons correlation co-efficients between MMP-1 and markers of LV function, volumes and remodelling both during in-patient stay and at follow up are shown in appendix tables 11.3. No consistent or significant correlations were seen between MMP-1 and markers of LV function, volumes or remodelling. No difference was seen in those subjects with (LVEF <40%) or without LV impairment either prior to discharge or at follow up (p all NS)

6.2.2: The Gelatinases

MMP-2 (Gelatinase-A)

There was no apparent correlation between MMP-2 at any time period with echocardiographic parameters of LV function, volumes or remodelling either pre-discharge or at follow up (appendix table 11.6)

MMP-9 (Gelatinase B)

Correlation between MMP-9 and echocardiographic markers are presented in appendix section 11.9. Peak MMP-9 was associated with more severe impairment of LV function during admission with a direct correlation with WMIS (r=0.217, p=0.048) and inverse correlation with LVEF (r=-0.316, p=0.004).
Patients with peak MMP-9 above the median (113ng/ml) had lower LVEF (Median LVEF 38%, range 22-66) compared to patients with MMP-9 $\leq$ 113ng/ml (46%, range 19-66, p=0.014)(Fig 6.2 upper panel). Similarly, subjects with LVEF <40% prior to discharge had significantly higher peak MMP-9 (154.3 v 91.2ng/ml, p=0.02)

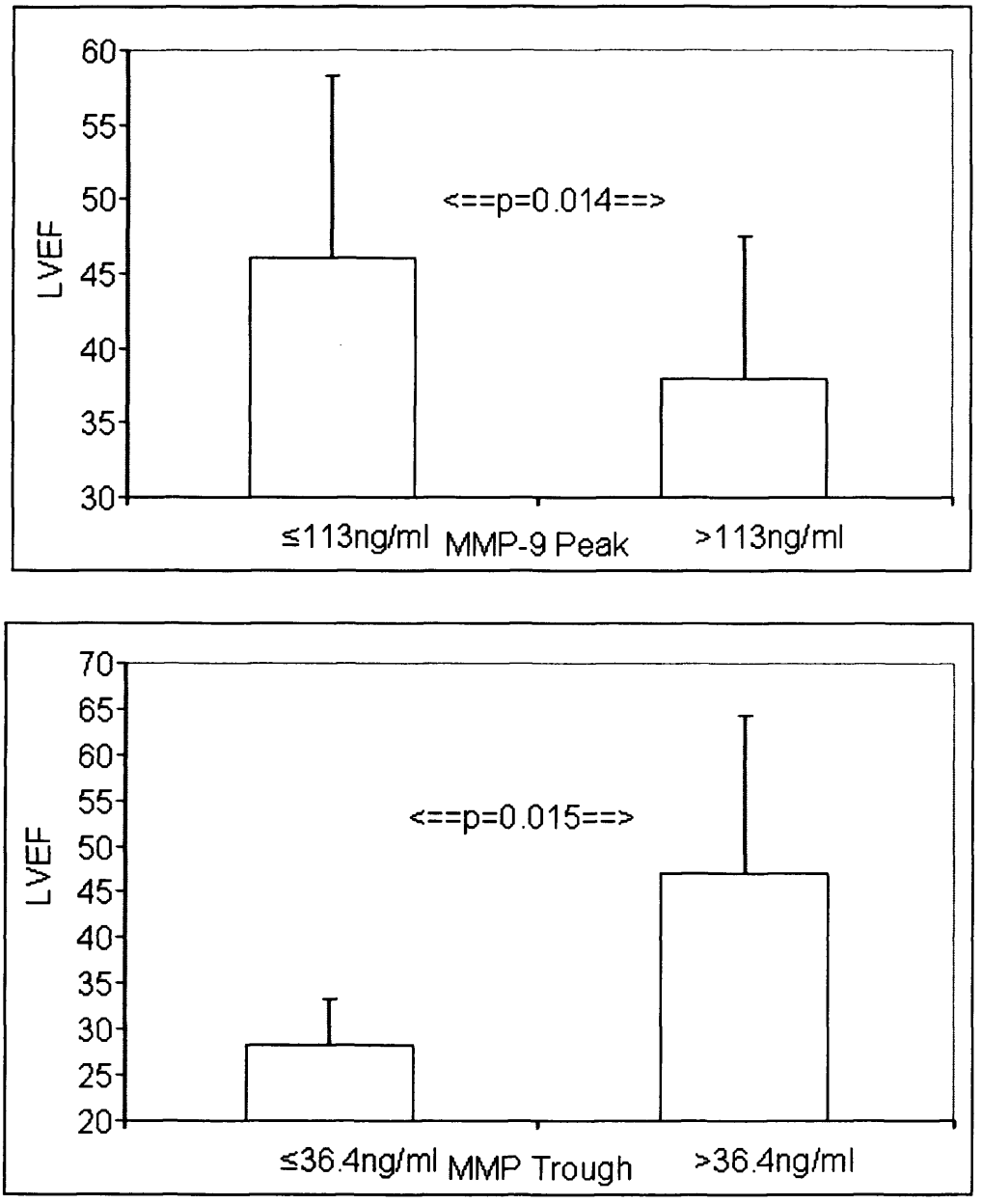
In contrast, higher **trough** levels of MMP-9 were associated with relative **preservation** of LV function at **follow-up** (appendix 11.9) with a direct correlation with LVEF (r=0.290, p=0.015) and inverse correlation with WMIS (r=-0.230, p=0.032).

At follow up examination LVEF was higher in those patients with trough MMP-9 levels above the median (36.4ng/ml) compared to those at or below the median (Median LVEF 52%, range 18-69 v 42%, range 16-62, p=0.035) (Fig.6.2 lower panel)

Figure. 6.2

Upper panel: Left ventricular ejection fraction according to peak MMP-9 below or above the median value (113ng/ml)

Lower panel: Left ventricular ejection fraction at follow up according to trough MMP-9 below or above the median value (36.4ng/ml)



Factors with univariable association with LV function were Age, territory of infarction, previous history of MI or angina and CK.

On multivariable linear regression analyses, only peak MMP-9 concentration ($p=0.026$) and anterior territory of AMI ($p=0.003$) retained independent predictive value for LVEF during admission. Factors with independent predictive value for WMIS were age ($p=0.014$), previous MI ($p=0.004$), anterior territory of AMI ($p<0.001$) and CK ($p=0.001$).

LV volumes and remodelling

There was no apparent relationship with LVEDV or LVESV during the index admission for MMP-9. Peak MMP-9 was indicative of the extent of LV remodelling after discharge. Peak MMP-9 correlated directly with the magnitude of change in EDV between admission and follow up (ΔEDV) ($r= 0.3$, $p=0.016$)(appendix table 11.9, Figure 6.3). Similarly, peak MMP-9 was higher in those patients in whom EDV increased (median 157.9ng/ml, range 45.0-398.1) compared to those in whom EDV decreased (135ng/ml, range 35.6-365.5, $p=0.047$).

In contrast, increasing trough MMP-9 concentrations were associated with relative preservation in LV volumes after discharge with an inverse correlation with Δ EDV ($r=-0.280$, $p=0.024$) (Figure 6.3) and ΔESV ($r=-0.4$, $p=0.001$)

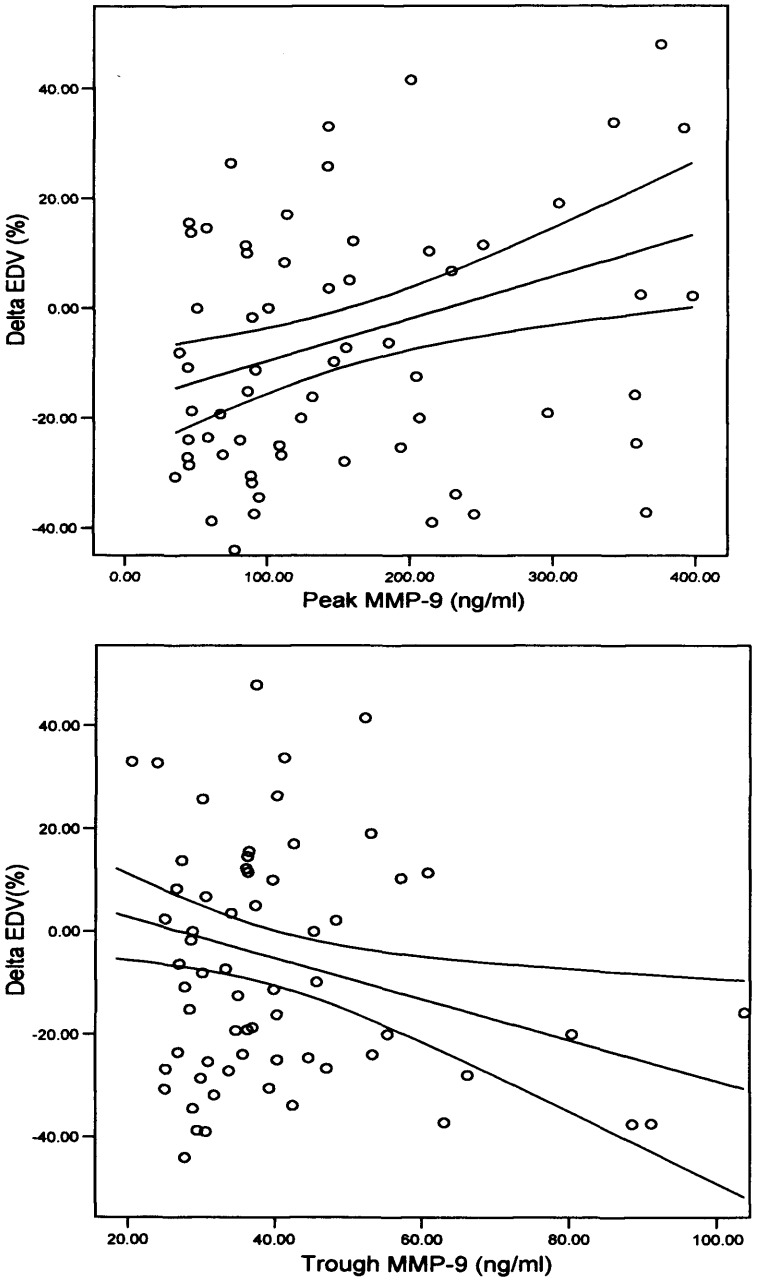
Factors with univariable association with ΔEDV were: history of hypertension (hypertension +4.5ml v no hypertension –13.0 ml, $p=0.027$), and diuretic use at discharge (diuretic +12.0ml v no diuretic –13.0ml, $p=0.003$). On multivariable

analysis peak MMP-9 concentration maintained independent association with ΔEDV ($p=0.007$) and trough MMP-9 with ΔESV ($p=0.005$).

No other pre-discharge medication was associated with the extent of remodelling.

There was no univariable or multivariable relationship between neutrophil counts and Δvolumes.

Figure. 6.3 Relationship between peak MMP-9 (upper) and trough MMP-9 (lower) and the change in left ventricular end diastolic volume (delta EDV)



6.2.3: The Stromelysins (MMP-3)

There was no apparent correlation between MMP-3 at any time period with echocardiographic parameters of LV function, volumes or remodelling either pre-discharge or at follow up (appendix table 11.12)

6.3

Discussion – Plasma Matrix metalloproteinases and left ventricular dysfunction and remodelling after acute myocardial infarction

Stage 1 aimed to examine the temporal profile of several MMPs and their relationship with markers of LV function, volumes, and remodelling in a limited population size. This stage would provide essential information on a variety of MMPs. The results from stage 1 have provided interesting and novel findings with regards to metalloproteinase activity post acute MI.

6.4

Temporal profiles and association with baseline demographics

We have demonstrated individual temporal profiles of MMP release post AMI. This may have several important implications in both the pathogenesis of acute myocardial infarction and the subsequent development of heart failure as well as the potential for metalloproteinase manipulation as a therapeutic target. Several previous studies have suggested that the metalloproteinase family are involved in both the development of coronary artery disease, plaque rupture leading to AMI and the remodelling process of

the LV leading to clinical heart failure. In addition MMP manipulation via selective or broad spectrum MMP inhibitors or knock out models has been shown to influence the pathogenesis of these diseases. These studies have been thoroughly discussed in the introduction to this thesis. Animal studies have shown that MMP manipulation may alter the remodelling process of the LV post experimental AMI (Spinale, Coker et al. 1999; Yarbrough, Mukherjee et al. 2003; Spinale, Escobar et al. 2006). However human studies have to date failed to see similar results (Hudson, Armstrong et al. 2006). The differing temporal profiles of the various enzymes in humans may be partly responsible for this lack of effect. Temporal profiles of the various MMPs in humans have to date not been widely described and most studies lack significant participant numbers. These data are essential in the investigation of both the effects of metalloproteinases post AMI and in the potential use in therapeutic practice. In addition we have demonstrated that individual subjects may have patterns of release of MMPs which does not reflect the median values seen. This again has important implications for the use of MMP manipulation as a therapeutic target and may suggests that individuals may have variable "therapeutic windows", in which MMP inhibition may have effect. We also observe correlations between the MMP subtypes. There is correlation seen between MMP-1 and MMP-3 at all time periods and correlation between MMP-1 and MMP-9 at all periods beyond 12 hours. This observation would be in keeping with the theory that MMP release forms a "cascade" of events with interrelationships between the various subtypes. Our information furthers our knowledge of the metalloproteinase family and may aid in the development of therapeutic targets.

6.4.1: Collagenases

Collagenase-1 (MMP-1) activity appears relatively stable from admission until discharge with no significant time dependent change seen over the 5 day in-patient stay. In addition levels of MMP-1 were significantly raised compared to our control population, this elevation reaching significance at 72-96hrs. Few previous studies have demonstrated the temporal profile of MMP-1. Papadopoulis et al (Papadopoulos, Moyssakis et al. 2005) observed a peak of MMP-1 activity in the first 24hrs post AMI followed by a later peak at 5-30days. However the study was limited by the small sample size. While we observed no significant peaks of MMP-1 from day 1-5, MMP-1 did correlate with troponin T, suggesting an association with infarct size. In apparent contrast to this, MMP-1 appeared elevated in inferior over anterior infarcts. While the explanation for this apparent discrepancy may be statistical, the possibility of differing regional release of MMP-1 is a possibility.

Results from an angiographic study by Inoue (Inoue, Kato et al. 2003) et al also showed MMP-1 to be elevated post MI and in subjects with unstable over stable angina and controls. Temporal profiles were not recorded. Hirohata et al (Hirohata, Kusachi et al. 1997) also observed a change in MMP-1 levels with time but in contrast to the above and our own study, levels were suppressed for the first 4 days post AMI compared to controls increasing thereafter. Soejima et al observed increased MMP-1 at 7 days and 2 weeks post AMI over that at admission (Soejima, Ogawa et al. 2003).

We also observe an interesting difference in MMP-1 levels between diabetics versus non diabetics. In vitro studies suggest that hyperglycaemia may induce MMP production (Death, Fisher et al. 2003; Maldonado, He et al. 2004; Kadoglou, Daskalopoulou et al. 2005) and animal studies have observed differential MMP activity in diabetic models over controls. Harris et al observed increased MMP-2 but decreased MMP-1 in a rat diabetes model compared to control animals. This group suggested that these effects may be causative in the cerebrovascular remodelling process contributing to the increased risk of stroke in diabetes (Harris, Hutchinson et al. 2005). Kamada et al observed increased MMP-9 in a rat model of cerebral ischaemia (Kamada, Yu et al. 2007). Similarly Westermann et al (Westermann, Rutschow et al. 2007) have demonstrated up-regulation of MMP-9 gene expression in rat models of diabetes induced heart failure, the effects of which are inhibited by angiotensin 1 receptor antagonists. Altered MMP activity has also been seen in diabetics with proliferative retinopathy (Kosano, Okano et al. 1999; Noda, Ishida et al. 2003), in children with Type I diabetes mellitus (Bister, Kolho et al. 2005; Derosa, Avanzini et al. 2005) and adult subjects with type I (Shiau, Tsai et al. 2006) and type II diabetes mellitus (Lee, Song et al. 2005; Sampson, Wall et al. 2006). In addition Goldstein et al have shown a reduction in MMP-9 following treatment with rosiglitazone (Goldstein, Weissman et al. 2006) and cardiovascular risk reduction in diabetic patients leads to a reduction in metalloproteinase activity (Tayebjee, Lim et al. 2004). Previous groups have hypothesized that altered activity of the MMP system may be responsible for the hyperglycaemia induced vascular changes seen in diabetes. While our data support this hypothesis, we cannot establish a cause-effect relationship. Children with microangiopathy secondary to diabetes express altered MMP-2 activity (Derosa, Avanzini et al. 2005) and Chung et al have recently shown

altered metalloproteinase activity in coronary artery sections taken at the time of CABG in diabetics over non diabetics (Chung, Luo et al. 2007). Our data also suggest that the altered activity in diabetics with ACS is independent of the acute plaque rupture or myocardial necrosis as both groups (diabetic v non diabetic) had similar degrees of myocardial damage as assessed by troponin and echo markers of LV function.

A prior diagnosis of diabetes is associated with increased risk of adverse outcome after AMI (Malmberg, Yusuf et al. 2000; McGuire, Emanuelsson et al. 2000; McGuire, Newby et al. 2004). "Diabetes" however is a diagnosis which may or may not have been made in subjects prior to their index admission. Elevated serum glucose is common post AMI as part of the stress response and elevated glucose during this period has been shown to be associated with adverse prognosis and the development of heart failure independent of a previous diagnosis of diabetes (Oswald, Corcoran et al. 1984; O'Sullivan, Conroy et al. 1991; McGuire, Emanuelsson et al. 2000; Capes, Hunt et al. 2001; Wahab, Cowden et al. 2002; McGuire, Newby et al. 2004; Ceriello 2005). In view of this preliminary data, we feel that the relationship between diabetes and hyperglycaemia with metalloproteinase expression warrants further investigation. Later in this thesis we will discuss the effects of admission glucose on metalloproteinase activity in addition to that of previous diabetes diagnosis.

6.4.2: Gelatinases

The differing temporal profiles of plasma MMP-9 and MMP-2 levels after AMI are in keeping with previous reports from our own (Squire, Evans et al. 2004) and other

(Tziakas, Chalikias et al. 2005; Wagner, Delagardelle et al. 2006; Webb, Bonnema et al. 2006) groups. We observe elevated levels of MMP-2 at all time periods post AMI compared to our control population whereas MMP-9 levels are only elevated in the immediate post infarction period (0-12hrs). Our own group (Squire, Evans et al. 2004) have previously reported the temporal profile of MMP-2 and MMP-9 in 50 patients post AMI, this work forming the basis for the studies included in this thesis. This study observed a peak of MMP-9 at day 1 post AMI with a further rise at day 4 with similar levels of MMP-2 seen from day 1-5. In contrast to the previous study did not observe a further peak at day 4 however levels were elevated at day 1. We also observed elevated levels of MMP-2 in anterior over inferior myocardial infarction whereas the previous observed elevated levels in inferior AMI. The explanation for this discrepancy may lie in the extent of infarction. In the initial study by Squire et al, mean CK was higher in inferior versus anterior infarcts indicating that in this cohort, subjects with inferior MI suffered greater myocardial damage. In our present study mean CK was higher in anterior over inferior infarcts hence potentially explaining the discrepancy between these 2 studies.

Previous clinical studies suggested acute plaque rupture to be the source of high plasma MMP-9 following AMI (Kai, Ikeda et al. 1998; Schmidt, Bultmann et al. 2006). Our data suggest that neutrophils may be an important or even the predominant source of plasma MMP-9 in this setting. This is in keeping with animal models of AMI (Lindsey, Wedin et al. 2001; Lindsey, Gannon et al. 2002; Tao, Cavasin et al. 2004), in which neutrophils appear to be the predominant source of MMP-9 in the early period of inflammation. In addition plaque-derived MMP-9 may facilitate

neutrophil infiltration and degranulation, and exacerbate the ischaemic insult (Frangogiannis, Smith et al. 2002).

Alternatively the early peak and the later plateau of plasma MMP-9 may come from separate sources, the early peak from acute plaque rupture, and the later plateau from neutrophils or elements of the myocardial matrix. Observations from the current study may lend support to this postulate.

6.4.3. Stromelysins

Serum levels of MMP-3 were also stable from admission to discharge and correlated with patient age and creatinine. Interestingly MMP-3 levels appear suppressed in the first 24hrs post MI compared to controls. Our results would be consistent with a previous study by Samnegard et al who observed similar levels of MMP-3 at admission and 48hrs post AMI with a subsequent rise at 3 months (Samnegard, Silveira et al. 2006). Levels seen in this study were similar to that seen in our population (Admission.10.5-21.8ng/ml & 48hrs.14.4-24.7ng/ml). There are no other previous data regarding the temporal profile of MMP-3 after AMI.

Our study has also identified other associations between plasma MMP levels and a number of clinical parameters. The observation in current smokers of lower MMP-3 and also MMP-2, in the early hours after AMI is of note. The matrix metalloproteinases have a role in the pathogenesis of smoking-related lung diseases, mediating smoke-induced inflammatory cell recruitment into lung tissue (Hautamaki, Kobayashi et al. 1997; Shapiro 2002). Moreover, both MMP-9 and MMP-2 are increased in a rat model of emphysema (Kirschvink, Vincke et al. 2005). Relevant to

the pathogenesis of AMI, cigarette smoking reduces nitric oxide production and increases that of reactive oxygen species (Perlstein and Lee 2006). We suggest that the observed association between smoking and variation in plasma gelatinase levels in the immediate post-AMI period is worthy of further study.

In addition MMP-3 levels appeared higher in those subjects with a history of hypertension which may be a result of increased myocardial strain or as a result of prior LV remodelling and left ventricular hypertrophy (LVH). Indeed Saglam et al have previously described increased MMP-3 in subjects with LVH secondary to hypertension (Saglam, Karakaya et al. 2006), Fielitz et al observed increased MMP-3 in subjects with aortic stenosis, again potential secondary to LVH (Fielitz, Leuschner et al. 2004) and gene expression of MMP-3 falls after surgical correction of aortic stenosis (Walther, Schubert et al. 2001) with associated regression of LVH. While these observations suggest pre-existing LV remodelling may contribute to higher MMP-3 concentrations, this is unlikely to be the sole, or even the main, explanation for differences in MMP-3 in our study.

Our observations of altered temporal profiles of MMP release or suppression in the case of MMP-3 may have significant impact on the use of MMP manipulation as a potential therapeutic target and would suggest that "broad spectrum" MMP inhibition may not be appropriate or even detrimental post AMI.

6.5

Metalloproteinases - LV function, volume and remodelling

Correlations between MMP levels and LV function are given in the results section.

6.5.1: Collagenases

Our results have both similarities and contrast to previous investigations of MMP-1. Papadopoulis et al (Papadopoulos, Moyssakis et al. 2005) observed higher levels of MMP-1 in subjects with left ventricular impairment post acute myocardial infarction. Hirohata et al (Hirohata, Kusachi et al. 1997) observed direct correlation between MMP-1 at day 5 with LV ejection fraction and negative correlation with left ventricular volume which is contradictory to the Papadopoulis study but consistent with a study by Thomas et al (Thomas, Coker et al. 1998) which observed suppressed expression of MMP-1 in subjects with dilated cardiomyopathy compared to controls. We observed no difference in MMP-1 activity in those subjects with echocardiographic evidence of LV systolic dysfunction either prior to discharge or at follow up. There were no significant correlations between MMP-1 and markers of LV volume or function and no correlation with LV remodelling. From our results it is not entirely possible to conclude that MMP-1 has no effect on LV function and remodelling. The in-vitro affinity of MMP-1 for types I, II and III collagens would certainly support a potential pathophysiological role. However our data present a more extensive investigation than these previous studies which were limited in participants.

6.5.2: Gelatinases

Our study has further elucidated the significance of the gelatinases with regards to LV function and remodelling post MI. There was no apparent correlation between MMP-2 at any time period with echocardiographic parameters of LV function, volumes or remodelling either pre-discharge or at follow up. In contrast we have demonstrated close relationships with MMP-9. Firstly peak MMP-9 correlated with more severe impairment of LV function during admission, patients with peak MMP-9 above the median had lower LVEF and subjects with LVEF < 40% had higher peak MMP-9. These results would be consistent with several previous studies. Our own group have demonstrated a correlation between MMP-9 and LV function as assessed by wall motion scoring (Squire, Evans et al. 2004) and similar results have been seen in both human (Reinhardt, Sigusch et al. 2002; Webb, Bonnema et al. 2006) and animal models (Ducharme, Frantz et al. 2000). Moreover, we also found higher MMP-9 in the early post-AMI period to indicate more extensive left ventricular remodelling in the following weeks and months. While these data may simply reflect an association between higher peak MMP-9 and the extent of myocardial infarction, they are in keeping with studies of experimental AMI (Heymans, Luttun et al. 1999; Ducharme, Frantz et al. 2000; Romanic, Burns-Kurtis et al. 2001; Chen, Tung et al. 2005). Indeed, increased MMP-9 activity during the very early post-AMI period may be responsible for early proteolytic degradation in the infarcted myocardium and thus for rapid ventricular dilatation at this time (Heymans, Luttun et al. 1999). Our data are also broadly in keeping with a recent clinical report by Webb et al (Webb, Bonnema et al. 2006) in approximately 50 patients with AMI, which suggested higher MMP-9

over the first few days after AMI to be associated with greater increases in LV volume. However the previous study considered changes in LV volume based upon dichotomised MMP-9 values. Our findings, showing linear relationships between MMP-9 on the one hand and LVEF, WMSI, LV volumes, and ΔLVEDV on the other, are logical and highly consistent, extending the results form the previous study and supporting a true pathophysiological role for MMP-9 after AMI.

In the same context, we report a further novel, and potentially clinically relevant observation. Intriguingly, while higher peak levels of plasma MMP-9 were associated with greater impairment of LV function during the index admission, higher trough levels of MMP-9 in the days following AMI were associated with *less* remodelling, and relative preservation of left ventricular function, thereafter. The nature of these associations indicates that higher peak levels, and also lower trough levels, of MMP-9 to be associated with greater increase in LV volumes. Once again experimental studies support this apparent contradiction. In the same study which showed that MMP-9 deficiency protects against myocardial rupture after AMI in mice, MMP-9 deficiency was also associated with impaired infarct healing, resulting in greater myocardial necrosis (Romanic, Burns-Kurtis et al. 2001). Indeed enhanced MMP activity soon after AMI results in proteolysis, allowing neutrophil infiltration of the infarct, which in the early period post AMI results in matrix degradation and increased likelihood of cardiac rupture (Heymans, Luttun et al. 1999; Romanic, Burns-Kurtis et al. 2001; Frangogiannis, Smith et al. 2002). Later in the process, this same proteolytic activity allows infiltration of other cell types which mediate wound healing (Schaffer and Nanney 1996). This observation would be in keeping with the possibility that the early peak and later troughs in plasma MMP-9 have different

sources. Our findings, when considered with previous experimental data, suggest that, in addition to contributing to adverse left ventricular remodelling, MMP-9 activity may also be important to wound healing after AMI. Our observation of an early peak, elevated over our control population followed by return to "normal" levels would also be suggestive that the temporal profile of MMP-9 release has significant pathophysiological effects on LV biology. There are potentially important clinical implications for these findings, in particular for the potential therapeutic manipulation of the metalloproteinase system via MMP inhibition. In the recent PREMIER (Prevention of Myocardial Infarction Early Remodelling) trial, the MMP inhibitor PG-116800 failed to attenuate adverse LV remodelling after AMI in man (Hudson, Armstrong et al. 2006). However PG-116800 was administered for 90 days with the first dose given on average >48 hours after the onset of AMI. In the context of the findings from the present study, we may postulate that long-term inhibition of MMP-9 may not be the most appropriate therapeutic manipulation of the MMP system after AMI. MMP-9 inhibition may be appropriate in the very early period after AMI, and potentially harmful thereafter.

6.5.3: Stromelysins

There was no apparent correlation between MMP-3 at any time period with echocardiographic parameters of LV function, volumes or remodelling either pre-discharge or at follow up. These results would again be consistent with previous studies. Firstly MMP-3 was not elevated in subjects with congestive heart failure compared to age matched controls (George, Patal et al. 2005) and MMP-3 is elevated in subjects with idiopathic dilated cardiomyopathy over those with cardiomyopathy

due to ischaemic heart disease (Spinale, Coker et al. 2000; Tziakas, Chalikias et al. 2005). Likewise the 5A/6A polymorphism of the MMP-3 gene has been shown to be an independent predictor of cardiac mortality in subjects with idiopathic but not ischaemic cardiomyopathy (Mizon-Gerard, de Groote et al. 2004). Indeed our data would suggest that MMP-3 is in fact suppressed in the post MI period and hence it is unlikely that measurement at this period would be associated with LV remodelling.

6.6

Chapter Summary

In summary we have observed individual temporal profiles of metalloproteinase expression post acute myocardial infarction. These temporal profiles are in keeping with some but not all previous studies however our data is more extensive than previous investigations. We have observed interesting and novel relationships between metalloproteinase activity and left ventricular structure, volume, function and remodelling especially with regards to the gelatinase MMP-9.

In the second stage of this study we will extend this data to investigate the relationship between metalloproteinase activity to prognosis and outcome post acute myocardial infarction which is intimately linked to the adverse ventricular function and dilatation which we have observed and discussed in this chapter.

Chapter 7

Association of plasma MMP-9, TIMP-1 & N-BNP with left ventricular dysfunction and remodelling, and with prognosis, post AMI

7.1

Aims and Original Hypothesis

Stage 2 of this project aimed to further our knowledge of metalloproteinase activity obtained from stage 1 in a larger population and to extend the work to the assessment of the relationship between MMP concentrations and prognosis. The development of left ventricular (LV) remodelling is among the most powerful indicators of adverse prognosis after myocardial infarction. Remodelling involves maladaptive changes to cellular and extracellular myocardium, involving both infarcted and non infarcted areas of the ventricle (Bolognese and Cerisano 1999). The extent of the resultant impairment of contractile function and progressive ventricular enlargement are closely associated with increased risk of adverse outcome (Pfeffer and Braunwald 1990) Our results from stage 1 indicate an association between MMP-9 at its peak level and both left ventricular function and remodelling. Our original hypothesis was therefore that MMP-9 would also be associated with adverse prognosis post AMI.

An important function of the TIMP enzymes is the regulation of physiological MMP activity. Based on previous studies discussed in chapter 2 and the known inhibitory

action of TIMP-1 on MMP-9 we also measured serum levels of this inhibitor in our extended population.

BNP and its N terminus are strong markers of both left ventricular dysfunction and prognosis post AMI (Chapter 3). We therefore also aimed to compare both MMP-9 and TIMP-1 with N terminal pro BNP in the prediction of prognosis post AMI.

7.2

Methods

All methods were as discussed in Chapter 4. We enrolled a total of 404 patients admitted to the Coronary Care unit (CCU) of our hospital between September 2004 and February 2006 with a confirmed diagnosis of AMI. The diagnosis was based on symptoms suggestive of myocardial ischaemia, together with appropriate ECG changes (dynamic ST segment elevation (STEMI, n=329) or ST segment / T wave changes (NSTEMI, n=75) and elevation in markers of myocardial necrosis (Creatine Kinase, Troponin I). We again excluded patients with active inflammatory disease or known malignancy.

All patients donated venous blood samples for measurement of MMP-9, TIMP-1 and N-BNP. MMP-9 was measured on daily samples with N-BNP and TIMP-1 measured on pre-discharge samples. All patients underwent echocardiographic examination during the index admission (N=404) and at follow-up (median 148, range 90-378 days) in 343 survivors. 26 subjects failed to attend for repeated echocardiography.

The pre-specified primary outcome was a composite of death or heart failure episode, the latter defined as clinical heart failure requiring hospital admission with high dose

diuretic, intravenous nitrate or inotropic support. Secondary outcomes included each individual component of the primary end point, and recurrent myocardial infarction.

All end-points were assessed at 5 months (150 days) post event, no patient was lost to clinical follow-up. Endpoints were identified via the hospital patient tracking system and survivors were also contacted at the end of follow-up to ensure complete capture of all hospital admission events.

7.3

Results

The admission demographic features of the study population are shown in Table 7.1. Approximately 75% of the population were male, ST-elevation was evident on the admission ECG in over 80%, and median creatine kinase was > 1300 I.U. Of the 329 patients presenting with STEMI, 203 (62%) received thrombolytic therapy. No patient received primary percutaneous revascularisation.

Echocardiography was undertaken in all patients prior to discharge and in 343 survivors attending for follow-up assessment. Prior to discharge, left ventricular ejection fraction and WMIS were measurable in 351 (86.9 %) and 356 (88.1%) patients respectively. Corresponding figures at follow-up echocardiographic examinations were 310 (90.3%) and 305 (88.9%). No patient was lost to clinical follow-up, which ranged from 1-150 days with a median of 150 days. For those subjects who survived to discharge the median follow up was 150 days (range 15-150)

Table. 7.1. Population demographics at admission

	Median	Range
Age (yrs)	62.1	24-91
CK (I.U., NR 0-200)	1391	41-7384
Troponin I (NR <0.06)	27.1	0.06-150
	Number (%)	
Male/Female	305/99 (75.5/24.5)	
STEMI	329 (81.4)	
Anterior / Inferior site	165/221 (40.8/54.7)	
Thrombolysis	203 (50.2)	
Current Smoker	147 (36.4)	
Diabetes	69 (17.1)	
Hypertension	169 (41.8)	
Previous MI	38 (9.4)	
Previous Revascularisation	10 (2.5)	
Medications		
	Admission	Discharge
Aspirin	82 (20.2)	331 (90.6)
Clopidogrel	14 (3.5)	86 (23.6)
Beta Blocker	82 (20.3)	336 (92.1)
ACE-I/ARB	98 (24.3)	363 (89.9)
Statin	86 (21.3)	354 (97)
Furosemide	44(10.9)	56 (13.9)

CK=Creatine Kinase; NR = Normal Range; ACE-I=Angiotensin Converting Enzyme Inhibitor

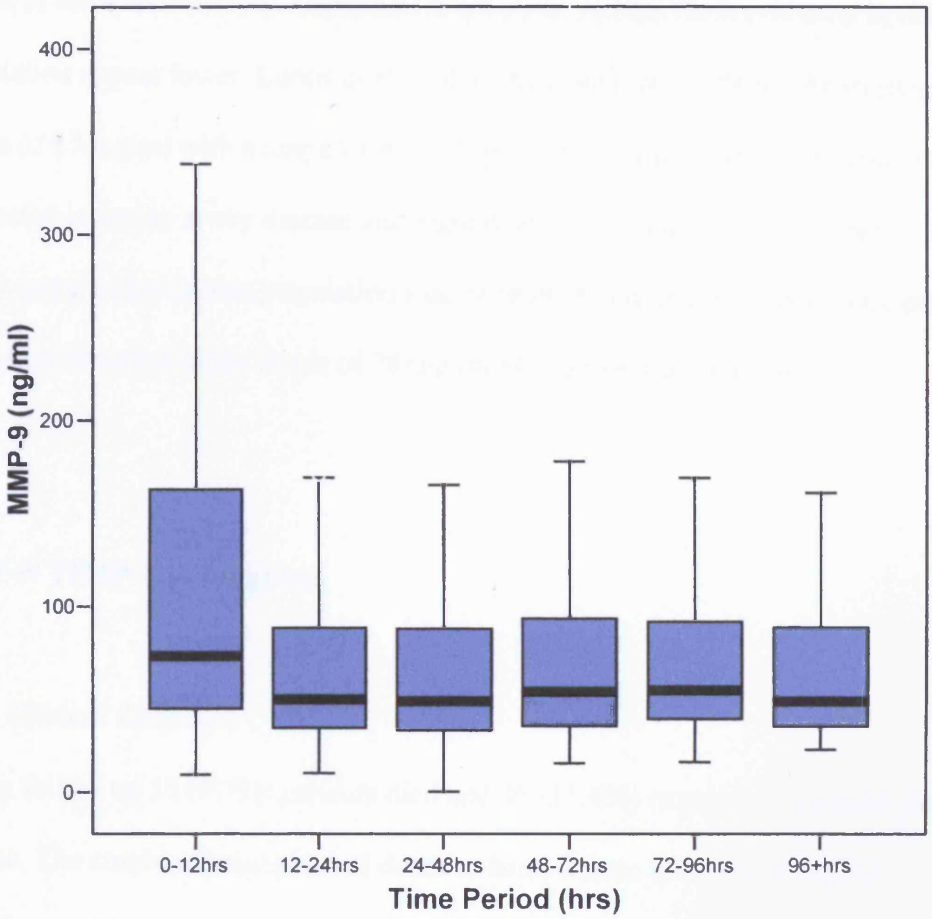
7.3.1: *Plasma MMP-9*

The temporal profile of MMP-9 was confirmed. The highest (peak) MMP-9 concentrations occurred at 0-12 hours post-AMI (median 73.14, range 9.52-652.13ng/ml). This level was elevated at this time compared to all later points (ANOVA - $p<0.001$)(Fig. 7.1) and was significantly higher than our control population (50.0, range 18.9-319.5ng/ml, $p<0.001$) . Beyond this time period there was no significant differences between cases and controls, 12-24hrs – 50.5ng/ml v 50.0ng/ml, p=0.860: 24-48hrs – 49.4ng/ml v 50.0ng/ml, p=0.905: 48-72hrs – 54.3ng/ml v 50.0ng/ml, p=0.211: 72-96hrs – 55.0ng/ml v 50.0ng/ml, p=0.102: 96+hrs – 48.7ng/ml v 50.0ng/ml, p=0.941

Initial MMP-9 levels were higher in patients with ST elevation, compared to non-ST elevation, AMI (median 141.8 ng/ml, range 19.5-652.1 v 95.6 ng/ml, range 19.61-615.1, p=0.005) and in patients with previous AMI (161.6 ng/ml, range 35.6-502.4 v 130.3, 19.5-652.1, p=0.005). There were no apparent differences in MMP-9 related to other demographic factors, gender, site of AMI, to pre-admission pharmacological treatment, or thrombolysis.

Peak MMP-9 correlated with CK (r=0.154, p=0.003) and with peripheral venous white cell (WCC r=0.244, $p<0.001$) and neutrophil count (r=0.245, $p<0.001$) at admission. The association with WCC (12-24 hrs r=0.142, p=0.01; 24-48 hrs 0.142, p=0.09; 48-72 hrs 0.128, p=0.02; 72-96 hrs 0.216, p=0.001) and neutrophil count (12-24 hrs r=0.113, p=0.04; 24-48 hrs 0.128, p=0.02; 48-72 hrs 0.123, p=0.03; 72-96 hrs 0.207, p=0.001) was maintained for MMP-9 at later time points.

Figure. 7.1. Temporal profile of MMP-9 post AMI



7.3.2: Plasma TIMP-1

TIMP-1 levels were higher in females compared to males (128.9ng/ml [range 41.4-465.9] v 107.9 ng/ml[7.7- 408.6], $p<0.001$) and in patients with a history of hypertension (123.3ng/ml [46.9- 465.9] v 107.9 ng/ml [7.7- 408.6], $p<0.001$). TIMP-1 correlated with patient age ($r=0.410$, $p<0.001$), CK ($r=0.161$, $p=0.001$), creatinine concentration ($r=0.331$, $p<0.001$) and neutrophil count ($r=0.183$, $p<0.001$). There was

only a weak correlation between plasma TIMP-1 and MMP-9 (r=0.082, p=0.103). Control population TIMP-1 levels were not available for our population due to cost issues of the TIMP-1 assay. Compared to previous studies the levels seen in our population appear lower. Lubos et al (Lubos, Schnabel, et al. 2006) observed median levels of 676ng/ml with a range from 6 – 2667ng/ml in a population of subjects with suspected coronary artery disease and Sundstrom et al (Sundstrom, J, Evans J et al. 2004) using a Framinham population free of heart failure or previous myocardial infarction observed mean levels of 781ng/ml SD 133 (see discussion)

7.4

MMP-9/ TIMP-1 & Prognosis

7.4.1: Clinical Endpoints

During follow up 35 (8.7%) patients died and 46 (11.4%) experienced a heart failure episode. The combined end point of death or heart failure was reached by 63 individual patients. Forty four (10.9%) experienced further myocardial infarction. Plasma MMP-9 at 0-12 hours and TIMP-1 were higher in the 65 patients reaching the primary end-point and in those reaching each individual component of the primary end-point (Table 7.2). There was no association of MMP-9 or TIMP-1 with the occurrence of re-infarction.

Table 7.2. MMP-9 (0-12hrs) (above) and TIMP-1 (below) in relation to the occurrence of clinical endpoints.

Endpoint	**MMP-9 (ng/ml), median [range]**		
	Event	**No event**	**p-value**
Death/heart failure (N=63)	114.9 [21.2-585.1]	67.1 [9.5-652.1]	**0.001**
Death (N=35)	87.0 [21.1-585.1]	70.0 [9.5-652.1]	**0.042**
Heart failure (N=46)	103.4 [30.0-450.7]	67.8 [9.5-652.1]	**0.011**
Re-infarction (N=44)	72.8 [22.3-307.7]	71.0 [9.52-652.1]	0.78

Endpoint	**TIMP-1 (ng/ml) median [range]**		
	Event	**No event**	**p-value**
Death/heart failure (N=63)	166.7 [46.5-465.9]	108.4 [7.7-408.6]	**<0.001**
Death (N=35)	173.9 [84.0-465.9]	110.8 [7.7-408.6]	**<0.001**
Heart failure (N=46)	163.4 [46.5-381.7]	110.6 [36.8-465.9]	**<0.001**
Re-infarction (N=44)	122.1 [37.5-299.2]	114.8 [36.8-465.9]	0.351

Multivariable predictors of outcome

Factors with univariable association with death or heart failure are shown in table 7.3.

Table 7.3 Prediction of death or heart failure episode, univariable associations (Logistic regression). Odds ratio for continuous variables (age (years), creatinine (μmol/L), glucose (mmol/L)) are per unit increase or per two fold increase in MMP-9/TIMP-1

Factor	**Odds Ratio [95%CI]**	**p-value**
Anterior territory	2.13 [1.21-3.76]	0.009
Hypertension	1.95 [1.14-3.36]	0.016
Age	1.084 [1.06-1.12]	0.001
Creatinine	1.022 [1.01-1.03]	0.001
Glucose	1.082 [1.03-1.14]	0.004
Log_2MMP-9	1.41 [1.12-1.78]	0.003
Log_2TIMP-1	7.76[4.40-13.69]	0.001

Factors with univariable association with death or heart failure (Table 7.3) were entered into multivariable binary logistic regression analyses for the prediction of outcome. When entered individually into the multivariable model, peak MMP-9 (OR 1.48: 95%CI 1.14-1.93 per unit increase in log transformed MMP-9, p=0.004) and TIMP-1 (OR 4.35: 95% CI 2.28-8.30 per unit increase in log transformed TIMP-1, p<0.001) each had independent association with the occurrence of death or heart failure. When entered simultaneously into the model, each retained independent predictive value (MMP-9, OR 1.44: 95%CI 1.09-1.91, p=0.011; TIMP-1, OR 3.78:

95% CI 1.90- 7.51, p<0.001). Age also had independent predictive value for the primary endpoint (OR 1.05: 95% CI 1.01-1.09, p=0.006).

Factors with independent association with death (MMP-9 and TIMP-1 entered individually) were TIMP-1 (OR 5.28, p<0.001), anterior territory of infarct (OR 2.59, p=0.023), age (OR1.1, p=0.001) and plasma glucose concentration (OR= 1.081, p=0.046). When MMP-9 and TIMP-1 were entered simultaneously, only TIMP-1 retained significance for the prediction of death (p=0.001). Factors with independent association with heart failure were MMP-9 (OR 1.33, p=0.05), TIMP-1 (OR 2.79, p=0.004), age (OR 1.05, p=0.008) and anterior territory of infarct (OR 2.40, p=0.017). Again when MMP-9 and TIMP-1 were entered together, only TIMP-1 retained independent predictive value (p=0.008)

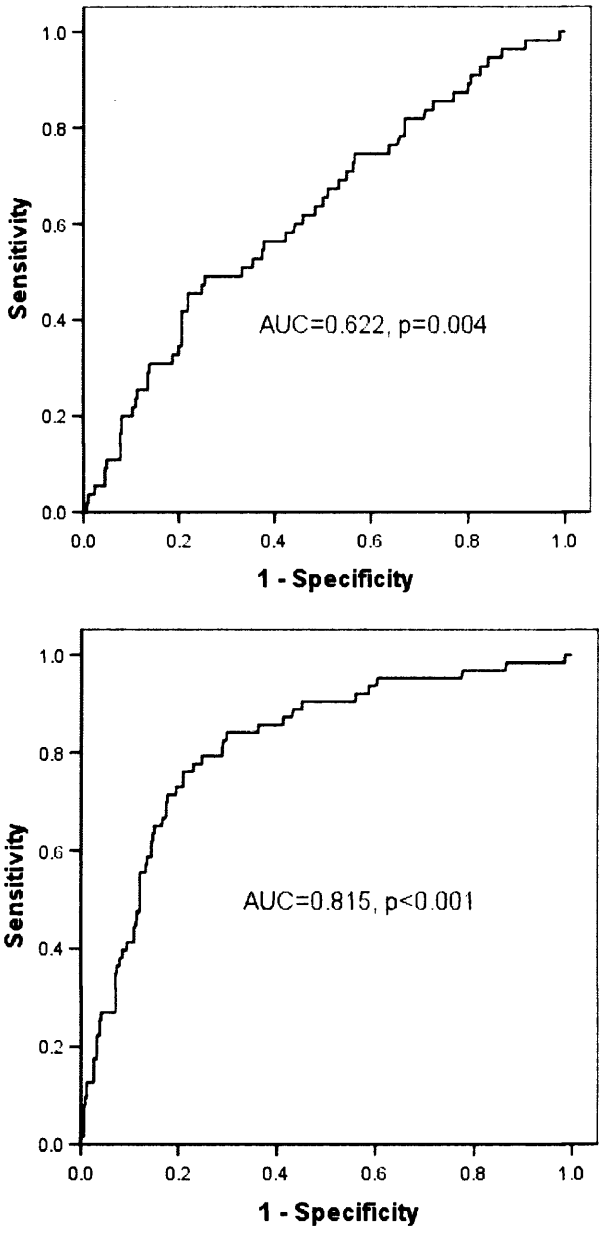
On Cox Proportional Hazards modelling (Table 7.4) independent predictors of death or heart failure were peak MMP-9, TIMP-1, age, admission blood glucose and creatinine.

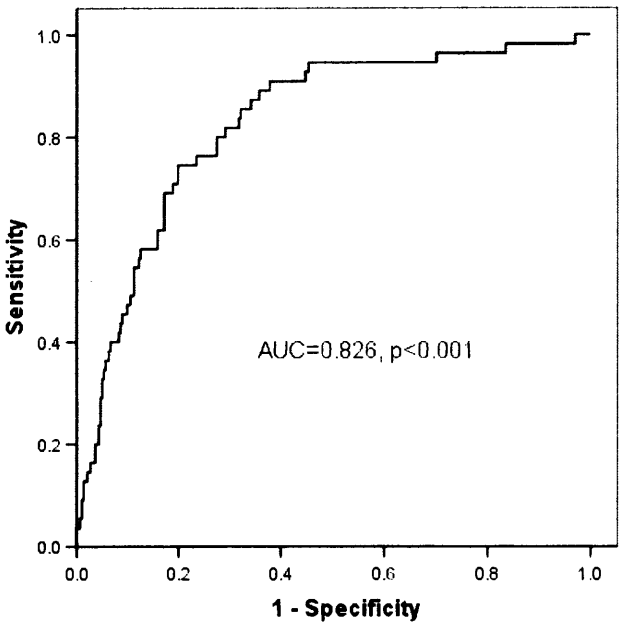
Table 7.4. Uni-variable and multi-variable predictors of death/heart failure(Cox regression)

Factor	**Univariable Hazards Ratio**	**p-value**	**Multivariable Hazards Ratio**	**p-value**
Anterior territory	1.98 [1.17-3.33]	0.010	1.59 [0.87-2.91]	0.129
History of Hypertension	1.85 [1.12-3.03]	0.016	1.60 [0.87-2.91]	0.128
Age	1.08 [1.05-1.10]	0.001	1.08 [1.04-1.11]	0.001
Creatinine	1.01 [1.01-1.02]	0.001	1.01 [0.99-1.01]	0.320
Glucose	1.07 [1.03-1.11]	0.001	1.07 [1.01-1.12]	0.008
Log MMP-9	1.47 [1.16- 1.86]	0.002	1.39 [1.11-1.74]	0 .008
Log TIMP-1	4.71 [3.26-6.81]	0.001	2.67 [1.60-4.46]	0.001

The area under the ROC curve for prediction of the primary outcome was 0.622 (95%CI 0.541-0.703; p=0.004) for MMP-9, and 0.815 (95% CI 0.757 – 0.872; p<0.001) for TIMP-1. A logistic model combining MMP-9 and TIMP-1 gave an area under the ROC of 0.826 (95% CI 0.768- 0.885, p<0.001)(Fig. 7.2).

Figure. 7.2: Receiver operator curves for the prediction of death or heart failure according to: MMP-9-above, TIMP-1- middle and combined regression model MMP-9/TIMP-1- below

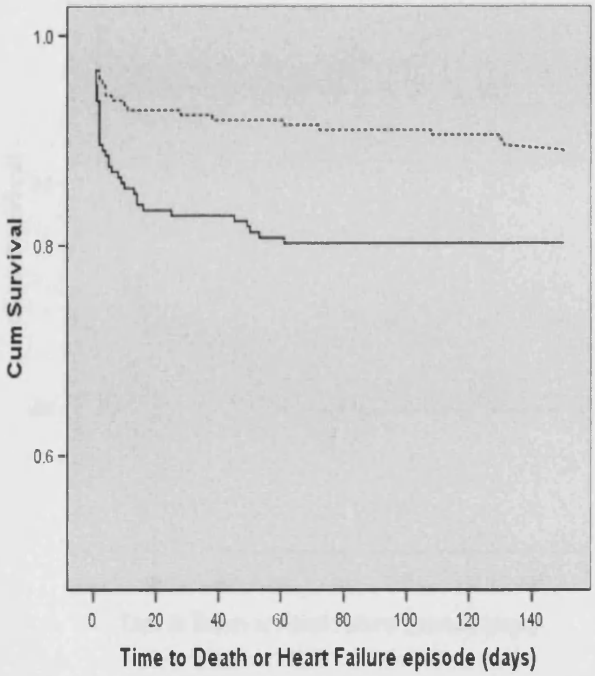




From the ROC curves we identified cut off points for the optimum combination of sensitivity and specificity for prediction of the primary endpoint for peak MMP-9 (76ng/ml) or TIMP-1 (135ng/ml). Patients for whom MMP-9 was above 76ng/ml had increased risk of death or heart failure (OR=1.91, 95% CI 1.10-3.33, Log Rank p=0.017). In comparison, the risk associated with elevation of TIMP-1 above 135ng/ml was much greater (HR 11.44, 95% CI 5.61-23.36, Log Rank p<0.001) (Fig. 7.3). The risk of adverse outcome was particularly elevated in patients for whom TIMP-1 and MMP-9 were both above the respective median (OR 15.38, 95% CI 6.09-38.5, p<0.001). Risk was lowest for patients with both MMP-9 and TIMP-1 below these value, with intermediate risk for those with either TIMP-1 or MMP-9 above the median (OR 3.77, 95% CI 1.5-9.43, p=0.003) (Fig. 7.4).

Figure. 7.3: Kaplan-Meier survival curves for patients stratified by optimum MMP-9 (76ng/ml) or TIMP-1(135ng/ml) concentrations.

(i) maximal MMP-9 ≤ or > optimum (ii) TIMP-1 ≤ or > optimum



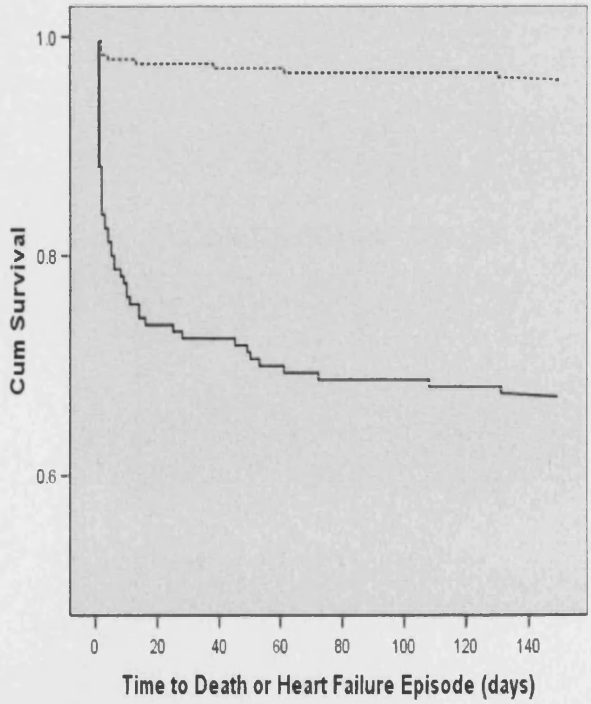
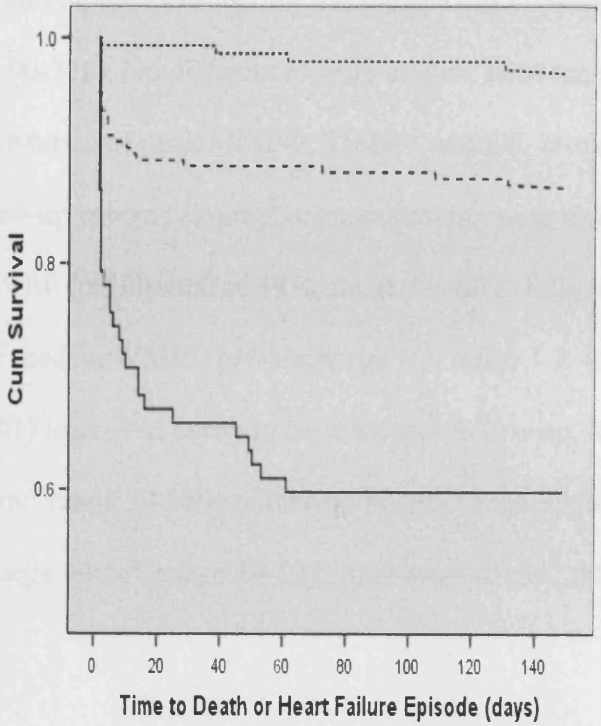


Figure. 7.4 Kaplan-Meier survival curves for patients stratified by optimum MMP-9 and TIMP-1 levels. Both levels above optimum (solid line) v either above optimum (middle broken line) v both below optimum (top broken line)



7.5

Left Ventricular structure and function.

Follow-up echocardiographic examination was carried out in 343 patients at a median of 148 days (range 90-378). No differences were evident between surviving attenders and non-attenders in terms of peak MMP-9, TIMP-1 and CK levels. Adequate pre-discharge and follow-up echocardiographic examinations were available in 292 patients. Median LVEF (pre-discharge 44%, range 15-78%; follow up 48%, range 15-82%, $p<0.001$) and median WMIS (pre-discharge 1.3, range 1-2.4; follow up 1.2, range 1-2.6, $p<0.001$) improved between baseline and follow up. Median LVEDV (pre-discharge 89cm^3, range 29-189; follow-up 87cm^3, range 32-204, $p=0.259$) and LVESV (pre-discharge 49cm^3, range 14-127; follow-up 45 cm^3, 15-147, $p=0.067$) fell slightly.

7.5.1: MMP-9 and LV volumes and function

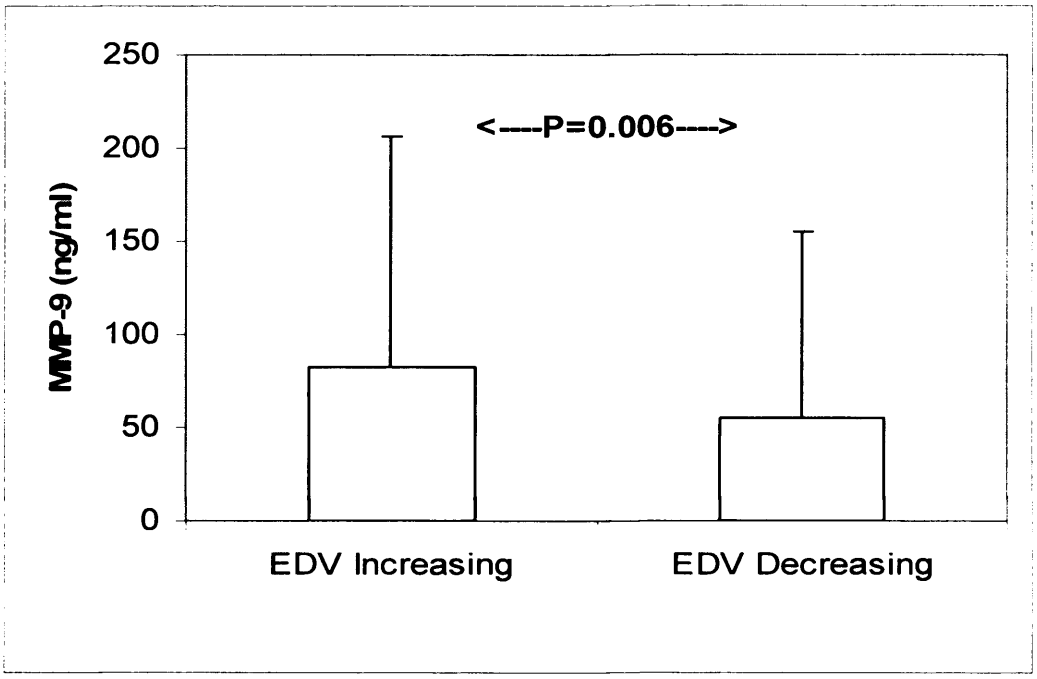
MMP-9 at 0-12 hours correlated with LVEDV and LVESV both pre-discharge (EDV1 & ESV1) and at follow up (EDV2 & ESV2). Similarly, MMP-9 at 0-12 hours was associated with more severe impairment of LV function pre-discharge, as indicated by direct and inverse correlation with WMIS and LVEF respectively (Table 7.5). There was a weak correlation between MMP-9 and WMIS at follow up (r=0.113, p=0.05)

Table 7.5 Correlation of MMP-9 (0-12hrs) with echocardiographic measures

MMP-9	EDV1	ESV1	LVEF	WMIS	EDV2	ESV2	LVEF2	WMIS2
r value	0.187	0.208	-0.179	0.160	0.191	0.147	-0.055	0.113
p value	<0.001	<0.001	0.001	0.003	0.001	0.01	0.339	0.05

Peak MMP-9 was associated with LV remodelling. For those patients showing an increase in LVEDV between pre-discharge and follow-up examinations (N=142), median MMP-9 was higher (82.5 ng/ml, range17.1-652.1) than in those for whom LVEDV fell (N=150; 55.0 ng/ml, range 9.5-489.6, p=0.006) (Fig. 7.5).

Figure. 7.5 MMP-9 levels in subjects with increasing v decreasing End Diastolic Volumes (EDV) between admission and follow up.



Similarly, MMP-9 was higher in patients in whom LVESV increased (N=162; 72.2 ng/ml, range 17.7- 652.1) compared to those in whom LVESV fell (N=127, 59.9 ng/ml, range 9.5 - 489.6, p=0.113).

Peak MMP-9 levels correlated in a linear fashion with the degree of LV remodelling expressed as ΔEDV (r=0.169, p=0.004). Factors showing uni-variable association ($p<0.1$) with ΔEDV were anterior territory of infarct (p=0.036), CK (p=0.004) and MMP-9 (p=0.005). On multivariable linear regression analyses both MMP-9 (Standardised beta=0.148, p=0.015) and CK (Standardised beta=0.138, p=0.026) maintained independent association with ΔEDV.

After excluding from analysis those patients with history of previous AMI, the univariable (p=0.005) and multivariable (p=0.037) relationship between MMP-9 and ΔEDV was maintained.

There were no statistically significant differences in MMP-9 levels between those patients in whom LVEF (p=0.145) or WMIS (p=0.818) increased or decreased. Similarly, there was no univariable correlation between MMP-9 and either changes in LVEF (ΔLVEF r=-0.048, p=0.42) or changes in WMIS (ΔWMIS r=0.038, p=0.528).

7.5.2: TIMP-1

Pre-discharge TIMP-1 levels correlated with the severity of LV impairment and with LV volume measured both pre-discharge and at follow up (Table 7.6).

Table 7.6 Correlation of TIMP-1 with echocardiographic measures

TIMP-1	EDV1	ESV1	LVEF	WMIS	EDV2	ESV2	LVEF2	WMIS2
r value	0.092	0.192	-0.246	0.298	0.149	0.238	-0.311	0.219
p value	0.088	<0.001	<0.001	<0.001	0.009	<0.001	<0.001	<0.001

Although TIMP-1 was higher in those patients in whom LV volumes increased between discharge and follow up, these differences did not reach statistical significance (LVEDV median 116.0 ng/ml, range 39-408.6 cf 108 ng/ml, range 36.8-300.2, p=0.099; LVESV 113.7 ng/ml, range 9-295.6 cf 114.5 ng/ml, range 36.8-408.6, p=0.375). There was a weak but statistically significant correlation between TIMP-1 and both ΔEDV and ΔESV (r=0.124, p=0.036 & r=0.116, p=0.05 respectively)

7.5.3: MMP-9:TIMP-1 ratio

In view of the biological interaction between MMP-9 and TIMP-1 we considered the ratio of MMP-9 to TIMP-1. There was no significant correlation between the MMP-9:TIMP-1 ratio either with any parameter of LV volume, function or remodelling.

7.6

Comparison of our "New" prognostic marker with N terminal pro BNP – the "Gold Standard", peptide marker.

As discussed in chapter 3, BNP is a strong marker of prognosis post AMI. In view of the strong association of TIMP-1 with prognosis we investigated the use of TIMP-1 both compared to and in association with N-BNP.

7.6.1: Plasma N-BNP

Median plasma N-BNP concentration was higher in females compared to males (1022.1 fmol/ml range[22.1- 9856.7] v 656.3 fmol/ml[0.3-9635.2], p=0.002), in non-smokers compared to current smokers (951.0 fmol/ml[0.30-9856.7] v 539.5 fmol/ml[0.3-9635.2], p=0.001), in patients with a history of hypertension (1011.8 fmol/ml [7.3-9856.7] v 592.1 fmol/ml [0.3-9598.4], p<0.001) and in anterior compared to inferior AMI (1051.7 fmol/ml [0.3 v 9635.2] v 588.4 fmol/ml [0.3-9856.7], p=0.002). Plasma N-BNP correlated with patient age (r=0.410, p<0.001), serum creatinine (r=0.232, p<0.001), plasma glucose (r=0.216, p<0.001), neutrophil

count (r=0.181, p<0.001), troponin I (r=0.168, p=0.002), and creatine kinase (r=0.113, p=0.03).

The nature of the relationships with echocardiographic parameters of LV function were similar for N-BNP than those seen for TIMP-1 (Table 7.7). N-BNP correlated with greater impairment of LV function, as indicated by direct correlation with WMIS and inverse correlation with both fractional shortening and LVEF. These relationships were evident at both pre-discharge and follow up echocardiographic assessment. Plasma N-BNP also correlated with LV volumes.

Table. 7.7 Univariable correlations between N-BNP and markers of LV function.

N-BNP	EDV1	ESV1	LVEF	WMIS	EDV2	ESV2	LVEF2	WMIS2
r value	0.076	**0.204**	**-0.338**	**0.389**	0.033	**0.158**	**-0.331**	**0.340**
p value	0.156	**<0.001**	**<0.001**	**<0.001**	0.562	**0.005**	**<0.001**	**<0.001**

7.6.2: Multivariable Model

Factors with univariable association with LV ejection fraction were: age, previous angina or MI, territory of infarct, CK and troponin I. When entered individually into a multivariable linear regression model both TIMP-1 and N-BNP had independent predictive value for LVEF both pre-discharge (TIMP-1, p=0.003; N-BNP, p<0.001) and at follow up (TIMP-1, p<0.001 N-BNP, p<0.001).

When TIMP-1 and N-BNP were entered into the model simultaneously, both retained independent predictive value for LVEF pre-discharge (TIMP-1 p=0. 030; N-BNP p=0.001) and at follow up (TIMP-1 p=0.001; N-BNP p=0.003).

7.6.3: Clinical Endpoints

Plasma N-BNP and TIMP-1 were higher in the 63 patients reaching the primary end-point (Table 7.2 for TIMP-1, Table 7.8 for N-BNP) and in those reaching each individual component of the primary end-point. There was no association of N-BNP or TIMP-1 with the occurrence of re-infarction

Table 7.8. N-BNP in relation to the occurrence of clinical endpoints

Endpoint	**N-BNP (fmol/ml) median [range]**		
	Event	**No event**	**p-value**
Death/heart failure (N=65)	2344.7 [9.0-9856.7]	595.8 [0.3-9635.2]	**<0.001**
Death (N=38)	2266.0 [9.0-9856.7]	672.3 [0.3-9635.2]	**<0.001**
Heart failure (N=49)	2162.9[507.3-9598.4]	633.0 [0.3-9856.7]	**<0.001**
Re-infarction (N=44)	586.3 [12.6-9635.2]	776.3 [0.3-9856.7]	0.746

Prediction of outcome

Factors with univariable association with death or heart failure (Table 7.3 above) were entered into multivariable logistic regression analyses for the prediction of outcome.

When entered individually into the multivariable model, N-BNP (OR [per unit increase in log transformed N-BNP] 1.66 [1.30-2.11], p<0.001) and TIMP-1 (OR 4.35: 95% CI 2.28-8.30 per unit increase in log transformed TIMP-1) each had independent association with the combined end-point of death or heart failure. When

entered simultaneously, each retained independent predictive value (N-BNP, p=0.001; TIMP-1, p=0.006).

Factors with independent association with death (N-BNP and TIMP-1 entered individually) were N-BNP (OR 1.58 [1.19-2.12], p=0.001), TIMP-1(OR 5.29 [2.35-11.91, p<0.001), anterior territory of infarct (OR 2.6[1.13-5.89], p=0.024) and age (OR=1.11[1.021-1.12] per year increase, p=0.005). When entered simultaneously, both N-BNP (OR= 1.44[1.07- 1.94], p=0.016) and TIMP-1 (OR=4.03[1.72- 9.31], p=0.001) retained independent significance for the prediction of death.

Factors with independent association with heart failure were N-BNP (OR 1.43 [1.13-1.82], p=0.003), TIMP-1 (OR 2.85[1.38-5.86], p=0.004), age (OR 1.04[1.01-1.08], p=0.017) and anterior territory of infarct (OR 2.1[1.01-4.36], p=0.046). Again when entered simultaneously, both N-BNP (OR= 1.35[1.06- 1.74], p=0.016) and TIMP-1 (OR= 2.24[1.07- 4.67], p=0.03) retained independent predictive value.

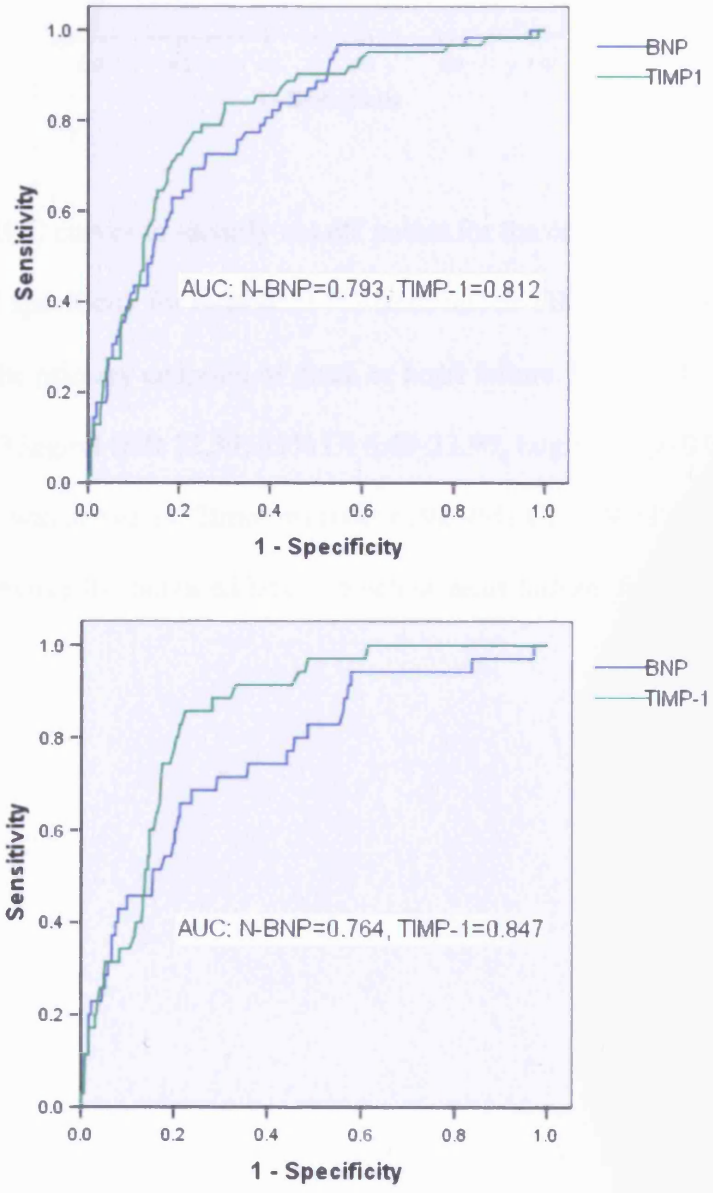
On Cox Proportional Hazards modelling (Table 7.9) independent predictors of death or heart failure were Age, Creatinine, Glucose, N-BNP and TIMP-1

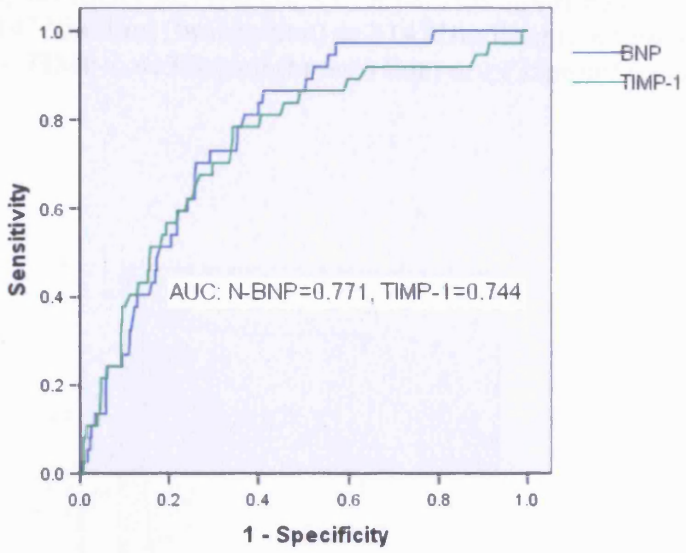
Table 7.9 Uni-variable and multi-variable predictors of death/heart failure(Cox regression)

Factor	**Univariable Hazards Ratio**	**p-value**	**Multivariable Hazards Ratio**	**p-value**
Anterior territory	1.98 [1.17-3.33]	0.010	1.43 [0.82-2.51]	0.210
History of Hypertension	1.85 [1.12-3.03]	0.016	1.38 [0.77-2.48]	0.275
Age	1.08 [1.05-1.10]	0.001	1.04 [1.01-1.07]	0.022
Creatinine	1.01 [1.01-1.02]	0.001	1.01 [1.00-1.01]	0.024
Glucose	1.07 [1.03-1.11]	0.001	1.04 [1.00-1.09]	0.06
Log NT-proBNP	1.71 [1.45-2.01]	0.001	1.52 [1.24-1.87]	0.001
Log TIMP-1	4.71 [3.26-6.81]	0.001	2.67 [1.60-4.46]	0.001

Figure 7.6 shows Receiver Operator characteristic (ROC) curves for the prediction of primary outcome. The area under each ROC was 0.793 (95% CI 0.735- 0.851; p<0.001) for N-BNP and 0.812 (95% CI 0.754 – 0.871; p<0.001) for TIMP-1.

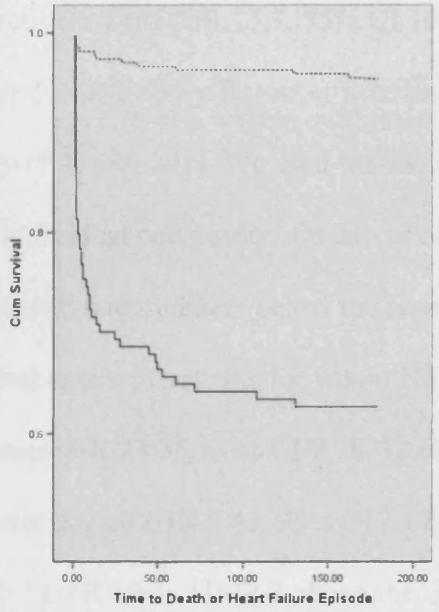
Figure. 7.6 Receiver Operator Curves (ROC) for the Prediction of Death or Heart Failure (Top), Death (middle) and Heart failure (bottom) by TIMP-1/N-BNP.

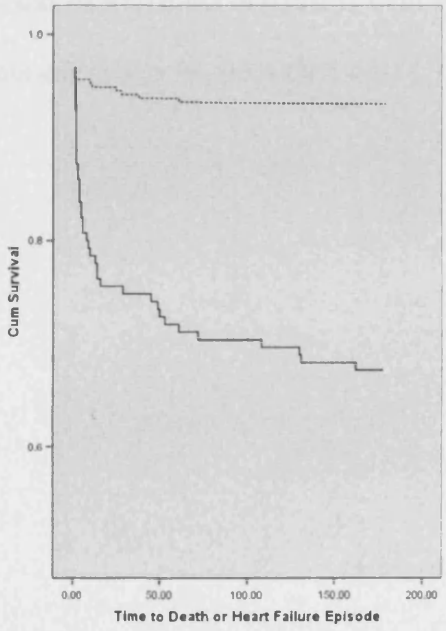




We used the ROC curves to identify cut off points for the optimum combination of sensitivity and specificity for N-BNP (1472 fmol/ml) or TIMP-1 (135ng/ml) for prediction of the primary endpoint of death or heart failure. Patients for whom TIMP-1 was above 135ng/ml (HR 12.39, 95% CI 6.43-23.90, Log Rank $p<0.001$) or for whom N-BNP was above 1472fmol/ml (HR=6.46, 95% CI 3.59-11.65, Log rank $p<0.001$) had markedly increased risk of death or heart failure (Figure 7.7)

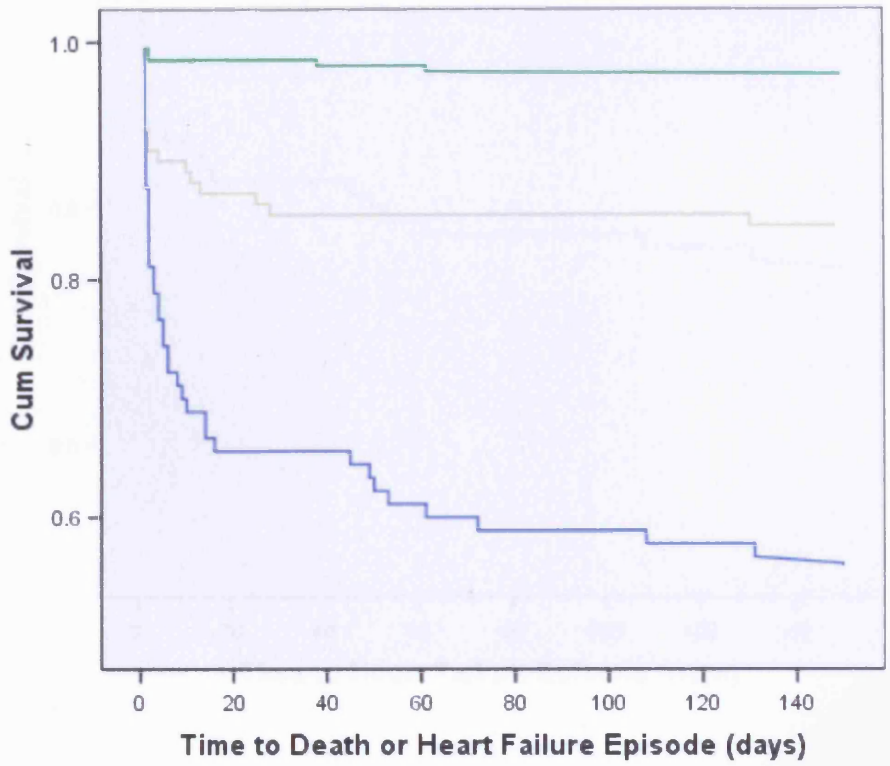
Figure 7.7: Kaplan-Meier survival curves for patients stratified by: Top panel - NTproBNP <1472fmol/ml (broken line) or ≥1472fmol/ml (continuous line) and Bottom panel – TIMP-1 <135ng/ml (broken line) or ≥135ng/ml (continuous line)

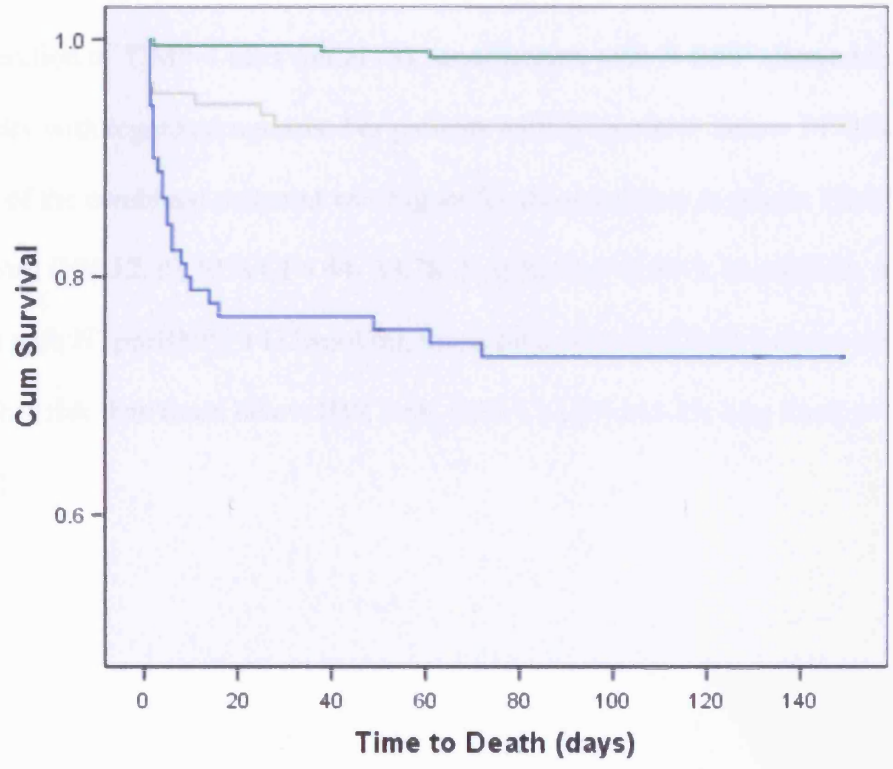


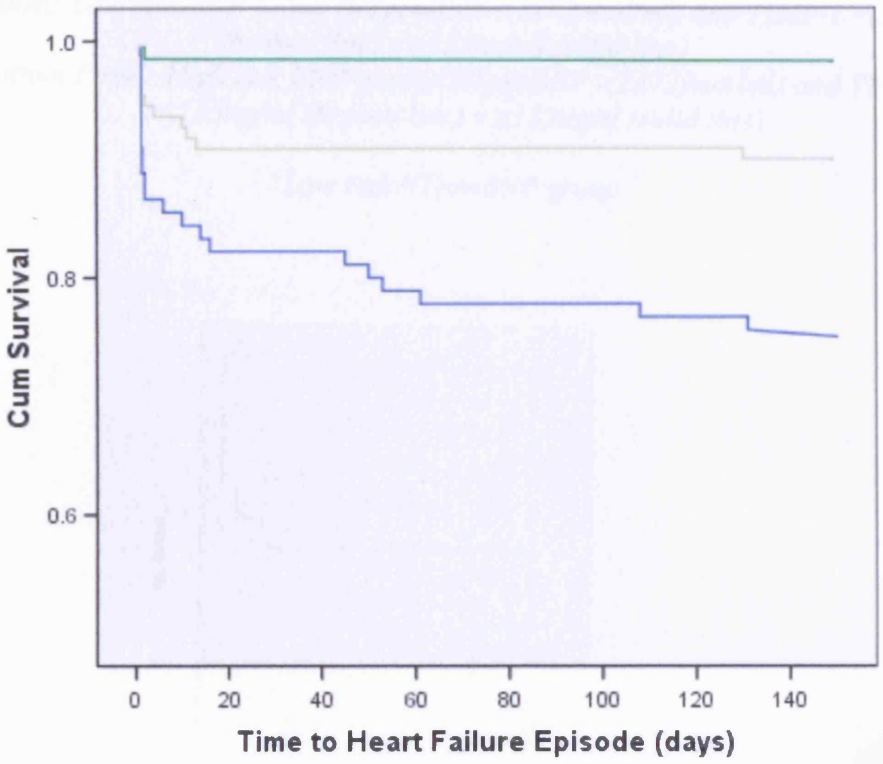


Consideration of both N-BNP and TIMP-1 improved the prediction of adverse outcome. The risk of the combined endpoint of death or heart failure was lowest for patients with both N-BNP and TIMP-1 below the cut-off. In comparison to those patients, risk was particularly elevated in patients for whom N-BNP and TIMP-1 were both above the respective cut-off (OR 25.2, 95% CI 10.3-65.6, $p<0.001$). Patients with either N-BNP or TIMP-1 above the cut-off median showed intermediate risk (OR 6.8, 95% CI 2.6-17.1, $p<0.001$). We used the same concentrations of NTproBNP and TIMP-1 for the individual end points of death or heart failure. For death, compared to patients with both markers below the respective cut-off, risk was elevated to the greatest extent in patients for whom NTproBNP and TIMP-1 were both above these values (HR 23.35, 95% CI 7.78–70.08; Log Rank $p<0.001$). Risk was elevated to a lesser degree (HR 5.46, 95% CI 1.67-17.84; Log Rank $p=0.003$) for patients with either NTproBNP or TIMP-1 above the cut-off. Similarly, compared to patients with both below the cut-off, risk of heart failure was highest in patients for whom NTproBNP and TIMP-1 were both elevated (HR 13.76, 95% CI 5.65-33.50; Log Rank $p<0.001$) and intermediate in patients with either NTproBNP N-BNP or TIMP-1 above the cut-off (HR 5.94, 95% CI 2.40-14.73, Log Rank $p=0.001$). (fig 7.8)

Figure 7.8. Kaplan Meier survival curves for the prediction of Death or Heart Failure (above), death (middle) and heart failure (lower) in subjects with both TIMP-1 and N-BNP above optimum (blue), versus both below optimum (green) versus either above optimum (grey).





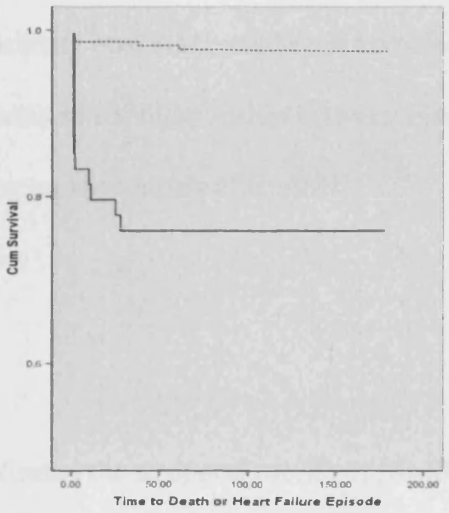


7.6.4: Does TIMP-1 add to N-BNP in the prediction of prognosis post AMI.

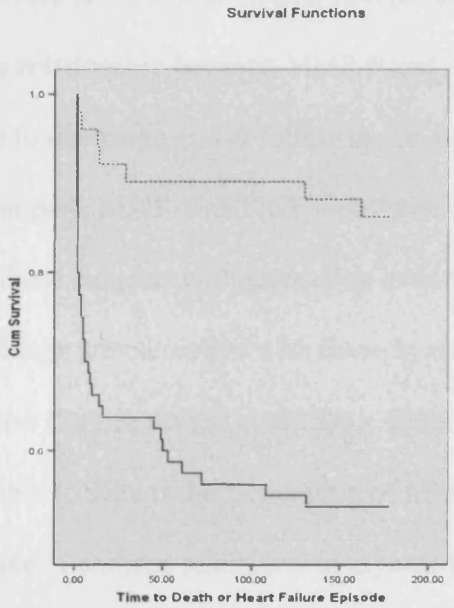
Consideration of TIMP-1 after initial risk stratification with N-BNP allowed further specificity with regards prognosis. For patients with NTproBNP below 1472fmol/ml, the risk of the combined endpoint was higher for those patients in whom TIMP-1 was ≥135ng/ml (HR 12.25, 95% CI 4.44- 33.78; Log Rank p<0.001). In addition, in patients with NTproBNP ≥1472fmol/ml, those subjects with TIMP-1 above optimum had higher risk than those below (HR 5.69, 95% CI 2.29-145.13; Log Rank p=0.001) (fig 7.9)

Figure 7.9. Kaplan-Meier survival curves for patients stratified first by NTproBNP, then by TIMP-1.
Top panel: Low risk BNP group (NTproBNP <1472fmol/ml) and TIMP-1 <135ngml (broken line) v ≥135ng/ml (solid line)
Bottom Panel: High risk BNP group (NTproBNP >1472fmol/ml) and TIMP-1<135ng/ml (broken line) v ≥135ngml (solid line)

"Low risk NTproBNP group"



"High risk NTproBNP group"



7.7

Discussion – Plasma MMP-9, TIMP-1 & N-BNP is associated with left ventricular dysfunction, remodelling and adverse prognosis post AMI

The second stage of this project has strengthened our investigation into the effects of metalloproteinase activity post AMI and to our knowledge is the most extensive examination in humans of the relationship between elements of the MMP system and both LV remodelling and prognosis after AMI.

7.7.1: MMP-9

We have again confirmed the temporal profile of MMP-9 as discussed in stage 1. We observed a peak at 0-12hrs which is significantly higher than our controls followed by fall to plateau and "normal" levels. We observed an association between MMP-9 and markers of increased myocardial necrosis; MMP-9 correlated with peak creatine kinase and was elevated in ST elevation over non ST elevation myocardial infarction. We again observe a relationship between MMP-9 and left ventricular volumes and function, both prior to discharge and at follow up. In addition we confirm the previous relationship between peak MMP-9 and left ventricular remodelling with MMP-9 being increased in those subjects with increasing over decreasing end diastolic volumes. These findings are consistent with those in stage 1 of this project and with other previous studies (Squire, Evans et al. 2004; Webb, Bonnema et al. 2006). From our data we are unable to state if the association of MMP-9 with increased necrosis is due to a direct release of enzyme secondary to myocardial cell death or as a response

to infarction. Our observation of correlation with neutrophils however indicates that these cells may be a source of plasma MMP-9 post infarction hence increasing the likelihood that MMP-9 activity is a response to infarction.

We now extended our findings of the relationship of MMP-9 with left ventricular function, volumes and remodelling to demonstrate an association with adverse prognosis. Elevated MMP-9 is associated with an increased incidence of our primary end-point of death or heart failure. These findings are logical and consistent with previous studies. One of the most prognostically significant consequences of AMI is the development of adverse left ventricular remodelling. The degree of LV remodelling and in particular the extent of changes in LV volume correlates closely with the severity of LV dysfunction and the likelihood of development of congestive heart failure and adverse outcome (Group 1983; White, Norris et al. 1987; Pfeffer and Braunwald 1990). MMP-9 above 76ng/ml was associated with an approximately 2-fold increase in the risk of death or hospitalisation with heart failure during follow-up. In contrast to the current report a previous study (Cavusoglu, Ruwende et al. 2006) failed to show any association with outcome of MMP-9. There are numerous possible explanations for these differences. Differences in population demographics, including the inclusion of only males in the previous study, in the co-variables assessed for prognosis, and in the assays used are likely contributors.

Our data has several important implications. Firstly we demonstrate a potential biomarker in prognostication post AMI. Identification of subjects at risk of adverse outcome is of up-most importance. This identification may steer patient management potentially leading to more aggressive therapy for those at high risk. It is unlikely that

MMP-9 alone would have sufficient sensitivity and specificity to be used as a prognostic biomarker alone however it may add additional data to a multi-variable approach to patient assessment.

In addition to prognostication, our data suggests that timed MMP-9 inhibition may reduce left ventricular remodelling post AMI and this reduction may also improve prognosis. As discussed in stage 1 several animal studies have observed a reduction in LV remodelling with MMP inhibition or in knock out models however human studies have to date failed to show any benefit. We again hypothesize that timing of MMP-9 inhibition is of up-most importance in the human subject.

MMP-9 is also associated with an increased incidence of all cause mortality indicating that the mechanism of death is not purely associated with heart failure. Indeed MMP-9 has previously been shown to be associated with adverse prognosis in non heart failure populations with an association of plasma MMP-9 with higher cardiovascular risk in patients with coronary artery disease (Blankenberg, Rupprecht et al. 2003). Our data would support these previous studies however we did not observe an association with re-infarction suggesting that further ischaemic events may not be the mechanism of death. To date no studies have shown association of MMPs with other mechanisms of cardiac death such as arrhythmia however investigation may be indicated.

Plasma levels of MMP-9 correlate with increased myocardial volume and greater LV dysfunction. Our study cannot inform as to whether the observed plasma levels represent a protective response to AMI or a deleterious consequence of this event. Animals deficient in the MMP-9 gene show attenuated ventricular dilatation, reduced

collagen accumulation (Ducharme, Frantz et al. 2000), reduced ventricular rupture rate (Heymans, Luttun et al. 1999), and smaller infarct size. However MMP-9 activation may equally be postulated to result in excessive matrix, and ventricular, remodelling. Support for this concept comes from the association of plasma MMP-9 with ventricular remodelling in a Framingham population (Sundstrom, Evans et al. 2004), and of the MMP-9 gene polymorphism C-1562T with adverse prognosis (Blankenberg, Rupprecht et al. 2003) in patients with cardiovascular disease. Our data may be interpreted in support of either a beneficial or a deleterious effect of elevated MMP-9 after AMI. Experimental studies, targeting inhibition of MMP-9 in the early (0-12 hour) period after AMI may help clarify this issue.

7.7.2: TIMP-1

The above observations extend to TIMP-1. This is the first study to describe the association of circulating TIMP-1 concentration and outcome after AMI in man. Our study clearly links plasma TIMP-1 not only to prognosis, but to one of its main determinants, namely the extent of LV dysfunction. We observe statistically strong relationships between TIMP-1 and LV volumes and function both prior to discharge and at follow up.

As discussed in the introduction to this thesis, the TIMPs are a group of low molecular weight proteins, the main action of which is to inhibit MMP proteolytic activity by binding to the active domain of MMPs. In addition TIMPs have other biological actions. There is increasing evidence to support the role of TIMPs in the maintenance of LV structure and function.

7.7.3: LV structure

Experimental studies, in which mice deficient in TIMP-1 demonstrate changes in LV geometry and increased LV remodelling after AMI (Roten, Nemoto et al. 2000) suggesting an intrinsic role for TIMP-1 in the maintenance of LV integrity. In addition TIMP-1 deficiency is also associated with left ventricular hypertrophy and increased remodelling after experimental AMI (Creemers, Cleutjens et al. 2001). Allaire et al (Allaire, Forough et al. 1998) observed that TIMP-1 over-expression reduced aneurysm formation n in a rat xenograft model of human aortic aneurysm suggesting that TIMP-1 may have beneficial effects in certain conditions. However we cannot simply extrapolate these findings to AMI. Moreover, in small clinical studies in community based (Sundstrom, Evans et al. 2004) and hypertensive (Lindsay, Maxwell et al. 2002; Timms, Wright et al. 2002) populations, plasma TIMP-1 concentrations correlate with greater LV mass, myocardial fibrosis, diastolic dysfunction and altered LV dimensions and treatment of hypertension with ACE inhibitors leads to a reduction in plasma TIMP-1 concentrations in humans (Laviades, Varo et al. 1998). Further data for the role of TIMP-1 in left ventricular remodelling comes from the study be Schwartskoptff in which subjects with dilated cardiomyopathy exhibited significantly higher levels of TIMP-1 compared to age matched controls (Schwartzkopff, Fassbach et al. 2002) and other studies have shown elevated plasma concentrations in this setting (Hirohata, Kusachi et al. 1997; Webb, Bonnema et al. 2006)

Our data are in keeping with the above studies. We observed a consistent although weak relationship between TIMP-1 and LV remodelling. This finding seems

biologically plausible. Although TIMP-1 has effects on metalloproteinase activity, the protein itself has no direct protease activity and is therefore not directly involved in ECM degradation. Thus, a weak relationship may be more likely and does not exclude a biological relationship. Unfortunately we did not measure TIMP-1 levels immediately after symptom onset, a time at which MMP-9 appears to exert its main effects on left ventricular function. Further studies to investigate the MMP:TIMP ratio at this time period will allow further examination of the effects of MMP inhibition via their natural inhibitors at this time period. Another possibility of the association between TIMP-1 and LV dysfunction may reflect myocardial collagen content and adverse remodelling which predates the AMI. The previously noted association of plasma TIMP-1 and LV hypertrophy and myocardial collagen turnover may support this possibility (Sundstrom, Evans et al. 2004). Increased TIMP-1 has previously been noted in association with a variety of cardiovascular risk factors (Sundstrom, Evans et al. 2004). Our data indicate higher levels in males, in smokers and those with a previous history of hypertension. Pre-existing remodelling is unlikely to explain the association of plasma TIMP-1 with LV function and prognosis post AMI.

The most likely biological explanation for our findings is that TIMP-1 levels are a reflection of the degree of disruption of the normally well balanced equilibrium between ECM synthesis and breakdown, the degree of elevation being an indicator of the extent of perturbation of this equilibrium in favour of proteolysis. In this context plasma TIMP-1 concentration may represent a physiological response to enhanced MMP activity and as such reflect an attempt to regulate excessive proteolytic activity. This simple answer may however not be the complete picture. Our previous chapter and publication (Kelly, Cockerill et al. 2007) demonstrated the associations between MMP-9 and both LV function and remodelling. The association between both MMP-9

and TIMP-1 and markers of LV dysfunction and volume are similar a few days post AMI. However the relationship some months down the line is somewhat different. At this stage the associations between TIMP-1 and LV function and volumes are much stronger than that of MMP-9. In addition TIMP-1 is the stronger predictor of adverse prognosis. As discussed previously, TIMPs have additional effects over and above that of MMP inhibition. Numerous studies have reported other activities such as cell growth promotion (Bertaux, Hornebeck et al. 1991), anti-apoptotic and anti-androgenic activity (Lambert, Dasse et al. 2004) which appear independent of their metalloproteinase inhibition activity. TIMP-1(Gasson, Golde et al. 1985) and TIMP-2 (Stetler-Stevenson, Bersch et al. 1992) have been shown to have erythroid potentiating activity and TIMP-2 stimulates the growth of lymphoma cells (Hayakawa, Yamashita et al. 1994). In addition recent experimental data suggests that TIMP-1 in certain settings may be pro-atherosclerotic (Silence, Collen et al. 2002). The mechanism by which TIMPs exert their effects independent of metalloproteinase inhibition is however to date poorly described and may explain to some degree our results. Of particular note in the context of our observations is the stimulation by TIMP-1 of fibroblast growth in vitro (Lovelock, Baker et al. 2005).

7.7.4: TIMP-1 and adverse prognosis

The current study represents the first demonstration of the prognostic utility of TIMP-1 after AMI, lending powerful support to our observations regarding LV dysfunction. Plasma TIMP-1 greater than 124ng/ml was associated with over 10-fold increase in the risk of adverse outcome. In keeping with this, the area under the ROC curve for

TIMP-1 for the prediction of death or heart failure was over 0.8, indicating a high predictive value for this outcome.

The strength of association between TIMP-1 and adverse outcome is demonstrated in its robust evaluation in our multi-variable models. In these models, when both MMP-9 and TIMP-1 were entered simultaneously, only TIMP-1 retained independent association with outcome indicating that this is the more significant marker of prognosis in this group. Moreover the combination of both MMP-9 and TIMP-1 above the optimum cut-off levels was even more informative, suggesting that plasma MMP-9 and TIMP-1 provide complementary prognostic information.

Previous studies have shown TIMP-1 to be elevated in the coronary circulation of patients with acute coronary syndrome. In a study by Inokubo et al (Inokubo, Hanada et al. 2001), plasma levels of TIMP-1 in the great cardiac vein obtained at the time of coronary angioplasty were significantly higher than a control population. Interestingly, levels in the aorta were no different to that of controls indicating that there is regional release of TIMP-1 with higher levels in the coronary circulation suggesting that TIMP-1 may originate either in the atherosclerotic plaque or from cellular infiltrates to this region.

Increased risk in association with higher plasma TIMP-1 has been demonstrated in the Framingham Heart Study (Sundstrom, Evans et al. 2004). In this population based study, TIMP-1 was associated with major cardiovascular risk factors such as male sex, age, body mass index and total/HDL cholesterol ratio, smoking history, diabetes and hypertension.. Plasma TIMP-1 levels have also been shown to fall after successful reduction in these risk factors such as via blood pressure control with ACE-Inhibitors

(Laviades, Varo et al. 1998). In another study by Lubos et al (Lubos, Schnabel et al. 2006), TIMP-1 was determined in 1979 subjects with suspected coronary disease with increased levels being seen in those subjects who subsequently progressed to a fatal cardiovascular event. The predictive ability of TIMP-1 was independent of other biomarkers such as BNP and CRP. A recent study also demonstrated increased risk of death or AMI in a cohort of patients undergoing coronary angiography (Cavusoglu, Ruwende et al. 2006). Our study would be consistent with these findings and now extends previous observations to patients with AMI and encompassing a wide range of LV dysfunction.

In contrast, George et al (George, Patal et al. 2005) investigating a heart failure population showed no association between TIMP-1 and mortality. This study was limited to only 88 patients with a relatively short follow up period.

Levels of TIMP-1 in our study appear lower to those seen in the above mention. The explanation for this may lie in the study population or assay analysis. An alternative explanation is that TIMP-1 may be suppressed post AMI and a "lack of suppression" and hence higher yet subnormal levels may be associated with adverse outcome.

Further studies are required to elucidate the nature of the relationship of TIMP-1 with remodelling and prognosis after AMI, in other stages of coronary heart disease, other cardiovascular pathology and the normal population.

7.7.5: Comparison of TIMP-1 and N-BNP

As discussed in chapter 3, N-BNP is well established as a marker of left ventricular dysfunction. More recently N-BNP has also been shown to a powerful marker of prognosis (Morita, Yasue et al. 1993) and increased levels early after AMI appear to be a strong marker of long term mortality (Arakawa, Nakamura et al. 1996; Darbar, Davidson et al. 1996; Omland, Persson et al. 2002; Richards, Nicholls et al. 2003; Mega, Morrow et al. 2004; Squire, O'Brien et al. 2004; Grabowski, Filipiak et al. 2005; Sun, Wang et al. 2006; Zhang and Wang 2006). Our data suggest that the prognostic ability of TIMP-1 is at least equivalent to that of N-BNP in our population. It may also be argued that plasma TIMP-1 provides very similar information to that obtained from plasma N-BNP. Certainly, the pattern and strength of the associations of both TIMP-1 and N-BNP with echocardiographic parameters of LV volume and LV function were very similar. However the use of both biomarkers in a multi-variable prognostic approach may yield additional information over either alone. Subjects with both TIMP-1 and N-BNP below our optimum cut off points had a greater than 95% event free survival compared to only 55% in those subjects with both peptides above the optimum. Intermediate risk is seen in those with either above optimum. Qualitatively similar differences, though quantitatively less powerful, were also seen in the prediction of the individual components of the end-point i.e. death or heart failure episodes. Moreover, after risk stratifying patients according to their initial N-BNP result into high or low risk groups, these 2 groups may then be further divided into those at very high risk or very low risk according to their TIMP-1 levels. We may postulate mechanistic reasons for these observations. Plasma concentrations of the B-type natriuretic peptides BNP and N-BNP are powerful markers of adverse outcome after AMI, in which setting plasma concentrations correlate inversely with LV function (Omland, Aakvaag et al. 1996; Richards, Nicholls et al. 1998). Indeed,

plasma concentrations of these peptides represent the "gold-standard", prognostic biomarker for patients after AMI. Natriuretic peptides are synthesised and released in response to increased myocardial wall stress. In the setting of AMI, the extent of release is proportional to the degree of LV damage, dysfunction and remodelling. As our study indicated a clinically relevant association between plasma TIMP-1 and the extent of LV remodelling, the observed association between TIMP-1 and N-BNP is biologically plausible, and indeed, logical.

7.8

Chapter Summary

In summary we have confirmed the temporal profile of release of MMP-9 post AMI. We have shown association between MMP-9 and TIMP-1 with LV dysfunction, LV volumes and LV remodelling post AMI. We have extended these findings to demonstrate a link between our biomarkers and prognosis post AMI.

Both MMP-9 and TIMP-1 may have a role in prognostication post AMI, indeed TIMP-1 may be a stronger predictor of outcome of the current "gold standard" of N-BNP. Both MMP-9 and TIMP-1 may herald a potential therapeutic target.

Chapter 8

The influence of diabetes and stress hyperglycaemia on matrix metalloproteinase activity post AMI

8.1

Introduction

Type 2 diabetes mellitus is a major risk factor for the development of cardiovascular disease (Institute 1999) and the increasing prevalence of this condition has implications for future coronary artery disease rates. Numerous studies have reported the association between diabetes and adverse prognosis after acute myocardial infarction (Oswald, Corcoran et al. 1984; Sewdarsen, Vythilingum et al. 1989; Capes, Hunt et al. 2000; Malmberg, Yusuf et al. 2000; McGuire, Emanuelsson et al. 2000; Berger, Breall et al. 2001; Mukamal, Nesto et al. 2001; Wahab, Cowden et al. 2002; Aguilar, Solomon et al. 2004; Ceriello 2005; Svensson, McGuire et al. 2005). Most of these studies have relied on an antecedent diagnosis of diabetes for the categorisation of patients. While diabetes has previously been established in approximately 20-30% of subjects prior to the index AMI (Vaccarino, Parsons et al. 2000; Berger, Breall et al. 2001; Mukamal, Nesto et al. 2001), a proportion of patients without this diagnosis have hitherto unidentified abnormalities of glucose metabolism.

Irrespective of antecedent diabetes status, blood glucose concentration at the time of admission for AMI is also associated with adverse prognosis (Oswald, Corcoran et al.

1984; Sewdarsen, Vythilingum et al. 1989; Capes, Hunt et al. 2000; Wahab, Cowden et al. 2002; Aguilar, Solomon et al. 2004; Ceriello 2005). The mechanisms by which diabetes or hyperglycaemia impact upon prognosis are unclear. Pathophysiological possibilities include altered endothelial function (Mulvihill, Foley et al. 2001), increased maladaptive apoptosis secondary to glucose induced gene transcription (Young, McNulty et al. 2002), and altered thrombosis and fibrinolysis (Beckman, Creager et al. 2002). In addition, patients with diabetes may be less likely to receive treatment with evidence-based therapies after AMI (Pfeffer, Moye et al. 1991; Chen, Marciniak et al. 1999; Mulvihill, Foley et al. 2001; Beckman, Creager et al. 2002; Young, McNulty et al. 2002).

The matrix metalloproteinase (MMP) family of endopeptidase enzymes has an important role in the maintenance of myocardial extracellular structure, and left ventricular function, in health and disease. Altered MMP activity has been observed in diabetic subjects and has been proposed to be associated with many pathological features associated with this condition. In vitro studies suggest that hyperglycaemia may induce MMP production (Death, Fisher et al. 2003; Maldonado, He et al. 2004; Kadoglou, Daskalopoulou et al. 2005) and differential MMP activity occurs in animal models of diabetes. It has been suggested that these effects may be causative in cerebrovascular remodelling thereby contributing to the increased risk of stroke and cerebral ischaemia in diabetes (Harris, Hutchinson et al. 2005). Altered MMP activity occurs in diabetic proliferative retinopathy (Kosano, Okano et al. 1999; Noda, Ishida et al. 2003), in children with type I diabetes mellitus (Bister, Kolho et al. 2005; Derosa, Avanzini et al. 2005) and adult subjects with type I (Shiau, Tsai et al. 2006) and type II diabetes mellitus (Lee, Song et al. 2005; Sampson, Wall et al. 2006). Indeed children with microangiopathy express altered MMP-2 activity (Derosa,

Avanzini et al. 2005) and altered MMP activity is seen in coronary artery sections taken at the time of CABG in patients with, compared to those without, diabetes (Chung, Luo et al. 2007).

In addition, there is evidence to indicate reduction in MMP activity after risk-factor treatment in diabetes. Treatment with the oral hypoglycaemic agent rosiglitazone is associated with a reduction in MMP-9 (Goldstein, Weissman et al. 2006) and cardiovascular risk reduction in diabetic patients is associated with reduced MMP activity (Tayebjee, Lim et al. 2004).

There is mounting evidence for the influence of the MMP system in AMI. First, evidence supports involvement in the remodelling process leading to coronary artery plaque formation and rupture (Galis, Sukhova et al. 1994; Galis, Muszynski et al. 1995; Moreau, Brocheriou et al. 1999; Schonbeck, Mach et al. 1999) (Nikkari, Geary et al. 1996) (Loftus, Naylor et al. 2000) (Brown, Hibbs et al. 1995), and in the subsequent platelet aggregation process (Sawicki, Salas et al. 1997; Sawicki, Sanders et al. 1998; Radomski, Stewart et al. 2001; Galt, Lindemann et al. 2002). Second, excess proteolysis, secondary to altered MMP activity, is a crucial contributor to the breakdown of myocardial extracellular matrix leading to LV remodelling and dysfunction (Rohde, Ducharme et al. 1999; Spinale, Coker et al. 1999; Peterson, Li et al. 2000; Etoh, Joffs et al. 2001; Squire, Evans et al. 2004; Webb, Bonnema et al. 2006; Kelly, Cockerill et al. 2007). These events are powerfully linked to increased risk of adverse prognosis. Thus, altered MMP activity may contribute to the vascular changes seen with hyperglycaemia in diabetes and to the development of LV remodelling and dysfunction after AMI.

8.2

Aims

The current study had two main aims. First, to explore the influence of antecedent diabetes and of blood glucose concentration on plasma MMP activity and on LV structure and function after AMI. Second, to examine the inter-relationship between blood glucose concentration, plasma MMP concentrations, and LV remodelling in this context.

8.3

Methods

Using the above population we performed retrospective analysis of the effects of antecedent diabetes diagnosis and admission glucose on plasma MMP levels, LV dysfunction, LV volumes and LV remodelling post AMI. Both previous diabetes status and admission glucose level were recorded at the time of admission. The remainder of methods are as per previous.

8.4

Results

Admission demographic features of the study population divided by prior diabetes status are shown in Table 8.1. Predictably, admission glucose concentration was higher in subjects with an antecedent diagnosis of diabetes. These patients were more likely to have a history of hypertension and to be receiving treatment with aspirin,

statin, ACE-inhibitor or angiotensin receptor blocker. CK was lower in subjects with antecedent diabetes.

Table 8.1. Comparison of patient demographics – diabetic v non diabetics

	Diabetes (N=69)	No Diabetes (N=335)	p-value
Age (yrs)	63.07	63.54	0.813
CK (i.u./L)	1056.38	1460.60	**0.013**
Troponin (μg/L)	22.35	28.13	0.390
Glucose (mmol/L)	11.6	7.5	**0.001**
Male %	75.4	75.4	0.967
Current Smoker %	44.9	34.4	0.098
Hypertension %	60.9	37.7	**0.001**
Previous MI %	11.6	8.9	0.484
Previous Revasc %	1.4	1.2	0.857
Medication %			
Aspirin	31.9	17.8	**0.011**
Clopidogrel	4.3	3.3	0.681
Beta Blocker	20.3	20.2	0.988
ACE-I/ARB	42.0	19.6	**0.001**
Statin	42.0	16.9	**0.001**
Furosemide	10.1	11.0	0.791
Oral Hypogly	50.7	0	**0.001**
Insulin	17.4	0	**0.001**
MMP concentration			
MMP-9 **(ng/ml)**	55.9 (16.46-515.26)	51.8 (17.79-512.30)	0.313
TIMP-1 **(ng/ml)**	124.85 (59.70-465.90)	113.55 (7.70-408.60)	0.084

Patient demographics according to admission glucose quartile are shown in Table 8.2. The mean age of the patients increased as glucose increased. Plasma levels of CK and troponin I were higher in glucose quartile 3 compared to other quartiles, including quartile 4.

Table 8.2. Patient demographics according to admission glucose quartile.

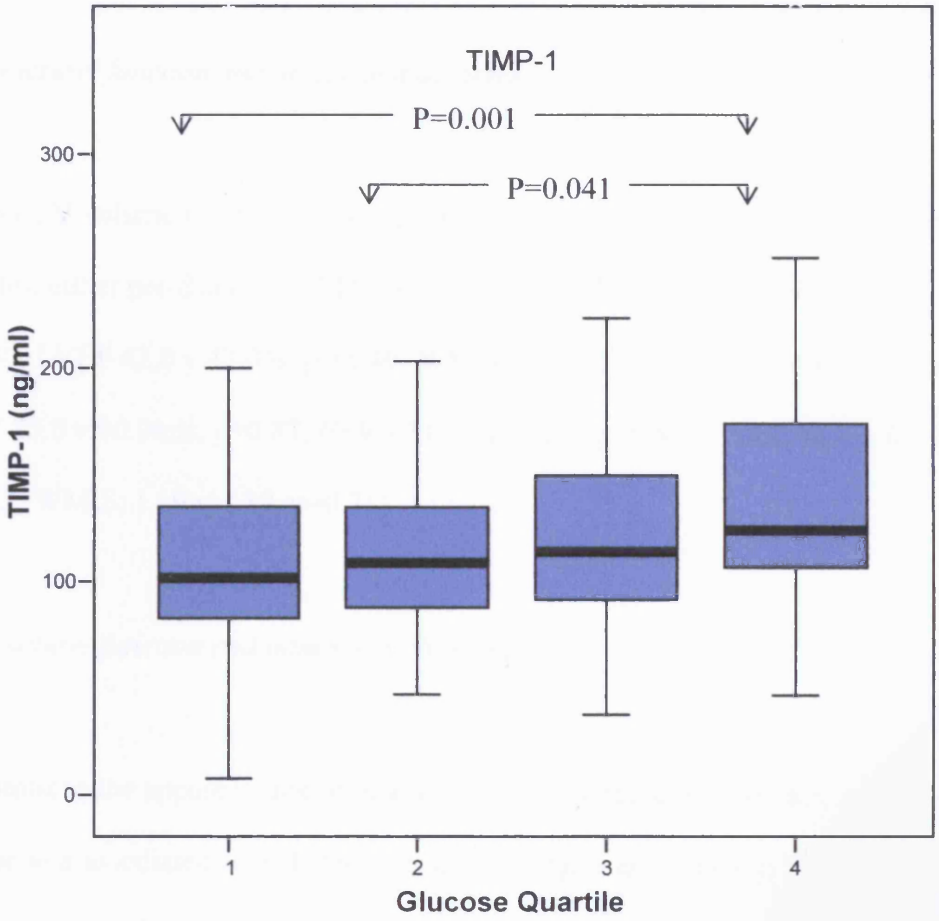
	Glucose Quartile				
	1st	2nd	3rd	4th	Sig.
Age (yrs)	60.7	63.3	64.1	66.1	0.163
CK (i.u./L)	764	998	1260*	731	**0.023**
Troponin (μg/L)	10.1	14.8	18.6*	11.1	**0.001**
Male %	78.6	76.3	80.2	66	0.086
Current Smoker %	32	41.9	34.4	37.1	0.519
Hypertension %	36.9	40.9	38.5	48.5	0.365
Previous MI %	12.6	8.6	7.3	9.3	0.614
Previous Revasc %	2.9	0	1	1	0.327
Medication %					
Aspirin	22.3	19.4	22.9	14.4	0.417
Clopidogrel	3.9	1.1	5.2	4.1	0.477
Beta Blocker	19.4	29	16.7	7.5	0.134
ACE-I/ARB	21.4	21.5	19.8	28.9	0.434
Statin	22.3	28.3	22.9	22.7	0.838
Furosemide	6.8	14	11.5	13.4	0.389
Oral Hypogly	1	3.2	7.3	21.6*	<0.001
Insulin %	2.9	2.2	3.1	9.3*	0.004

* $p<0.001$ cf other quartiles

Metalloproteinase concentrations and diabetes/admission glucose

There were no meaningful differences between patients with and without antecedent diabetes (Table 8.1). There were direct, linear correlations between glucose and TIMP-1 ($r= 0.231$, $p<0.001$). No such relationship was seen between admission glucose and MMP-9. Admission plasma glucose concentration was divided by quartiles (quartile 1 <6.5; quartile 2 6.5-7.9; quartile 3 8.0-10; quartile 4 >10mmol/L). Plasma TIMP-1 differed by glucose quartile (ANOVA, $p<0.001$), TIMP-1 being higher in quartile 4 compared to quartile 1 ($p<0.001$) and quartile 2 ($p=0.041$) [Figure 9.1]. No difference was seen in MMP-9 among glucose quartiles.

Figure. 8.1. TIMP-1 concentration according to glucose quartile.



We assessed the relationship of individual MMP concentrations with co-variables using multivariable analyses. Factors showing univariable association with increased TIMP-1 (increasing age $p<0.001$, female sex $p=0.01$, history of hypertension $p<0.001$, glucose (quartile) $p<0.001$, troponin I $p=0.006$, creatinine $p<0.001$) were entered into multivariable linear regression analyses for determinants of plasma TIMP-1 concentration. Factors with independent association with TIMP-1 levels were glucose quartile ($p=0.013$), age ($p<0.001$), troponin I ($p=0.006$) and creatinine ($p<0.001$). When antecedent diabetes was added to the models, only glucose quartile retained statistical significance for the prediction of plasma TIMP-1 ($p=0.039$).

8.4.1: Echocardiographic assessment

LV structure/function and antecedent diabetes

Neither LV volume nor function differed between patients with or without antecedent diabetes, either pre-discharge (EDV 86.5 v 92.0 mls, p=0.71; ESV 51.0 v 49.0 mls, p=0.40; LVEF 42.0 v 43.0%, p=0. 40; WMIS 1.37 v 1.31, p=0.50) or at follow up (EDV 85.0 v 90.0mls, p=0.87; ESV 47.0 v 46.0 mls, p=0.88 ; LVEF 44.0 v 48.0%, p=0.13; WMIS, 1.19 v 1.19, p=0.71)

LV structure/function and admission blood glucose

In contrast to the apparent lack of relationship with antecedent diabetes, admission glucose was associated with differences in LV volume and function both prior to discharge and at follow up (Table 8.3).

Table 8.3. LV function and volumes according to glucose quartile

	Glucose Quartile				p-value (ANOVA)
	1	2	3	4	
$LVEF_1$ (%)	48.0	44.0	41.0	42.0	**0.001 * #**
$LVEF_2$ (%)	52.0	48.0	46.0	43.0	**0.016 #**
$WMIS_1$	1.19	1.31	1.38	1.50	**0.001 * #**
$WMIS_2$	1.0	1.25	1.25	1.31	**0.001 # •**
$LVEDV_1$ (ml)	87.0	92.0	94.0	87.0	0.865
$LVEDV_2$ (ml)	87.0	87.0	94.0	87.0	0.596
$LVESV_1$ (ml)	44.0	50.4	53.0	50.5	**0.046 #**
$LVESV_2$ (ml)	42.0	45.5	49.0	46.0	0.076

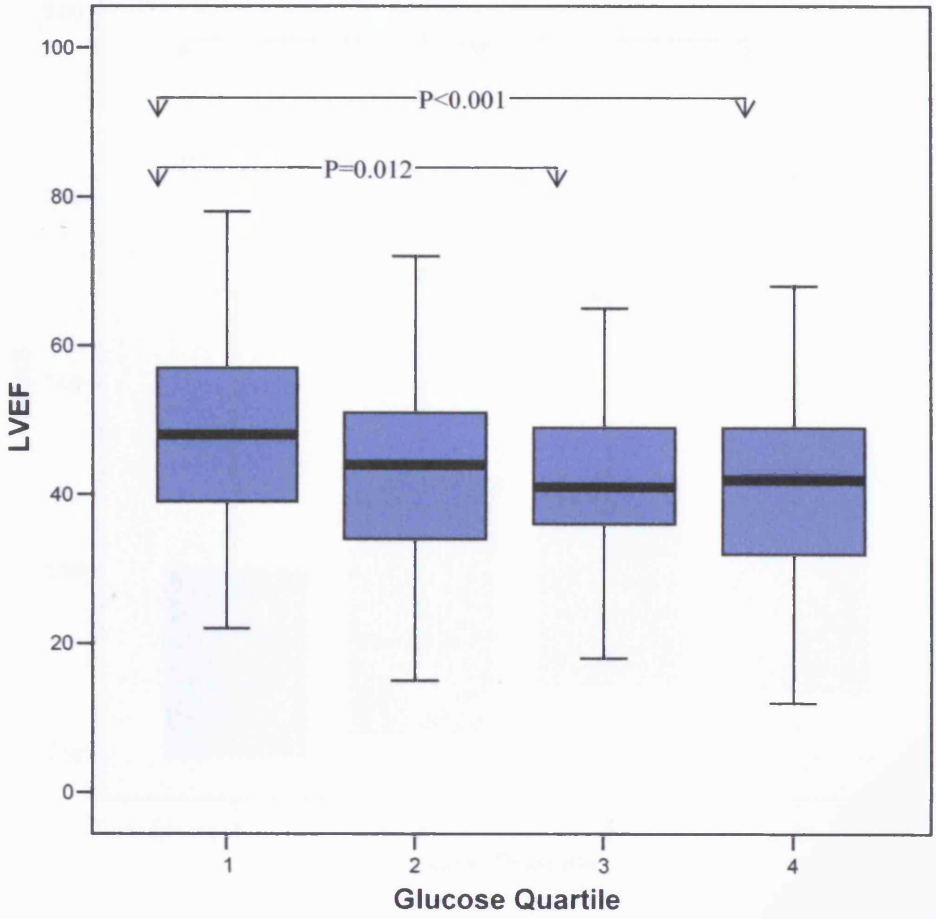
* $p<0.05$ Quartile 1 v 3

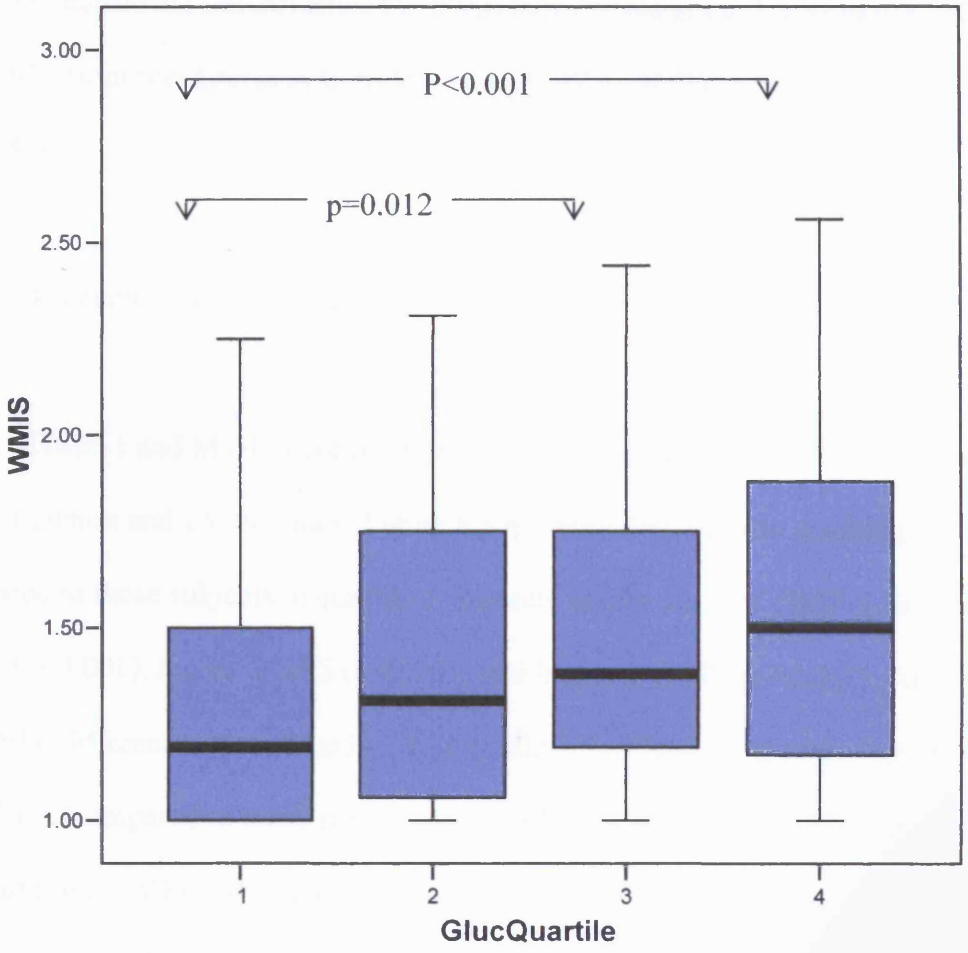
\# $p<0.05$ Quartile 1 v 4

• $p<0.05$ Quartile 1 v 2

Differences were apparent between glucose quartiles in LVEF ($p=0.001$), WMIS, ($p<0.001$) and end systolic volume ($p=0.046$) prior to discharge, and in LVEF ($p=0.016$) and WMIS ($p=0.001$) at follow up. Prior to discharge, compared to those subjects in quartile 1, LVEF was lower in quartile 3 ($p=0.012$) and quartile 4 ($p<0.001$) [Figure 8.2], WMIS was higher in quartile 3 ($p=0.012$) and quartile 4 ($p<0.001$) and ESV was higher in quartile 4 ($p=0.04$). At follow up, compared to those subjects in quartile 1, LVEF remained lower and WMIS higher in subjects in quartile 4 ($p=0.01$ & 0.001 respectively).

Figure. 8.2. LV function (LVEF above & WMIS below) according to glucose quartile



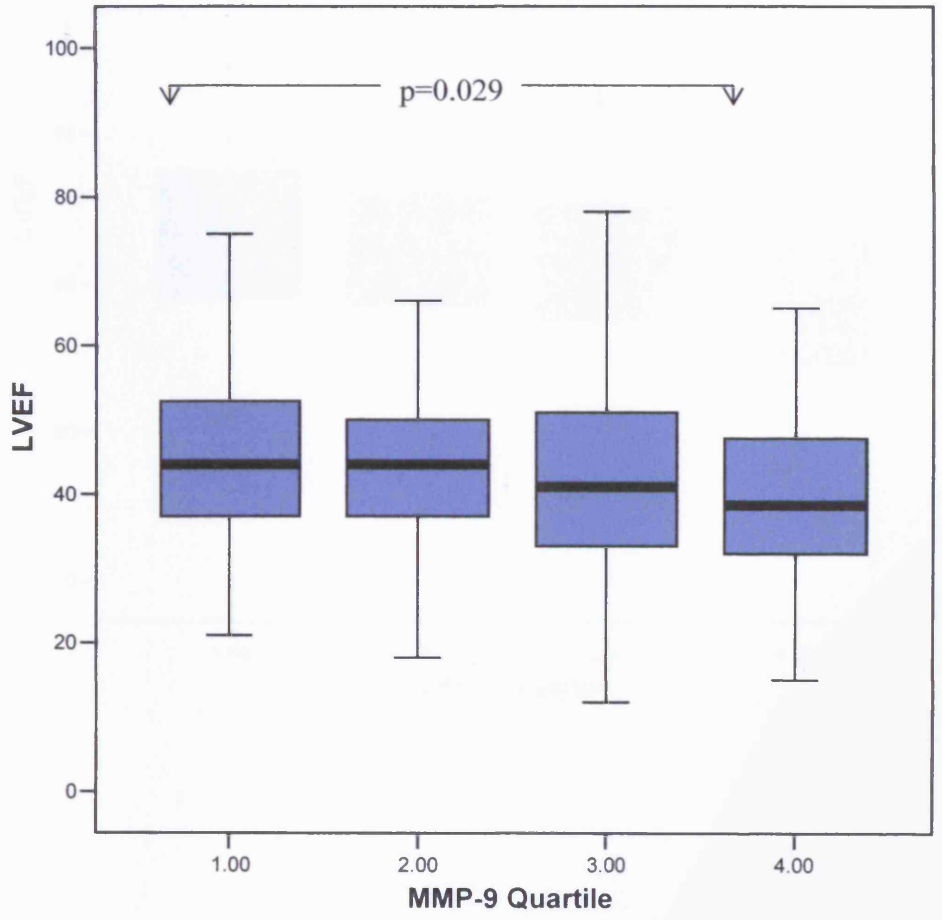

P<0.001
p=0.012
3.00
2.50
2.00
1.50
1.00
WMIS
1
2
3
4
GlucQuartile

Glucose quartile did not influence the magnitude of changes in LV volumes (ΔEDV or ΔESV) from pre-discharge to follow up (ANOVA: ΔEDV, p=0.335; ΔESV, p=0.583).

8.4.2: LV structure/function and MMP concentration

Higher TIMP-1 and MMP-9 concentrations were associated with greater impairment of LV function and LV volumes (Figure 8.3 & Table 8.4). Prior to discharge compared to those subjects in quartile 1, subjects in quartile 4 of TIMP-1 had lower LVEF ($p<0.001$), higher WMIS ($p<0.001$) and higher LVESV ($p<0.001$). At follow up similar differences were seen in LVEF ($p<0.001$), WMIS ($p<0.001$) and LVESV ($p<0.001$). Compared to those subjects in quartile 1 of MMP-9, those in quartile 4 also had lower LVEF (p=0.029) and higher LVESV (p=0.015) prior to discharge. No statistically significant differences were evident at follow up.

Figure. 8.3. LVEF according to MMP-9 (above) and TIMP-1 (below) Quartile



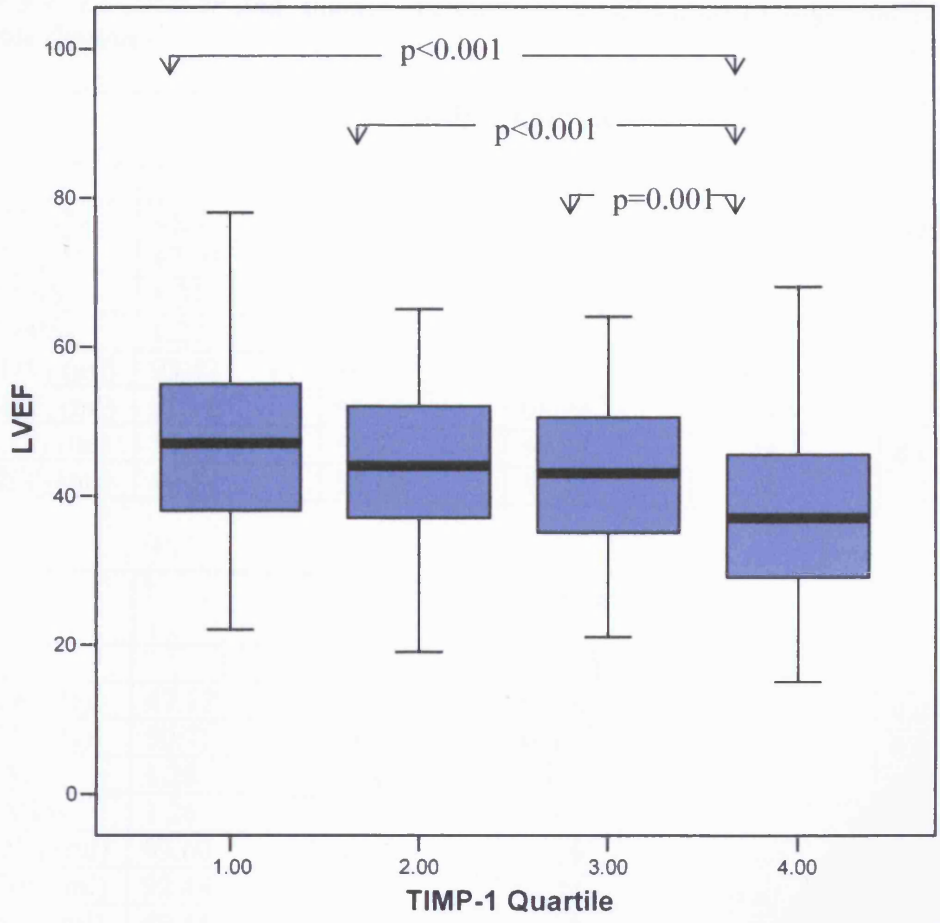

p<0.001
p<0.001
p=0.001
100
80
60
40
20
0
LVEF
1.00
2.00
3.00
4.00
TIMP-1 Quartile

Table 8.4. LV function and volumes according to MMP-9 quartile (top) and TIMP-1 quartile (bottom)

	MMP-9 Quartile				p-value (ANOVA)
	1	2	3	4	
$LVEF_1$ (%)	45.32	43.62	41.64	40.21	**0.019***
$LVEF_2$ (%)	47.94	44.76	46.59	45.64	0.497
$WMIS_1$	1.37	1.43	1.45	1.52	0.112
$WMIS_2$	1.27	1.33	1.32	1.39	0.395
$LVEDV_1$ (ml)	93.42	89.96	98.56	108.27	**0.004* #**
$LVEDV_2$ (ml)	91.18	95.55	95.24	107.87	0.077
$LVESV_1$ (ml)	52.40	51.27	59.27	65.78	**0.005* #**
$LVESV_2$ (ml)	49.17	54.18	53.13	59.39	0.297

	TIMP-1 Quartile				p-value (ANOVA)
	1	2	3	4	
$LVEF_1$ (%)	47.17	44.66	43.26	37.43	**0.001* # α**
$LVEF_2$ (%)	50.71	49.12	46.02	39.16	**0.001* # α**
$WMIS_1$	1.28	1.38	1.44	1.63	**0.001* # α**
$WMIS_2$	1.26	1.21	1.36	1.49	**0.001* # •**
$LVEDV_1$ (ml)	93.60	94.63	92.39	103.69	0.104
$LVEDV_2$ (ml)	92.44	91.07	94.74	108.65	0.068
$LVESV_1$ (ml)	49.41	53.85	53.78	66.92	**0.001* # α**
$LVESV_2$ (ml)	46.61	47.92	52.58	67.32	**0.001* # α**

* $p<0.05$ Quartile 1 v 4

\# $p<0.05$ Quartile 2 v 4

α $p<0.05$ Quartile 3 v 4

• $p<0.05$ Quartile 2 v 3

Multivariable analysis

Factors showing univariable association with LV function were entered into multi-variable linear regression analyses for determinants of LV function. Univariable factors associated with LVEF were increasing age ($p=0.001$), previous angina ($p=0.017$), previous MI ($p<0.001$), anterior territory of infarct ($p<0.001$), troponin I ($p=0.022$), TIMP-1 quartile and glucose quartile. Factors retaining independent

association with LV dysfunction as assessed by LVEF were glucose quartile ($p=0.014$), TIMP-1 quartile ($p<0.001$), anterior territory of infarct ($p<0.001$) and history of previous MI ($p<0.001$) Factors retaining independent association with WMIS were as above with both TIMP-1 ($p<0.001$) and glucose quartile ($p=0.026$) retaining independent association.

8.5

Discussion

This retrospective analysis has several novel and clinically important findings. Firstly, we observed linear association between admission blood glucose concentration and the severity of LV dysfunction and dilatation after AMI. Second, blood glucose concentration correlated with plasma concentrations of specific MMP and TIMP enzymes in the sub-acute phase of AMI. Moreover, our data shows that it is the degree of dysglycaemia, rather than the diagnosis of "diabetes" which correlates with LV structure and function. Third, our data indicate a relationship between plasma concentration of both MMP-9 and TIMP-1 with the severity of LV dysfunction both soon after, and some months following, AMI. This relationship was independent of that between blood glucose and LV dysfunction. Our data suggest that both blood glucose concentration and MMP activity influence independently the development of LV dysfunction after AMI.

The extent of LV remodelling, the precursor of LV dysfunction and heart failure, represents one of the most powerful determinants of adverse prognosis after AMI (White, Norris et al. 1987; Pfeffer and Braunwald 1990). Augmented MMP

expression appears to be central to the process of left ventricular dysfunction and remodelling post AMI as discussed in the previous chapters. Plasma MMP-9 and TIMP-1 were associated with higher blood glucose concentration, which was in its own right correlated with LV structure and dysfunction. Stress hyperglycaemia has been associated with increased risk of congestive cardiac failure post AMI (Zeller, Verges et al. 2006). Moreover, a previous study in 162 patients with AMI demonstrated an association of stress hyperglycaemia with adverse ventricular remodelling after AMI (Bauters, Ennezat et al. 2007). The previous study was conducted in patients without a prior history of diabetes, and was restricted to those with anterior AMI (Bauters, Ennezat et al. 2007). In addition, stress hyperglycaemia was defined as admission blood glucose of $\geq$7mmol/L. The current study extends the finding of an association between blood glucose concentration and the extent of LV remodelling to a larger cohort of patients, without restriction regarding site or type (ST elevation or non ST elevation) of infarct. More importantly, our study included patients with antecedent diabetes, as well as those without this diagnosis. Indeed, therein lies the greatest strength of this study. When antecedent diabetes and blood glucose concentration were considered together, only the latter was found to hold independent association with two indicators of greater LV dysfunction, namely LVEF and WMIS. Moreover, our data suggest a graded association between blood glucose concentration and the extent of LV dysfunction after AMI (Figure 8.2).

Numerous potential pathophysiological mechanisms have been proposed to explain the association with adverse outcome after AMI for abnormalities of glucose metabolism (Mulvihill, Foley et al. 2001). In addition subjects with diabetes are less likely to receive thrombolysis (Pfeffer, Moye et al. 1991) or beta-blocker therapy

(Chen, Marciniak et al. 1999) post AMI. The current study provides evidence for a mechanistic link between elevated blood glucose concentration and adverse LV remodelling, one which would reasonably be expected to impact upon outcome, namely augmented MMP expression. Our observations of association between admission glucose and augmented MMP activity present an interesting and novel hypothesis for the mechanism by which stress hyperglycaemia leads to adverse prognosis.

In-vitro and animal studies support a link between hyperglycaemia and augmented MMP expression. Hyperglycaemia may induce MMP production in-vitro (Death, Fisher et al. 2003; Maldonado, He et al. 2004; Kadoglou, Daskalopoulou et al. 2005). Prolonged pre-exposure of histiocytes to high glucose in-vitro markedly increased lipopolysaccharide-stimulated MMP-1 secretion and mRNA expression through the nuclear factor kappaB (NFkappaB) and mitogen-activated protein kinase (MAPK) cascades (Maldonado, He et al. 2004). Further, glucose may induce endothelial cell expression and activity of MMP-1 and MMP-2, whilst reducing expression MMP-3 and may increase expression and activity of MMP-9 from monocyte-derived macrophages in vitro (Death, Fisher et al. 2003)

Animal studies also indicate that hyperglycaemia may induce MMP activity. Increased MMP-2 and decreased MMP-1 occur in a rat model of diabetes and may be causative in cerebrovascular remodelling and increased risk of stroke in this model of diabetes (Harris, Hutchinson et al. 2005). In addition MMP expression and activity are also altered in a variety of tissues in humans with diabetes (Kosano, Okano et al. 1999; Noda, Ishida et al. 2003; Bister, Kolho et al. 2005; Derosa, Avanzini et al. 2005; Lee, Song et al. 2005; Sampson, Wall et al. 2006; Shiau, Tsai et al. 2006).

To our knowledge, our observation of altered MMP activity associated with stress hyperglycaemia after AMI is novel, and may have potentially important clinical implications. Our data raise the possibility that strict blood glucose control may improve left ventricular dysfunction and prognosis post AMI. Indeed the failure of blood glucose concentration to fall after AMI has been associated with adverse prognosis (Goyal, Mahaffey et al. 2006). However only some (Malmberg, Ryden et al. 1995) but not all (Malmberg, Ryden et al. 2005) previous studies have demonstrated clinical benefit from strict glucose control after AMI.

Our observations also have implications for the potential therapeutic use of MMP inhibition after AMI. In the recent PREMIER (Prevention of Myocardial Infarction Early Remodelling) trial, the MMP inhibitor PG-116800 failed to attenuate adverse LV remodelling after AMI in man (Hudson, Armstrong et al. 2006). This study used a selective MMP inhibitor and did not evaluate medium or long term prognosis. Other human studies of MMP inhibition are currently lacking. In addition the observations of altered MMP activity in relation to glucose levels may extend to other disorders associated with diabetes and add insight into the pathogenesis of these conditions.

8.6

Chapter Summary

In conclusion, we have demonstrated an association between admission blood glucose concentration and the extent of LV dysfunction both in the early post AMI period and

in the medium term. Blood glucose concentration is also associated with specific MMP and TIMP enzyme expression. Studies are required to confirm our findings and to investigate whether strict blood glucose control leads to changes in MMP expression which may impact upon LV remodelling and prognosis.

Chapter 9

Final remarks and study limitations

9.1

Summary

In summary this thesis presents data regarding the levels of matrix metalloproteinases post acute myocardial infarction in humans. In our first stage we present data regarding the temporal profile of several of the important MMPs in humans. We demonstrate individual patterns to these temporal profiles between MMPs. We have demonstrated an effect of both peak and trough levels of MMP-9 on left ventricular function, volumes and remodelling. In stage 2 we further this data and included the effect of metalloproteinase levels on post MI prognosis in addition to LV function and remodelling. We have established MMP-9 and in particular TIMP-1 to be a significant prognostic marker post AMI. Indeed TIMP-1 appears at least equivalent to the current "gold standard" prognostic marker in N-BNP. We suggest that TIMP-1 may be used as a prognostic marker both in its own rights and in conjunction with N-BNP to provide specific prognostic data. This essential information will allow risk stratification of patients post AMI and may therefore potentially lead to altered patient management.

Moreover we have hypothesized (with scientific evidence) potential mechanisms by which both MMPs and TIMPs are associated with left ventricular dysfunction and adverse prognosis. In addition we present data to link stress hyperglycaemia post MI with altered MMP activation. We hypothesize that this altered activity may be

responsible for the adverse prognosis and greater LV dysfunction seen in subjects with stress hyperglycaemia or diabetes mellitus.

Our data suggests that metalloproteinase manipulation via MMP inhibitors, TIMP enhancement or indeed genetic alteration of MMP pathways may represent a significant therapeutic target in the post MI patient.

My Contribution

I performed the majority of work towards the data produced for this thesis. I was responsible for all patient recruitment and blood sampling and transthoracic echocardiography prior to discharge and at follow up. I was also responsible for clinical follow up including telephone contact with patients and review of medical notes. Lab work was performed at 2 sites. N-BNP, MMP-9 and MMP-2 were assayed at Leicester University and assays were performed by myself and 1 lab technician. MMP-1, MMP-3, and TIMP-1 were assayed at St Georges' Hospital Medical School by a funded lab technician using commercially available kits. In order to gain experience in the use of these kits I spent 1 week at St Georges's with this technician.

9.2

Study Limitations.

We acknowledge that our study does have several limitations.

Our findings are from a single-centre study, and should be investigated in other populations, we have however included a relatively large cohort with a wide spectrum

of ethnic and social background with a variety of degrees of cardiac damage and LV dysfunction. We cannot be sure that the observed levels of MMPs and TIMPs are solely the result of the index AMI however we have excluded to the best of our ability, patients with conditions that may lead to elevation of MMP such as previous heart failure or inflammatory conditions and prior studies have shown that the myocardium appears to be the source of variations in plasma MMP levels after AMI (Joffs, Gunasinghe et al. 2001; Bradham, Gunasinghe et al. 2002). We acknowledge that diurnal variation may occur in MMP levels however the majority of our samples were taken between 7 and 9am. Also CK and troponin measurements were taken from medical notes hence we cannot be entirely confident that we have captured the true peak of these markers.

Differences in pharmacological therapy both prior to admission and following discharge may add bias to our results. Several cardiovascular therapies are known to alter metalloproteinase activity such as ACE inhibitors (Sakata, Yamamoto et al. 2004; Schieffer, Bunte et al. 2004), Angiotensin II receptor blockers (Luchtefeld, Grote et al. 2005) and statins (Grothusen, Bley et al. 2005; Leu, Chen et al. 2005; Nakaya, Uzui et al. 2005; Wang, Lee et al. 2006; Yasuda, Miyazaki et al. 2007) however prior to admission relatively few of our subjects were receiving such medications. Likewise pharmacological therapy at discharge was largely uniform across our cohort hence should have little effect on both remodelling characteristics and prognosis.

We recognise the relatively weak statistical association between MMP levels and the assessed echocardiographic parameters in both stage 1 and 2 of this thesis however

such correlations are common place in "real life", human studies. Our data appears to be internally consistent despite these weak statistical associations suggesting that a genuine relationship exists. Sensitive imaging methodology, such as cardiac magnetic resonance imaging, may provide more detailed information on the relationship between plasma MMP concentrations and remodelling. With regard to our findings regarding remodelling, we considered echocardiographic parameters only for those patients with both pre-discharge and follow-up examinations. However as patients with the most extensive, early remodelling are likely to be among non-survivors, our data are likely to underestimate the strength of the relationship between MMP-9 or TIMP-1 and the extent of remodelling. Likewise, subjects with significant morbidity may not have attended for follow-up echocardiography however our drop out rate was very low and unlikely to truly bias results. In addition although every effort was made to perform pre-discharge echocardiography at day 4-5 post event, logistical difficulties resulted in some scans falling outside this time window although these were few and only deviated from this time point by 1-2 days and is unlikely to truly bias our results. The relationship of MMP levels to remodelling in relation to patency of the infarct related artery, in the context of primary PCI or thrombolysis, may further enhance our understanding of the pathophysiology of the MMP system in AMI.

We recognise the possibility of inflation of error associated with multiple measures of metalloproteinase levels. In this context we took representative measures of each MMP profile and entered only single values into our analyses hence reducing the likelihood of false positive results in stage 2.

We also acknowledge that some degree of MMP degradation may have occurred during the storage of samples however previous in vitro studies have suggested that MMPs are stable at -70°C for a relatively long period of time (Rouy, Ernens et al. 2005)and hence we feel that batch measurement of levels is less likely to be subject to laboratory error. We do acknowledge that some degree of laboratory error is unavoidable however samples were measured in duplicate using well established techniques and assays to ensure accurate results.

Lastly, we measured plasma levels of MMP/TIMP. From our data we are unable to state that these levels are truly representative of the metalloproteinase activity seen within the myocardium however such data would be difficult to obtain in human subjects. Samples taken at the time of coronary angiography or primary PCI may add further information as to the spatial distribution of MMPs. For this study however we have assumed that plasma levels are representative of myocardial levels of MMP.

With regards to our review of the effects of diabetes and glucose levels on MMP activity, we acknowledge that analysis was performed in a retrospective manner. We are unable to confirm a causal relationship either between blood glucose and plasma MMP concentration or between MMP concentration and left ventricular dysfunction. However as discussed above, experimental and clinical data support both possibilities.

We again recognise the relatively weak, although statistically significant, nature of some of our observed associations. It may be argued that admission glucose is simply a surrogate for the extent of myocardial damage post AMI, however we note the smaller rise in CK and Troponin I in quartile 4 of blood glucose, the group for whom

associations of blood glucose with TIMP-1 and with LV dysfunction were strongest. Indeed, worse prognosis and lower LVEF despite lower levels of CK were observed in patients with diabetes in a large, multi-centre post MI study.(Aguilar, Solomon et al. 2004)

9.3

Further study

Based on the findings from this thesis we recommend further studies to investigate the effects of MMPs on ventricular function and remodelling as well as prognosis post AMI.

Firstly multi-centre studies similar to the above may demonstrate similar results in larger and more diverse populations. More precise imaging techniques such as cardiac MRI would allow more accurate assessment of LV remodelling and may demonstrate closer relationships between MMPs and these parameters. Analysis of a broader range of MMPs may allow further elucidation of the "cascade" of metalloproteinase activity.

Further studies are merited regarding the mechanisms behind these observations. MMP-9, TIMP-1 and other elements of the MMP system should be the focus of prognostic studies after AMI, other acute coronary syndromes, and in chronic heart failure. In addition studies are required to confirm our findings regarding stress hyperglycaemia to investigate whether strict blood glucose control leads to changes in MMP expression which may impact upon LV remodelling and prognosis

Chapter 10

References

(1986). "Randomised trial of intravenous atenolol among 16 027 cases of suspected acute myocardial infarction: ISIS-1. First International Study of Infarct Survival Collaborative Group." Lancet **2**(8498): 57-66.

(1993). "An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction. The GUSTO investigators." N Engl J Med **329**(10): 673-82.

(1997). "A clinical trial comparing primary coronary angioplasty with tissue plasminogen activator for acute myocardial infarction. The Global Use of Strategies to Open Occluded Coronary Arteries in Acute Coronary Syndromes (GUSTO IIb) Angioplasty Substudy Investigators." N Engl J Med **336**(23): 1621-8.

Aguilar, D., S. D. Solomon, et al. (2004). "Newly diagnosed and previously known diabetes mellitus and 1-year outcomes of acute myocardial infarction: the VALsartan In Acute myocardial iNfarcTion (VALIANT) trial." Circulation **110**(12): 1572-8.

Aguirre, F. V., L. T. Younis, et al. (1995). "Early and 1-year clinical outcome of patients' evolving non-Q-wave versus Q-wave myocardial infarction after thrombolysis. Results from The TIMI II Study." Circulation **91**(10): 2541-8.

Ahmed, S. H., L. L. Clark, et al. (2006). "Matrix metalloproteinases/tissue inhibitors of metalloproteinases: relationship between changes in proteolytic determinants of matrix composition and structural, functional, and clinical manifestations of hypertensive heart disease." Circulation **113**(17): 2089-96.

Ahokas, K., T. Skoog, et al. (2005). "Matrilysin-2 (matrix metalloproteinase-26) is upregulated in keratinocytes during wound repair and early skin carcinogenesis." J Invest Dermatol **124**(4): 849-56.

Allaire, E., R. Forough, et al. (1998). "Local overexpression of TIMP-1 prevents aortic aneurysm degeneration and rupture in a rat model." J Clin Invest **102**(7): 1413-20.

Allender, S., V. Peto, et al. (2006). Coronary Heart Disease Statsistics 2006. British Heart Foundation Health Promotion Group Publication, Univerity of Oxford.

Anversa, P., C. Beghi, et al. (1985). "Myocardial response to infarction in the rat. Morphometric measurement of infarct size and myocyte cellular hypertrophy." Am J Pathol **118**(3): 484-92.

Arakawa, N., M. Nakamura, et al. (1996). "Plasma brain natriuretic peptide concentrations predict survival after acute myocardial infarction." J Am Coll Cardiol **27**(7): 1656-61.

Aukrust, P., T. Ueland, et al. (1999). "Cytokine network in congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy." Am J Cardiol **83**(3): 376-82.

Banfi, C., V. Cavalca, et al. (2005). "Neurohormonal activation is associated with increased levels of plasma matrix metalloproteinase-2 in human heart failure." Eur Heart J **26**(5): 481-8.

Banyai, L. and L. Patthy (1999). "The NTR module: domains of netrins, secreted frizzled related proteins, and type I procollagen C-proteinase enhancer protein are homologous with tissue inhibitors of metalloproteases." Protein Sci **8**(8): 1636-42.

Bauters, C., P. V. Ennezat, et al. (2007). "Stress hyperglycaemia is an independent predictor of left ventricular remodelling after first anterior myocardial infarction in non-diabetic patients." Eur Heart J **28**(5): 546-52.

Beckman, J. A., M. A. Creager, et al. (2002). "Diabetes and atherosclerosis: epidemiology, pathophysiology, and management." Jama **287**(19): 2570-81.

Benedict, C. R., B. Shelton, et al. (1996). "Prognostic significance of plasma norepinephrine in patients with asymptomatic left ventricular dysfunction. SOLVD Investigators." Circulation **94**(4): 690-7.

Ben-Yosef, Y., N. Lahat, et al. (2002). "Regulation of endothelial matrix metalloproteinase-2 by hypoxia/reoxygenation." Circ Res **90**(7): 784-91.

Berger, A. K., J. A. Breall, et al. (2001). "Effect of diabetes mellitus and insulin use on survival after acute myocardial infarction in the elderly (the Cooperative Cardiovascular Project)." Am J Cardiol **87**(3): 272-7.

Bertaux, B., W. Hornebeck, et al. (1991). "Growth stimulation of human keratinocytes by tissue inhibitor of metalloproteinases." J Invest Dermatol **97**(4): 679-85.

Beyzade, S., S. Zhang, et al. (2003). "Influences of matrix metalloproteinase-3 gene variation on extent of coronary atherosclerosis and risk of myocardial infarction." J Am Coll Cardiol **41**(12): 2130-7.

Bibbins-Domingo, K., M. Ansari, et al. (2003). "B-type natriuretic peptide and ischemia in patients with stable coronary disease: data from the Heart and Soul study." Circulation **108**(24): 2987-92.

Billinghurst, R. C., L. Dahlberg, et al. (1997). "Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage." J Clin Invest **99**(7): 1534-45.

Birkedal-Hansen, H. (1993). "Role of matrix metalloproteinases in human periodontal diseases." J Periodontol **64**(5 Suppl): 474-84.

Bister, V., K. L. Kolho, et al. (2005). "Metalloelastase (MMP-12) is upregulated in the gut of pediatric patients with potential celiac disease and in type 1 diabetes." Scand J Gastroenterol **40**(12): 1413-22.

Blankenberg, S., H. J. Rupprecht, et al. (2003). "Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease." Circulation **107**(12): 1579-85.

Bolognese, L. and G. Cerisano (1999). "Early predictors of left ventricular remodeling after acute myocardial infarction." Am Heart J **138**(2 Pt 2): S79-83.

Borg. T , B. (1993). "Holding it together: Organisation and function(s) of the xtracellular matrix of the heart." Heart Failure(9): 230-8.

Brack, A., V. Martinez-Taboada, et al. (1999). "Disease pattern in cranial and large-vessel giant cell arteritis." Arthritis Rheum **42**(2): 311-7.

Bradham, W. S., H. Gunasinghe, et al. (2002). "Release of matrix metalloproteinases following alcohol septal ablation in hypertrophic obstructive cardiomyopathy." J Am Coll Cardiol **40**(12): 2165-73.

Brown, D. L., M. S. Hibbs, et al. (1995). "Identification of 92-kD gelatinase in human coronary atherosclerotic lesions. Association of active enzyme synthesis with unstable angina." Circulation **91**(8): 2125-31.

Burrage, P. S., K. S. Mix, et al. (2006). "Matrix metalloproteinases: role in arthritis." Front Biosci **11**: 529-43.

Butler, G. S., H. Will, et al. (1997). "Membrane-type-2 matrix metalloproteinase can initiate the processing of progelatinase A and is regulated by the tissue inhibitors of metalloproteinases." Eur J Biochem **244**(2): 653-7.
Calabro, A., C. Grappone, et al. (2004). "Spatial and temporal pattern of expression of interstitial collagenase, stromelysin/transin, gelatinase A, and TIMP-1 during experimental gastric ulcer healing." Digestion **70**(2): 127-38.
Califf, R. M., H. D. White, et al. (1996). "One-year results from the Global Utilization of Streptokinase and TPA for Occluded Coronary Arteries (GUSTO-I) trial. GUSTO-I Investigators." Circulation **94**(6): 1233-8.
Capes, S. E., D. Hunt, et al. (2000). "Stress hyperglycaemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview." Lancet **355**(9206): 773-8.
Capes, S. E., D. Hunt, et al. (2001). "Stress hyperglycemia and prognosis of stroke in nondiabetic and diabetic patients: a systematic overview." Stroke **32**(10): 2426-32.
Castaner, A., A. Betriu, et al. (1984). "Natural history of severe left ventricular dysfunction after myocardial infarction." Chest **85**(6): 744-50.
Cavusoglu, E., C. Ruwende, et al. (2006). "Tissue inhibitor of metalloproteinase-1 (TIMP-1) is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction." Am Heart J **151**(5): 1101 e1-8.
Ceriello, A. (2005). "Acute hyperglycaemia: a 'new' risk factor during myocardial infarction." Eur Heart J **26**(4): 328-31.
Chancey, A. L., G. L. Brower, et al. (2002). "Effects of matrix metalloproteinase inhibition on ventricular remodeling due to volume overload." Circulation **105**(16): 1983-8.
Chase, A. J., M. Bond, et al. (2002). "Role of nuclear factor-kappa B activation in metalloproteinase-1, -3, and -9 secretion by human macrophages in vitro and rabbit foam cells produced in vivo." Arterioscler Thromb Vasc Biol **22**(5): 765-71.
Chatterjee, K. (2005). "Neurohormonal activation in congestive heart failure and the role of vasopressin." Am J Cardiol **95**(9A): 8B-13B.
Chen, J., T. A. Marciniak, et al. (1999). "Beta-blocker therapy for secondary prevention of myocardial infarction in elderly diabetic patients. Results from the National Cooperative Cardiovascular Project." J Am Coll Cardiol **34**(5): 1388-94.
Chen, J., C. H. Tung, et al. (2005). "Near-infrared fluorescent imaging of matrix metalloproteinase activity after myocardial infarction." Circulation **111**(14): 1800-5.
Chen, Z. M., H. C. Pan, et al. (2005). "Early intravenous then oral metoprolol in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial." Lancet **366**(9497): 1622-32.
Cho, H. J., I. H. Chae, et al. (2002). "Functional polymorphism in the promoter region of the gelatinase B gene in relation to coronary artery disease and restenosis after percutaneous coronary intervention." J Hum Genet **47**(2): 88-91.
Chung, A. W., H. Luo, et al. (2007). "Enhanced cell cycle entry and mitogen-activated protein kinase-signaling and downregulation of matrix metalloproteinase-1 and -3 in human diabetic arterial vasculature." Atherosclerosis.
Cleutjens, J. P., J. C. Kandala, et al. (1995). "Regulation of collagen degradation in the rat myocardium after infarction." J Mol Cell Cardiol **27**(6): 1281-92.

Close, D. R. (2001). "Matrix metalloproteinase inhibitors in rheumatic diseases." Ann Rheum Dis **60 Suppl 3**: iii62-7.

CONSENSUS (1987). "Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). The CONSENSUS Trial Study Group." N Engl J Med **316**(23): 1429-35.

Corcoran, M. L., W. G. Stetler-Stevenson, et al. (1994). "Effect of cholera toxin and pertussis toxin on prostaglandin H synthase-2, prostaglandin E2, and matrix metalloproteinase production by human monocytes." Arch Biochem Biophys **310**(2): 481-8.

Creemers, E. E., J. P. Cleutjens, et al. (2001). "Matrix metalloproteinase inhibition after myocardial infarction: a new approach to prevent heart failure?" Circ Res **89**(3): 201-10.

Crilley, J. G. and M. Farrer (2001). "Left ventricular remodelling and brain natriuretic peptide after first myocardial infarction." Heart **86**(6): 638-42.

Crowther, M., S. Goodall, et al. (2000). "Localization of matrix metalloproteinase 2 within the aneurysmal and normal aortic wall." Br J Surg **87**(10): 1391-400.

Darbar, D., N. C. Davidson, et al. (1996). "Diagnostic value of B-type natriuretic peptide concentrations in patients with acute myocardial infarction." Am J Cardiol **78**(3): 284-7.

Dargie, H. J. (2001). "Effect of carvedilol on outcome after myocardial infarction in patients with left-ventricular dysfunction: the CAPRICORN randomised trial." Lancet **357**(9266): 1385-90.

Davies, M., F. Hobbs, et al. (2001). "Prevalence of left-ventricular systolic dysfunction and heart failure in the Echocardiographic Heart of England Screening study: a population based study." Lancet **358**(9280): 439-44.

Davies, M. J. and A. Thomas (1984). "Thrombosis and acute coronary-artery lesions in sudden cardiac ischemic death." N Engl J Med **310**(18): 1137-40.

Davies, M. J. and A. C. Thomas (1985). "Plaque fissuring--the cause of acute myocardial infarction, sudden ischaemic death, and crescendo angina." Br Heart J **53**(4): 363-73.

de Lemos, J. A., D. A. Morrow, et al. (2001). "The prognostic value of B-type natriuretic peptide in patients with acute coronary syndromes." N Engl J Med **345**(14): 1014-21.

De Luca, G., H. Suryapranata, et al. (2004). "Time delay to treatment and mortality in primary angioplasty for acute myocardial infarction: every minute of delay counts." Circulation **109**(10): 1223-5.

Death, A. K., E. J. Fisher, et al. (2003). "High glucose alters matrix metalloproteinase expression in two key vascular cells: potential impact on atherosclerosis in diabetes." Atherosclerosis **168**(2): 263-9.

Derosa, G., M. A. Avanzini, et al. (2005). "Matrix metalloproteinase 2 may be a marker of microangiopathy in children and adolescents with type 1 diabetes mellitus." Diabetes Res Clin Pract **70**(2): 119-25.

Ducharme, A., S. Frantz, et al. (2000). "Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction." J Clin Invest **106**(1): 55-62.

Duhamel-Clerin, E., C. Orvain, et al. (1997). "Thrombin receptor-mediated increase of two matrix metalloproteinases, MMP-1 and MMP-3, in human endothelial cells." Arterioscler Thromb Vasc Biol **17**(10): 1931-8.

Egeblad, M. and Z. Werb (2002). "New functions for the matrix metalloproteinases in cancer progression." Nat Rev Cancer **2**(3): 161-74.
Erlebacher, J. A., J. L. Weiss, et al. (1984). "Early dilation of the infarcted segment in acute transmural myocardial infarction: role of infarct expansion in acute left ventricular enlargement." J Am Coll Cardiol **4**(2): 201-8.
Etoh, T., C. Joffs, et al. (2001). "Myocardial and interstitial matrix metalloproteinase activity after acute myocardial infarction in pigs." Am J Physiol Heart Circ Physiol **281**(3): H987-94.
Fabunmi, R. P., A. H. Baker, et al. (1996). "Divergent regulation by growth factors and cytokines of 95 kDa and 72 kDa gelatinases and tissue inhibitors or metalloproteinases-1, -2, and -3 in rabbit aortic smooth muscle cells." Biochem J **315 (Pt 1)**: 335-42.
Fabunmi, R. P., G. K. Sukhova, et al. (1998). "Expression of tissue inhibitor of metalloproteinases-3 in human atheroma and regulation in lesion-associated cells: a potential protective mechanism in plaque stability." Circ Res **83**(3): 270-8.
Falk, E., P. K. Shah, et al. (1995). "Coronary plaque disruption." Circulation **92**(3): 657-71.
Fedak, P. W., S. M. Altamentova, et al. (2003). "Matrix remodeling in experimental and human heart failure: a possible regulatory role for TIMP-3." Am J Physiol Heart Circ Physiol **284**(2): H626-34.
Fedak, P. W., D. S. Smookler, et al. (2004). "TIMP-3 deficiency leads to dilated cardiomyopathy." Circulation **110**(16): 2401-9.
Fernandez-Patron, C., M. A. Martinez-Cuesta, et al. (1999). "Differential regulation of platelet aggregation by matrix metalloproteinases-9 and -2." Thromb Haemost **82**(6): 1730-5.
Fielitz, J., M. Leuschner, et al. (2004). "Regulation of matrix metalloproteinases and their inhibitors in the left ventricular myocardium of patients with aortic stenosis." J Mol Med **82**(12): 809-20.
Fox, K. F., M. R. Cowie, et al. (2001). "Coronary artery disease as the cause of incident heart failure in the population." Eur Heart J **22**(3): 228-36.
Fox, K. M. (2003). "Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study)." Lancet **362**(9386): 782-8.
Frangogiannis, N. G., C. W. Smith, et al. (2002). "The inflammatory response in myocardial infarction." Cardiovasc Res **53**(1): 31-47.
Freije, J. M., I. Diez-Itza, et al. (1994). "Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas." J Biol Chem **269**(24): 16766-73.
Gadher, S. J., T. M. Schmid, et al. (1989). "Cleavage of collagen type X by human synovial collagenase and neutrophil elastase." Matrix **9**(2): 109-15.
Galasko, G. I., S. Basu, et al. (2004). "Is echocardiography a valid tool to screen for left ventricular systolic dysfunction in chronic survivors of acute myocardial infarction? A comparison with radionuclide ventriculography." Heart **90**(12): 1422-6.
Galis, Z. S., M. Muszynski, et al. (1995). "Enhanced expression of vascular matrix metalloproteinases induced in vitro by cytokines and in regions of human atherosclerotic lesions." Ann N Y Acad Sci **748**: 501-7.

Galis, Z. S., G. K. Sukhova, et al. (1995). "Macrophage foam cells from experimental atheroma constitutively produce matrix-degrading proteinases." Proc Natl Acad Sci U S A **92**(2): 402-6.
Galis, Z. S., G. K. Sukhova, et al. (1994). "Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques." J Clin Invest **94**(6): 2493-503.
Galloway, W. A., G. Murphy, et al. (1983). "Purification and characterization of a rabbit bone metalloproteinase that degrades proteoglycan and other connective-tissue components." Biochem J **209**(3): 741-52.
Galt, S. W., S. Lindemann, et al. (2002). "Outside-in signals delivered by matrix metalloproteinase-1 regulate platelet function." Circ Res **90**(10): 1093-9.
Gardner, R. S., F. Ozalp, et al. (2003). "N-terminal pro-brain natriuretic peptide. A new gold standard in predicting mortality in patients with advanced heart failure." Eur Heart J **24**(19): 1735-43.
Gasson, J. C., D. W. Golde, et al. (1985). "Molecular characterization and expression of the gene encoding human erythroid-potentiating activity." Nature **315**(6022): 768-71.
George, J., S. Patal, et al. (2005). "Circulating matrix metalloproteinase-2 but not matrix metalloproteinase-3, matrix metalloproteinase-9, or tissue inhibitor of metalloproteinase-1 predicts outcome in patients with congestive heart failure." Am Heart J **150**(3): 484-7.
Goldstein, B. J., P. N. Weissman, et al. (2006). "Reductions in biomarkers of cardiovascular risk in type 2 diabetes with rosiglitazone added to metformin compared with dose escalation of metformin: an EMPIRE trial sub-study." Curr Med Res Opin **22**(9): 1715-23.
Gomez, D. E., D. F. Alonso, et al. (1997). "Tissue inhibitors of metalloproteinases: structure, regulation and biological functions." Eur J Cell Biol **74**(2): 111-22.
Goodall, S., M. Crowther, et al. (2001). "Ubiquitous elevation of matrix metalloproteinase-2 expression in the vasculature of patients with abdominal aneurysms." Circulation **104**(3): 304-9.
Goyal, A., K. W. Mahaffey, et al. (2006). "Prognostic significance of the change in glucose level in the first 24 h after acute myocardial infarction: results from the CARDINAL study." Eur Heart J **27**(11): 1289-97.
Grabowski, M., K. J. Filipiak, et al. (2005). "Prognostic value of B-type natriuretic peptide levels on admission in patients with acute ST elevation myocardial infarction." Acta Cardiol **60**(5): 537-42.
Grines, C. L., K. F. Browne, et al. (1993). "A comparison of immediate angioplasty with thrombolytic therapy for acute myocardial infarction. The Primary Angioplasty in Myocardial Infarction Study Group." N Engl J Med **328**(10): 673-9.
Gronski, T. J., Jr., R. L. Martin, et al. (1997). "Hydrolysis of a broad spectrum of extracellular matrix proteins by human macrophage elastase." J Biol Chem **272**(18): 12189-94.
Gross, J. and C. M. Lapiere (1962). "Collagenolytic activity in amphibian tissues: a tissue culture assay." Proc Natl Acad Sci U S A **48**: 1014-22.
Grothusen, C., S. Bley, et al. (2005). "Combined effects of HMG-CoA-reductase inhibition and renin-angiotensin system blockade on experimental atherosclerosis." Atherosclerosis **182**(1): 57-69.

Group, I.-C. (1986). "Effectiveness of intravenous thrombolytic treatment in acute myocardial infarction. Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico (GISSI)." Lancet **1**(8478): 397-402.
Group, T. M. P. R. (1983). "Risk Stratification and survival after myocardial infarction." N Engl J Med **309**: 331-336.
Guidry, U. C., J. C. Evans, et al. (1999). "Temporal trends in event rates after Q-wave myocardial infarction: the Framingham Heart Study." Circulation **100**(20): 2054-9.
Gurkan, A., G. Emingil, et al. (2007). "Matrix Metalloproteinase-2, -9, and -12 Gene Polymorphisms in Generalized Aggressive Periodontitis." J Periodontol **78**(12): 2338-2347.
Habib, G. B., J. Heibig, et al. (1991). "Influence of coronary collateral vessels on myocardial infarct size in humans. Results of phase I thrombolysis in myocardial infarction (TIMI) trial. The TIMI Investigators." Circulation **83**(3): 739-46.
Hall, C. (2005). "NT-ProBNP: the mechanism behind the marker." J Card Fail **11**(5 Suppl): S81-3.
Hama, N., H. Itoh, et al. (1995). "Rapid ventricular induction of brain natriuretic peptide gene expression in experimental acute myocardial infarction." Circulation **92**(6): 1558-64.
Hamer, A. W., M. Takayama, et al. (1994). "End-systolic volume and long-term survival after coronary artery bypass graft surgery in patients with impaired left ventricular function." Circulation **90**(6): 2899-904.
Harris, A. K., J. R. Hutchinson, et al. (2005). "Type 2 diabetes causes remodeling of cerebrovasculature via differential regulation of matrix metalloproteinases and collagen synthesis: role of endothelin-1." Diabetes **54**(9): 2638-44.
Hautamaki, R. D., D. K. Kobayashi, et al. (1997). "Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice." Science **277**(5334): 2002-4.
Hayakawa, T., K. Yamashita, et al. (1994). "Cell growth-promoting activity of tissue inhibitor of metalloproteinases-2 (TIMP-2)." J Cell Sci **107 (Pt 9)**: 2373-9.
Health, H. G. D. (1999). Our Healthier Nation**:** Chapter 5.
Heeschen, C., C. W. Hamm, et al. (2004). "N-terminal pro-B-type natriuretic peptide levels for dynamic risk stratification of patients with acute coronary syndromes." Circulation **110**(20): 3206-12.
Hellermann, J. P., T. Y. Goraya, et al. (2003). "Incidence of heart failure after myocardial infarction: is it changing over time?" Am J Epidemiol **157**(12): 1101-7.
Henney, A. M., P. R. Wakeley, et al. (1991). "Localization of stromelysin gene expression in atherosclerotic plaques by in situ hybridization." Proc Natl Acad Sci U S A **88**(18): 8154-8.
Herman, M. P., G. K. Sukhova, et al. (2001). "Tissue factor pathway inhibitor-2 is a novel inhibitor of matrix metalloproteinases with implications for atherosclerosis." J Clin Invest **107**(9): 1117-26.
Herman, M. P., G. K. Sukhova, et al. (2001). "Expression of neutrophil collagenase (matrix metalloproteinase-8) in human atheroma: a novel collagenolytic pathway suggested by transcriptional profiling." Circulation **104**(16): 1899-904.

Heymans, S., A. Luttun, et al. (1999). "Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure." Nat Med **5**(10): 1135-42.
Heymans, S., B. Schroen, et al. (2005). "Increased cardiac expression of tissue inhibitor of metalloproteinase-1 and tissue inhibitor of metalloproteinase-2 is related to cardiac fibrosis and dysfunction in the chronic pressure-overloaded human heart." Circulation **112**(8): 1136-44.
Hibbs, M. S., J. R. Hoidal, et al. (1987). "Expression of a metalloproteinase that degrades native type V collagen and denatured collagens by cultured human alveolar macrophages." J Clin Invest **80**(6): 1644-50.
Hirata, N., S. Nakano, et al. (1992). "[The long-term prognosis of coronary artery bypass surgery--the influence of preoperative left ventricular volume on the prognosis of cardiac death]." Nippon Kyobu Geka Gakkai Zasshi **40**(2): 184-8.
Hirohata, S., S. Kusachi, et al. (1997). "Time dependent alterations of serum matrix metalloproteinase-1 and metalloproteinase-1 tissue inhibitor after successful reperfusion of acute myocardial infarction." Heart **78**(3): 278-84.
Hirsch, A. T., C. E. Talsness, et al. (1991). "Tissue-specific activation of cardiac angiotensin converting enzyme in experimental heart failure." Circ Res **69**(2): 475-82.
Hjalmarson, A., J. Herlitz, et al. (1983). "The Goteborg metoprolol trial. Effects on mortality and morbidity in acute myocardial infarction." Circulation **67**(6 Pt 2): I26-32.
Ho, K. K., K. M. Anderson, et al. (1993). "Survival after the onset of congestive heart failure in Framingham Heart Study subjects." Circulation **88**(1): 107-15.
Hojo, Y., U. Ikeda, et al. (2001). "Expression of matrix metalloproteinases in patients with acute myocardial infarction." Jpn Circ J **65**(2): 71-5.
Hopkins, W. E., Z. Chen, et al. (2004). "Increased atrial and brain natriuretic peptides in adults with cyanotic congenital heart disease: enhanced understanding of the relationship between hypoxia and natriuretic peptide secretion." Circulation **109**(23): 2872-7.
Hudson, M. P., P. W. Armstrong, et al. (2006). "Effects of selective matrix metalloproteinase inhibitor (PG-116800) to prevent ventricular remodeling after myocardial infarction: results of the PREMIER (Prevention of Myocardial Infarction Early Remodeling) trial." J Am Coll Cardiol **48**(1): 15-20.
Hulsmann, M., R. Berger, et al. (2002). "Prediction of outcome by neurohumoral activation, the six-minute walk test and the Minnesota Living with Heart Failure Questionnaire in an outpatient cohort with congestive heart failure." Eur Heart J **23**(11): 886-91.
Hunt, P. J., A. M. Richards, et al. (1997). "Immunoreactive amino-terminal pro-brain natriuretic peptide (NT-PROBNP): a new marker of cardiac impairment." Clin Endocrinol (Oxf) **47**(3): 287-96.
Inokubo, Y., H. Hanada, et al. (2001). "Plasma levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 are increased in the coronary circulation in patients with acute coronary syndrome." Am Heart J **141**(2): 211-7.
Inoue, T., T. Kato, et al. (2003). "Circulating matrix metalloproteinase-1 and -3 in patients with an acute coronary syndrome." Am J Cardiol **92**(12): 1461-4.
Institute, T. N. H. a. L. (1999). "Diabetes mellitus: a major risk factor for cardiovascular disease. A joint editorial statement by the American Diabetes

Association; The National Heart, Lung, and Blood Institute; The Juvenile Diabetes Foundation International; The National Institute of Diabetes and Digestive and Kidney Diseases; and The American Heart Association." Circulation **100**(10): 1132-3.

James, S. K., B. Lindahl, et al. (2006). "Usefulness of biomarkers for predicting long-term mortality in patients with diabetes mellitus and non-ST-elevation acute coronary syndromes (a GUSTO IV substudy)." Am J Cardiol **97**(2): 167-72.

Jernberg, T., B. Lindahl, et al. (2003). "N-terminal pro-brain natriuretic peptide in relation to inflammation, myocardial necrosis, and the effect of an invasive strategy in unstable coronary artery disease." J Am Coll Cardiol **42**(11): 1909-16.

Jernberg, T., M. Stridsberg, et al. (2002). "N-terminal pro brain natriuretic peptide on admission for early risk stratification of patients with chest pain and no ST-segment elevation." J Am Coll Cardiol **40**(3): 437-45.

Joffs, C., H. R. Gunasinghe, et al. (2001). "Cardiopulmonary bypass induces the synthesis and release of matrix metalloproteinases." Ann Thorac Surg **71**(5): 1518-23.

Jormsjo, S., S. Ye, et al. (2000). "Allele-specific regulation of matrix metalloproteinase-12 gene activity is associated with coronary artery luminal dimensions in diabetic patients with manifest coronary artery disease." Circ Res **86**(9): 998-1003.

Ju, H., S. Zhao, et al. (1998). "Expression of Gq alpha and PLC-beta in scar and border tissue in heart failure due to myocardial infarction." Circulation **97**(9): 892-9.

Kadoglou, N. P., S. S. Daskalopoulou, et al. (2005). "Matrix metalloproteinases and diabetic vascular complications." Angiology **56**(2): 173-89.

Kahari, V. M. and U. Saarialho-Kere (1999). "Matrix metalloproteinases and their inhibitors in tumour growth and invasion." Ann Med **31**(1): 34-45.

Kai, H., H. Ikeda, et al. (1998). "Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes." J Am Coll Cardiol **32**(2): 368-72.

Kamada, H., F. Yu, et al. (2007). "Influence of hyperglycemia on oxidative stress and matrix metalloproteinase-9 activation after focal cerebral ischemia/reperfusion in rats: relation to blood-brain barrier dysfunction." Stroke **38**(3): 1044-9.

Kawakami, R., Y. Saito, et al. (2004). "Overexpression of brain natriuretic peptide facilitates neutrophil infiltration and cardiac matrix metalloproteinase-9 expression after acute myocardial infarction." Circulation **110**(21): 3306-12.

Keeley, E. C., J. A. Boura, et al. (2003). "Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials." Lancet **361**(9351): 13-20.

Kelly, D., G. Cockerill, et al. (2007). "Plasma matrix metalloproteinase-9 and left ventricular remodelling after acute myocardial infarction in man: a prospective cohort study." Eur Heart J **28**(6): 711-8.

Kim, J. S., H. Y. Park, et al. (2002). "The roles of stromelysin-1 and the gelatinase B gene polymorphism in stable angina." Yonsei Med J **43**(4): 473-81.

Kirschvink, N., G. Vincke, et al. (2005). "Repeated cadmium nebulizations induce pulmonary MMP-2 and MMP-9 production and emphysema in rats." Toxicology **211**(1-2): 36-48.

Kleiner, D. E. and W. G. Stetler-Stevenson (1999). "Matrix metalloproteinases and metastasis." Cancer Chemother Pharmacol **43 Suppl**: S42-51.

Knauper, V., A. Osthues, et al. (1993). "Fragmentation of human polymorphonuclear-leucocyte collagenase." Biochem J **291 (Pt 3)**: 847-54.
Knauper, V., S. M. Wilhelm, et al. (1993). "Direct activation of human neutrophil procollagenase by recombinant stromelysin." Biochem J **295 (Pt 2)**: 581-6.
Koglin, J., S. Pehlivanli, et al. (2001). "Role of brain natriuretic peptide in risk stratification of patients with congestive heart failure." J Am Coll Cardiol **38**(7): 1934-41.
Kosano, H., T. Okano, et al. (1999). "ProMMP-9 (92 kDa gelatinase) in vitreous fluid of patients with proliferative diabetic retinopathy." Life Sci **64**(25): 2307-15.
Krampert, M., W. Bloch, et al. (2004). "Activities of the matrix metalloproteinase stromelysin-2 (MMP-10) in matrix degradation and keratinocyte organization in wounded skin." Mol Biol Cell **15**(12): 5242-54.
Krum, H. and R. E. Gilbert (2003). "Demographics and concomitant disorders in heart failure." Lancet **362**(9378): 147-58.
Lambert, E., E. Dasse, et al. (2004). "TIMPs as multifacial proteins." Crit Rev Oncol Hematol **49**(3): 187-98.
Lamblin, N., C. Bauters, et al. (2002). "Polymorphisms in the promoter regions of MMP-2, MMP-3, MMP-9 and MMP-12 genes as determinants of aneurysmal coronary artery disease." J Am Coll Cardiol **40**(1): 43-8.
Laviades, C., N. Varo, et al. (1998). "Abnormalities of the extracellular degradation of collagen type I in essential hypertension." Circulation **98**(6): 535-40.
Lazarus, G. S., R. S. Brown, et al. (1968). "Human granulocyte collagenase." Science **159**(822): 1483-5.
Leco, K. J., R. Khokha, et al. (1994). "Tissue inhibitor of metalloproteinases-3 (TIMP-3) is an extracellular matrix-associated protein with a distinctive pattern of expression in mouse cells and tissues." J Biol Chem **269**(12): 9352-60.
Lee, S. W., K. E. Song, et al. (2005). "Alterations in peripheral blood levels of TIMP-1, MMP-2, and MMP-9 in patients with type-2 diabetes." Diabetes Res Clin Pract **69**(2): 175-9.
Lees, M., D. J. Taylor, et al. (1994). "Mast cell proteinases activate precursor forms of collagenase and stromelysin, but not of gelatinases A and B." Eur J Biochem **223**(1): 171-7.
Lemaitre, V., P. D. Soloway, et al. (2003). "Increased medial degradation with pseudo-aneurysm formation in apolipoprotein E-knockout mice deficient in tissue inhibitor of metalloproteinases-1." Circulation **107**(2): 333-8.
Leppert, D., R. L. Lindberg, et al. (2001). "Matrix metalloproteinases: multifunctional effectors of inflammation in multiple sclerosis and bacterial meningitis." Brain Res Brain Res Rev **36**(2-3): 249-57.
Leu, H. B., J. W. Chen, et al. (2005). "Effects of fluvastatin, an HMG-CoA reductase inhibitor, on serum levels of interleukin-18 and matrix metalloproteinase-9 in patients with hypercholesterolemia." Clin Cardiol **28**(9): 423-8.
Levin, E. R., D. G. Gardner, et al. (1998). "Natriuretic peptides." N Engl J Med **339**(5): 321-8.
Levine, B., J. Kalman, et al. (1990). "Elevated circulating levels of tumor necrosis factor in severe chronic heart failure." N Engl J Med **323**(4): 236-41.
Lew, W. Y., Z. Y. Chen, et al. (1985). "Mechanisms of augmented segment shortening in nonischemic areas during acute ischemia of the canine left ventricle." Circ Res **56**(3): 351-8.

Li, H., H. Simon, et al. (2000). "MMP/TIMP expression in spontaneously hypertensive heart failure rats: the effect of ACE- and MMP-inhibition." Cardiovasc Res **46**(2): 298-306.
Li, Y. Y., A. M. Feldman, et al. (1998). "Differential expression of tissue inhibitors of metalloproteinases in the failing human heart." Circulation **98**(17): 1728-34.
Li, Y. Y., Y. Feng, et al. (2001). "Downregulation of matrix metalloproteinases and reduction in collagen damage in the failing human heart after support with left ventricular assist devices." Circulation **104**(10): 1147-52.
Liekens, S., E. De Clercq, et al. (2001). "Angiogenesis: regulators and clinical applications." Biochem Pharmacol **61**(3): 253-70.
Lijnen, H. R., F. Lupu, et al. (1999). "Temporal and topographic matrix metalloproteinase expression after vascular injury in mice." Thromb Haemost **81**(5): 799-807.
Lindsay, M. M., P. Maxwell, et al. (2002). "TIMP-1: a marker of left ventricular diastolic dysfunction and fibrosis in hypertension." Hypertension **40**(2): 136-41.
Lindsey, M., K. Wedin, et al. (2001). "Matrix-dependent mechanism of neutrophil-mediated release and activation of matrix metalloproteinase 9 in myocardial ischemia/reperfusion." Circulation **103**(17): 2181-7.
Lindsey, M. L., J. Gannon, et al. (2002). "Selective matrix metalloproteinase inhibition reduces left ventricular remodeling but does not inhibit angiogenesis after myocardial infarction." Circulation **105**(6): 753-8.
Liotta, L. A., S. Abe, et al. (1979). "Preferential digestion of basement membrane collagen by an enzyme derived from a metastatic murine tumor." Proc Natl Acad Sci U S A **76**(5): 2268-72.
Liu, D., L. Gao, et al. (2006). "Neuronal angiotensin II type 1 receptor upregulation in heart failure: activation of activator protein 1 and Jun N-terminal kinase." Circ Res **99**(9): 1004-11.
Liu, P. Y., J. H. Chen, et al. (2003). "Synergistic effect of stromelysin-1 (matrix metallo-proteinase-3) promoter 5A/6A polymorphism with smoking on the onset of young acute myocardial infarction." Thromb Haemost **90**(1): 132-9.
Loftus, I. M., A. R. Naylor, et al. (2000). "Increased matrix metalloproteinase-9 activity in unstable carotid plaques. A potential role in acute plaque disruption." Stroke **31**(1): 40-7.
Lovelock, J. D., A. H. Baker, et al. (2005). "Heterogeneous effects of tissue inhibitors of matrix metalloproteinases on cardiac fibroblasts." Am J Physiol Heart Circ Physiol **288**(2): H461-8.
Lu, L., J. Q. Zhang, et al. (2004). "Molecular and cellular events at the site of myocardial infarction: from the perspective of rebuilding myocardial tissue." Biochem Biophys Res Commun **320**(3): 907-13.
Lubos, E., R. Schnabel, et al. (2006). "Prognostic value of tissue inhibitor of metalloproteinase-1 for cardiovascular death among patients with cardiovascular disease: results from the AtheroGene study." Eur Heart J **27**(2): 150-6.
Luchtefeld, M., K. Grote, et al. (2005). "Angiotensin II induces MMP-2 in a p47phox-dependent manner." Biochem Biophys Res Commun **328**(1): 183-8.
Lund, L. R., J. Romer, et al. (1999). "Functional overlap between two classes of matrix-degrading proteases in wound healing." Embo J **18**(17): 4645-56.
Luttun, A., E. Lutgens, et al. (2004). "Loss of matrix metalloproteinase-9 or matrix metalloproteinase-12 protects apolipoprotein E-deficient mice against

atherosclerotic media destruction but differentially affects plaque growth." Circulation **109**(11): 1408-14.
Maldonado, A., L. He, et al. (2004). "Pre-exposure to high glucose augments lipopolysaccharide-stimulated matrix metalloproteinase-1 expression by human U937 histiocytes." J Periodontal Res **39**(6): 415-23.
Malmberg, K., L. Ryden, et al. (1995). "Randomized trial of insulin-glucose infusion followed by subcutaneous insulin treatment in diabetic patients with acute myocardial infarction (DIGAMI study): effects on mortality at 1 year." J Am Coll Cardiol **26**(1): 57-65.
Malmberg, K., L. Ryden, et al. (2005). "Intense metabolic control by means of insulin in patients with diabetes mellitus and acute myocardial infarction (DIGAMI 2): effects on mortality and morbidity." Eur Heart J **26**(7): 650-61.
Malmberg, K., S. Yusuf, et al. (2000). "Impact of diabetes on long-term prognosis in patients with unstable angina and non-Q-wave myocardial infarction: results of the OASIS (Organization to Assess Strategies for Ischemic Syndromes) Registry." Circulation **102**(9): 1014-9.
Marumoto, K., M. Hamada, et al. (1995). "Augmented secretion of atrial and brain natriuretic peptides during dynamic exercise in patients with old myocardial infarction." Jpn Circ J **59**(11): 715-24.
Matsunaga, T., N. Abe, et al. (2005). "Circulating level of gelatinase activity predicts ventricular remodeling in patients with acute myocardial infarction." Int J Cardiol **105**(2): 203-8.
Matsuyama, A., N. Sakai, et al. (2003). "Matrix metalloproteinases as novel disease markers in Takayasu arteritis." Circulation **108**(12): 1469-73.
McDonagh, T. A., C. E. Morrison, et al. (1997). "Symptomatic and asymptomatic left-ventricular systolic dysfunction in an urban population." Lancet **350**(9081): 829-33.
McGuire, D. K., H. Emanuelsson, et al. (2000). "Influence of diabetes mellitus on clinical outcomes across the spectrum of acute coronary syndromes. Findings from the GUSTO-IIb study. GUSTO IIb Investigators." Eur Heart J **21**(21): 1750-8.
McGuire, D. K., L. K. Newby, et al. (2004). "Association of diabetes mellitus and glycemic control strategies with clinical outcomes after acute coronary syndromes." Am Heart J **147**(2): 246-52.
Mega, J. L., D. A. Morrow, et al. (2004). "B-type natriuretic peptide at presentation and prognosis in patients with ST-segment elevation myocardial infarction: an ENTIRE-TIMI-23 substudy." J Am Coll Cardiol **44**(2): 335-9.
MIAMI (1985). "Metoprolol in acute myocardial infarction (MIAMI). A randomised placebo-controlled international trial. The MIAMI Trial Research Group." Eur Heart J **6**(3): 199-226.
Miller, E. J., E. D. Harris, Jr., et al. (1976). "Cleavage of Type II and III collagens with mammalian collagenase: site of cleavage and primary structure at the NH2-terminal portion of the smaller fragment released from both collagens." Biochemistry **15**(4): 787-92.
Mizon-Gerard, F., P. de Groote, et al. (2004). "Prognostic impact of matrix metalloproteinase gene polymorphisms in patients with heart failure according to the aetiology of left ventricular systolic dysfunction." Eur Heart J **25**(8): 688-93.
Moreau, M., I. Brocheriou, et al. (1999). "Interleukin-8 mediates downregulation of tissue inhibitor of metalloproteinase-1 expression in cholesterol-loaded human

macrophages: relevance to stability of atherosclerotic plaque." Circulation **99**(3): 420-6.
Morgan, A. R., B. Zhang, et al. (2003). "Haplotypic analysis of the MMP-9 gene in relation to coronary artery disease." J Mol Med **81**(5): 321-6.
Morita, E., H. Yasue, et al. (1993). "Increased plasma levels of brain natriuretic peptide in patients with acute myocardial infarction." Circulation **88**(1): 82-91.
Morrow, D. A., J. A. de Lemos, et al. (2005). "Prognostic value of serial B-type natriuretic peptide testing during follow-up of patients with unstable coronary artery disease." Jama **294**(22): 2866-71.
Morrow, D. A., J. A. de Lemos, et al. (2003). "Evaluation of B-type natriuretic peptide for risk assessment in unstable angina/non-ST-elevation myocardial infarction: B-type natriuretic peptide and prognosis in TACTICS-TIMI 18." J Am Coll Cardiol **41**(8): 1264-72.
Mukamal, K. J., R. W. Nesto, et al. (2001). "Impact of diabetes on long-term survival after acute myocardial infarction: comparability of risk with prior myocardial infarction." Diabetes Care **24**(8): 1422-7.
Mukherjee, R., T. A. Brinsa, et al. (2003). "Myocardial infarct expansion and matrix metalloproteinase inhibition." Circulation **107**(4): 618-25.
Mukoyama, M., K. Nakao, et al. (1991). "Brain natriuretic peptide as a novel cardiac hormone in humans. Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide." J Clin Invest **87**(4): 1402-12.
Mulvihill, N. T., J. B. Foley, et al. (2001). "Enhanced endothelial activation in diabetic patients with unstable angina and non-Q-wave myocardial infarction." Diabet Med **18**(12): 979-83.
Murphy, G., T. E. Cawston, et al. (1981). "Metalloproteinases from rabbit bone culture medium degrade types IV and V collagens, laminin and fibronectin." Biochem J **199**(3): 807-11.
Murphy, G., J. J. Reynolds, et al. (1982). "Partial purification of collagenase and gelatinase from human polymorphonuclear leucocytes. Analysis of their actions on soluble and insoluble collagens." Biochem J **203**(1): 209-21.
Murphy, G., R. Ward, et al. (1989). "Characterization of gelatinase from pig polymorphonuclear leucocytes. A metalloproteinase resembling tumour type IV collagenase." Biochem J **258**(2): 463-72.
Murry, C. E., R. B. Jennings, et al. (1986). "Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium." Circulation **74**(5): 1124-36.
Nakaya, R., H. Uzui, et al. (2005). "Pravastatin suppresses the increase in matrix metalloproteinase-2 levels after acute myocardial infarction." Int J Cardiol **105**(1): 67-73.
NHS (2000). Coronary Heart Disease. National Service Frameworks.
Nikkari, S. T., R. L. Geary, et al. (1996). "Expression of collagen, interstitial collagenase, and tissue inhibitor of metalloproteinases-1 in restenosis after carotid endarterectomy." Am J Pathol **148**(3): 777-83.
Noda, K., S. Ishida, et al. (2003). "Production and activation of matrix metalloproteinase-2 in proliferative diabetic retinopathy." Invest Ophthalmol Vis Sci **44**(5): 2163-70.
Noji, Y., K. Kajinami, et al. (2001). "Circulating matrix metalloproteinases and their inhibitors in premature coronary atherosclerosis." Clin Chem Lab Med **39**(5): 380-4.

Nojiri, T., H. Morita, et al. (2003). "Genetic variations of matrix metalloproteinase-1 and -3 promoter regions and their associations with susceptibility to myocardial infarction in Japanese." Int J Cardiol **92**(2-3): 181-6.
Norris, R. M., H. D. White, et al. (1992). "Prognosis after recovery from myocardial infarction: the relative importance of cardiac dilatation and coronary stenoses." Eur Heart J **13**(12): 1611-8.
Ogata, Y., J. J. Enghild, et al. (1992). "Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9." J Biol Chem **267**(6): 3581-4.
Ogiwara, K., N. Takano, et al. (2005). "Gelatinase A and membrane-type matrix metalloproteinases 1 and 2 are responsible for follicle rupture during ovulation in the medaka." Proc Natl Acad Sci U S A **102**(24): 8442-7.
O'Grady, A., C. Dunne, et al. (2007). "Differential expression of matrix metalloproteinase (MMP)-2, MMP-9 and tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 in non-melanoma skin cancer: implications for tumour progression." Histopathology **51**(6): 793-804.
Oie, E., L. E. Vinge, et al. (2000). "Induction of a myocardial adrenomedullin signaling system during ischemic heart failure in rats." Circulation **101**(4): 415-22.
Okada, Y., H. Nagase, et al. (1986). "A metalloproteinase from human rheumatoid synovial fibroblasts that digests connective tissue matrix components. Purification and characterization." J Biol Chem **261**(30): 14245-55.
Okumura, Y., H. Sato, et al. (1997). "Proteolytic activation of the precursor of membrane type 1 matrix metalloproteinase by human plasmin. A possible cell surface activator." FEBS Lett **402**(2-3): 181-4.
Omland, T., A. Aakvaag, et al. (1996). "Plasma brain natriuretic peptide as an indicator of left ventricular systolic function and long-term survival after acute myocardial infarction. Comparison with plasma atrial natriuretic peptide and N-terminal proatrial natriuretic peptide." Circulation **93**(11): 1963-9.
Omland, T., A. Persson, et al. (2002). "N-terminal pro-B-type natriuretic peptide and long-term mortality in acute coronary syndromes." Circulation **106**(23): 2913-8.
Opdenakker, G., I. Nelissen, et al. (2003). "Functional roles and therapeutic targeting of gelatinase B and chemokines in multiple sclerosis." Lancet Neurol **2**(12): 747-56.
O'Sullivan, J. J., R. M. Conroy, et al. (1991). "In-hospital prognosis of patients with fasting hyperglycemia after first myocardial infarction." Diabetes Care **14**(8): 758-60.
Oswald, G. A., S. Corcoran, et al. (1984). "Prevalence and risks of hyperglycaemia and undiagnosed diabetes in patients with acute myocardial infarction." Lancet **1**(8389): 1264-7.
Otterstad, J. E., M. G. St John Sutton, et al. (2002). "Prognostic value of two-dimensional echocardiography and N-terminal proatrial natriuretic peptide following an acute myocardial infarction. Assessment of baseline values (2-7 days) and changes at 3 months in patients with a preserved systolic function." Eur Heart J **23**(13): 1011-20.
Papadopoulos, D. P., I. Moyssakis, et al. (2005). "Clinical significance of matrix metalloproteinases activity in acute myocardial infarction." Eur Cytokine Netw **16**(2): 152-60.

Parsons, S. L., S. A. Watson, et al. (1997). "Matrix metalloproteinases." Br J Surg **84**(2): 160-6.
Pei, D. and S. J. Weiss (1996). "Transmembrane-deletion mutants of the membrane-type matrix metalloproteinase-1 process progelatinase A and express intrinsic matrix-degrading activity." J Biol Chem **271**(15): 9135-40.
Perlstein, T. S. and R. T. Lee (2006). "Smoking, metalloproteinases, and vascular disease." Arterioscler Thromb Vasc Biol **26**(2): 250-6.
Peterson, J. T., H. Li, et al. (2000). "Evolution of matrix metalloprotease and tissue inhibitor expression during heart failure progression in the infarcted rat." Cardiovasc Res **46**(2): 307-15.
Pfeffer, M. A. and E. Braunwald (1990). "Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications." Circulation **81**(4): 1161-72.
Pfeffer, M. A., L. A. Moye, et al. (1991). "Selection bias in the use of thrombolytic therapy in acute myocardial infarction. The SAVE Investigators." Jama **266**(4): 528-32.
Pitt, B., W. Remme, et al. (2003). "Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction." N Engl J Med **348**(14): 1309-21.
Pollanen, P. J., P. J. Karhunen, et al. (2001). "Coronary artery complicated lesion area is related to functional polymorphism of matrix metalloproteinase 9 gene: an autopsy study." Arterioscler Thromb Vasc Biol **21**(9): 1446-50.
Quaranta, M., A. Daniele, et al. (2007). "MMP-2, MMP-9, VEGF and CA 15.3 in breast cancer." Anticancer Res **27**(5B): 3593-600.
Radomski, A., M. W. Stewart, et al. (2001). "Pharmacological characteristics of solid-phase von Willebrand factor in human platelets." Br J Pharmacol **134**(5): 1013-20.
Rajagopalan, S., X. P. Meng, et al. (1996). "Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability." J Clin Invest **98**(11): 2572-9.
Rajavashisth, T. B., J. K. Liao, et al. (1999). "Inflammatory cytokines and oxidized low density lipoproteins increase endothelial cell expression of membrane type 1-matrix metalloproteinase." J Biol Chem **274**(17): 11924-9.
Reinhardt, D., H. H. Sigusch, et al. (2002). "Cardiac remodelling in end stage heart failure: upregulation of matrix metalloproteinase (MMP) irrespective of the underlying disease, and evidence for a direct inhibitory effect of ACE inhibitors on MMP." Heart **88**(5): 525-30.
Richards, A. M., R. Doughty, et al. (2001). "Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: prognostic utility and prediction of benefit from carvedilol in chronic ischemic left ventricular dysfunction. Australia-New Zealand Heart Failure Group." J Am Coll Cardiol **37**(7): 1781-7.
Richards, A. M., M. G. Nicholls, et al. (2003). "B-type natriuretic peptides and ejection fraction for prognosis after myocardial infarction." Circulation **107**(22): 2786-92.
Richards, A. M., M. G. Nicholls, et al. (1998). "Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: new neurohormonal predictors of left ventricular function and prognosis after myocardial infarction." Circulation **97**(19): 1921-9.

Richards, A. M., M. G. Nicholls, et al. (1999). "Neuroendocrine prediction of left ventricular function and heart failure after acute myocardial infarction. The Christchurch Cardioendocrine Research Group." Heart **81**(2): 114-20.

Roberts, R., W. J. Rogers, et al. (1991). "Immediate versus deferred beta-blockade following thrombolytic therapy in patients with acute myocardial infarction. Results of the Thrombolysis in Myocardial Infarction (TIMI) II-B Study." Circulation **83**(2): 422-37.

Rodriguez-Pla, A., J. A. Bosch-Gil, et al. (2005). "Metalloproteinase-2 and -9 in giant cell arteritis: involvement in vascular remodeling." Circulation **112**(2): 264-9.

Rohde, L. E., A. Ducharme, et al. (1999). "Matrix metalloproteinase inhibition attenuates early left ventricular enlargement after experimental myocardial infarction in mice." Circulation **99**(23): 3063-70.

Romanic, A. M., C. L. Burns-Kurtis, et al. (2001). "Matrix metalloproteinase expression in cardiac myocytes following myocardial infarction in the rabbit." Life Sci **68**(7): 799-814.

Ross, R. and J. A. Glomset (1973). "Atherosclerosis and the arterial smooth muscle cell: Proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis." Science **180**(93): 1332-9.

Roten, L., S. Nemoto, et al. (2000). "Effects of gene deletion of the tissue inhibitor of the matrix metalloproteinase-type 1 (TIMP-1) on left ventricular geometry and function in mice." J Mol Cell Cardiol **32**(1): 109-20.

Rouis, M., C. Adamy, et al. (1999). "Adenovirus-mediated overexpression of tissue inhibitor of metalloproteinase-1 reduces atherosclerotic lesions in apolipoprotein E-deficient mice." Circulation **100**(5): 533-40.

Rouleau, J. L., J. de Champlain, et al. (1993). "Activation of neurohumoral systems in postinfarction left ventricular dysfunction." J Am Coll Cardiol **22**(2): 390-8.

Rouy, D., I. Ernens, et al. (2005). "Plasma storage at -80 degrees C does not protect matrix metalloproteinase-9 from degradation." Anal Biochem **338**(2): 294-8.

Rundhaug, J. E. (2005). "Matrix metalloproteinases and angiogenesis." J Cell Mol Med **9**(2): 267-85.

Sabatine, M. S., C. P. Cannon, et al. (2005). "Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation." N Engl J Med **352**(12): 1179-89.

Sadoshima, J., L. Jahn, et al. (1992). "Molecular characterization of the stretch-induced adaptation of cultured cardiac cells. An in vitro model of load-induced cardiac hypertrophy." J Biol Chem **267**(15): 10551-60.

Saglam, M., O. Karakaya, et al. (2006). "Contribution of plasma matrix metalloproteinases to development of left ventricular hypertrophy and diastolic dysfunction in hypertensive subjects." Tohoku J Exp Med **208**(2): 117-22.

Sakata, Y., K. Yamamoto, et al. (2004). "Activation of matrix metalloproteinases precedes left ventricular remodeling in hypertensive heart failure rats: its inhibition as a primary effect of Angiotensin-converting enzyme inhibitor." Circulation **109**(17): 2143-9.

Salmela, M. T., S. L. Pender, et al. (2004). "Collagenase-1 (MMP-1), matrilysin-1 (MMP-7), and stromelysin-2 (MMP-10) are expressed by migrating enterocytes during intestinal wound healing." Scand J Gastroenterol **39**(11): 1095-104.

Samnegard, A., A. Silveira, et al. (2006). "Lower serum concentration of matrix metalloproteinase-3 in the acute stage of myocardial infarction." J Intern Med **259**(5): 530-6.
Sampson, M., S. Wall, et al. (2006). "Monocyte matrix and ADAM metalloproteinase expression in type 2 diabetes after aspirin therapy." Diabetes Res Clin Pract **71**(1): 45-51.
Sanchez-Lopez, R., C. M. Alexander, et al. (1993). "Role of zinc-binding- and hemopexin domain-encoded sequences in the substrate specificity of collagenase and stromelysin-2 as revealed by chimeric proteins." J Biol Chem **268**(10): 7238-47.
Sawicki, G., E. Salas, et al. (1997). "Release of gelatinase A during platelet activation mediates aggregation." Nature **386**(6625): 616-9.
Sawicki, G., E. J. Sanders, et al. (1998). "Localization and translocation of MMP-2 during aggregation of human platelets." Thromb Haemost **80**(5): 836-9.
Schaffer, C. J. and L. B. Nanney (1996). "Cell biology of wound healing." Int Rev Cytol **169**: 151-81.
Schieffer, B., C. Bunte, et al. (2004). "Comparative effects of AT1-antagonism and angiotensin-converting enzyme inhibition on markers of inflammation and platelet aggregation in patients with coronary artery disease." J Am Coll Cardiol **44**(2): 362-8.
Schiller, N. B., P. M. Shah, et al. (1989). "Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms." J Am Soc Echocardiogr **2**(5): 358-67.
Schmidt, R., A. Bultmann, et al. (2006). "Extracellular matrix metalloproteinase inducer regulates matrix metalloproteinase activity in cardiovascular cells: implications in acute myocardial infarction." Circulation **113**(6): 834-41.
Schonbeck, U., F. Mach, et al. (1999). "Expression of stromelysin-3 in atherosclerotic lesions: regulation via CD40-CD40 ligand signaling in vitro and in vivo." J Exp Med **189**(5): 843-53.
Schrier, R. W. and W. T. Abraham (1999). "Hormones and hemodynamics in heart failure." N Engl J Med **341**(8): 577-85.
Schwartzkopff, B., M. Fassbach, et al. (2002). "Elevated serum markers of collagen degradation in patients with mild to moderate dilated cardiomyopathy." Eur J Heart Fail **4**(4): 439-4.
Scott-Burden, T. (1994). Extracellular matrix: the cellular environment, NiPS: 110-5.
Seltzer, J. L., K. T. Akers, et al. (1990). "Cleavage specificity of human skin type IV collagenase (gelatinase). Identification of cleavage sites in type I gelatin, with confirmation using synthetic peptides." J Biol Chem **265**(33): 20409-13.
Seltzer, J. L., A. Z. Eisen, et al. (1989). "Cleavage of type VII collagen by interstitial collagenase and type IV collagenase (gelatinase) derived from human skin." J Biol Chem **264**(7): 3822-6.
Senior, R. M., G. L. Griffin, et al. (1991). "Human 92- and 72-kilodalton type IV collagenases are elastases." J Biol Chem **266**(12): 7870-5.
Senzaki, H., S. Masutani, et al. (2001). "Circulating matrix metalloproteinases and their inhibitors in patients with Kawasaki disease." Circulation **104**(8): 860-3.
Sewdarsen, M., S. Vythilingum, et al. (1989). "Prognostic importance of admission plasma glucose in diabetic and non-diabetic patients with acute myocardial infarction." Q J Med **71**(265): 461-6.

Shapiro, S. D. (2002). "Proteinases in chronic obstructive pulmonary disease." Biochem Soc Trans **30**(2): 98-102.
Shapiro, S. D., D. K. Kobayashi, et al. (1993). "Cloning and characterization of a unique elastolytic metalloproteinase produced by human alveolar macrophages." J Biol Chem **268**(32): 23824-9.
Shiau, M. Y., S. T. Tsai, et al. (2006). "Increased circulatory MMP-2 and MMP-9 levels and activities in patients with type 1 diabetes mellitus." Mt Sinai J Med **73**(7): 1024-8.
Silence, J., D. Collen, et al. (2002). "Reduced atherosclerotic plaque but enhanced aneurysm formation in mice with inactivation of the tissue inhibitor of metalloproteinase-1 (TIMP-1) gene." Circ Res **90**(8): 897-903.
Silence, J., F. Lupu, et al. (2001). "Persistence of atherosclerotic plaque but reduced aneurysm formation in mice with stromelysin-1 (MMP-3) gene inactivation." Arterioscler Thromb Vasc Biol **21**(9): 1440-5.
Smith, M. F., E. W. McIntush, et al. (1999). "Regulation of ovarian extracellular matrix remodelling by metalloproteinases and their tissue inhibitors: effects on follicular development, ovulation and luteal function." J Reprod Fertil Suppl **54**: 367-81.
Sobue, T., Y. Hakeda, et al. (2001). "Tissue inhibitor of metalloproteinases 1 and 2 directly stimulate the bone-resorbing activity of isolated mature osteoclasts." J Bone Miner Res **16**(12): 2205-14.
Soejima, H., H. Ogawa, et al. (2003). "Increased serum matrix metalloproteinase-1 concentration predicts advanced left ventricular remodeling in patients with acute myocardial infarction." Circ J **67**(4): 301-4.
SOLVD (1991). "Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. The SOLVD Investigators." N Engl J Med **325**(5): 293-302.
Sopata, I. and A. M. Dancewicz (1974). "Presence of a gelatin-specific proteinase and its latent form in human leucocytes." Biochim Biophys Acta **370**(2): 510-23.
Sorsa, T., L. Tjaderhane, et al. (2004). "Matrix metalloproteinases (MMPs) in oral diseases." Oral Dis **10**(6): 311-8.
Spencer, F. A., T. E. Meyer, et al. (1999). "Twenty year trends (1975-1995) in the incidence, in-hospital and long-term death rates associated with heart failure complicating acute myocardial infarction: a community-wide perspective." J Am Coll Cardiol **34**(5): 1378-87.
Spinale, F. G., M. L. Coker, et al. (2000). "A matrix metalloproteinase induction/activation system exists in the human left ventricular myocardium and is upregulated in heart failure." Circulation **102**(16): 1944-9.
Spinale, F. G., M. L. Coker, et al. (1999). "Matrix metalloproteinase inhibition during the development of congestive heart failure : effects on left ventricular dimensions and function." Circ Res **85**(4): 364-76.
Spinale, F. G., G. P. Escobar, et al. (2006). "Chronic matrix metalloproteinase inhibition following myocardial infarction in mice: differential effects on short and long-term survival." J Pharmacol Exp Ther **318**(3): 966-73.
Squire, I. B., J. Evans, et al. (2004). "Plasma MMP-9 and MMP-2 following acute myocardial infarction in man: correlation with echocardiographic and neurohumoral parameters of left ventricular dysfunction." J Card Fail **10**(4): 328-33.
Squire, I. B., R. J. O'Brien, et al. (2004). "N-terminal pro-atrial natriuretic peptide (N-ANP) and N-terminal pro-B-type natriuretic peptide (N-BNP) in the prediction

of death and heart failure in unselected patients following acute myocardial infarction." Clin Sci (Lond) **107**(3): 309-16.
Squire, I. B., S. Orn, et al. (2005). "Plasma natriuretic peptides up to 2 years after acute myocardial infarction and relation to prognosis: an OPTIMAAL substudy." J Card Fail **11**(7): 492-7.
St John Sutton, M., M. A. Pfeffer, et al. (1994). "Quantitative two-dimensional echocardiographic measurements are major predictors of adverse cardiovascular events after acute myocardial infarction. The protective effects of captopril." Circulation **89**(1): 68-75.
Steinberg, D., S. Parthasarathy, et al. (1989). "Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity." N Engl J Med **320**(14): 915-24.
Sternlicht, M. D. and Z. Werb (2001). "How matrix metalloproteinases regulate cell behavior." Annu Rev Cell Dev Biol **17**: 463-516.
Stetler-Stevenson, W. G. (1999). "Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention." J Clin Invest **103**(9): 1237-41.
Stetler-Stevenson, W. G., N. Bersch, et al. (1992). "Tissue inhibitor of metalloproteinase-2 (TIMP-2) has erythroid-potentiating activity." FEBS Lett **296**(2): 231-4.
Stricklin, G. P., H. G. Welgus, et al. (1982). "Human skin collagenase in recessive dystrophic epidermolysis bullosa. Purification of a mutant enzyme from fibroblast cultures." J Clin Invest **69**(6): 1373-83.
Strongin, A. Y., I. Collier, et al. (1995). "Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease." J Biol Chem **270**(10): 5331-8.
Sudoh, T., K. Kangawa, et al. (1988). "A new natriuretic peptide in porcine brain." Nature **332**(6159): 78-81.
Sun, M., F. Dawood, et al. (2004). "Excessive tumor necrosis factor activation after infarction contributes to susceptibility of myocardial rupture and left ventricular dysfunction." Circulation **110**(20): 3221-8.
Sun, T., L. Wang, et al. (2006). "Prognostic value of B-type natriuretic peptide in patients with acute coronary syndromes." Arch Med Res **37**(4): 502-5.
Sundstrom, J., J. C. Evans, et al. (2004). "Relations of plasma matrix metalloproteinase-9 to clinical cardiovascular risk factors and echocardiographic left ventricular measures: the Framingham Heart Study." Circulation **109**(23): 2850-6.
Sundstrom, J., J. C. Evans, et al. (2004). "Relations of plasma total TIMP-1 levels to cardiovascular risk factors and echocardiographic measures: the Framingham heart study." Eur Heart J **25**(17): 1509-16.
Sutton, M. G. and N. Sharpe (2000). "Left ventricular remodeling after myocardial infarction: pathophysiology and therapy." Circulation **101**(25): 2981-8.
Svensson, A. M., D. K. McGuire, et al. (2005). "Association between hyper- and hypoglycaemia and 2 year all-cause mortality risk in diabetic patients with acute coronary events." Eur Heart J **26**(13): 1255-61.
Tao, Z. Y., M. A. Cavasin, et al. (2004). "Temporal changes in matrix metalloproteinase expression and inflammatory response associated with cardiac rupture after myocardial infarction in mice." Life Sci **74**(12): 1561-72.
Tateishi, J., M. Masutani, et al. (2000). "Transient increase in plasma brain (B-type) natriuretic peptide after percutaneous transluminal coronary angioplasty." Clin Cardiol **23**(10): 776-80.

Tayebjee, M. H., H. S. Lim, et al. (2004). "Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 and -2 in type 2 diabetes: effect of 1 year's cardiovascular risk reduction therapy." Diabetes Care **27**(8): 2049-51.

ten Wolde, M., Tulevski, II, et al. (2003). "Brain natriuretic peptide as a predictor of adverse outcome in patients with pulmonary embolism." Circulation **107**(16): 2082-4.

Terashima, M., H. Akita, et al. (1999). "Stromelysin promoter 5A/6A polymorphism is associated with acute myocardial infarction." Circulation **99**(21): 2717-9.

Thomas, C. V., M. L. Coker, et al. (1998). "Increased matrix metalloproteinase activity and selective upregulation in LV myocardium from patients with end-stage dilated cardiomyopathy." Circulation **97**(17): 1708-15.

Timms, P. M., A. Wright, et al. (2002). "Plasma tissue inhibitor of metalloproteinase-1 levels are elevated in essential hypertension and related to left ventricular hypertrophy." Am J Hypertens **15**(3): 269-72.

Torre-Amione, G., S. Kapadia, et al. (1996). "Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD)." J Am Coll Cardiol **27**(5): 1201-6.

Tsutamoto, T., A. Wada, et al. (1997). "Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure: prognostic role of plasma brain natriuretic peptide concentration in patients with chronic symptomatic left ventricular dysfunction." Circulation **96**(2): 509-16.

Tulevski, II, M. T. Wolde, et al. (2006). "Combined utility of brain natriuretic peptide and cardiac troponine T may improve rapid triage and risk stratification in normotensive patients with pulmonary embolism." Int J Cardiol.

Tziakas, D. N., G. K. Chalikias, et al. (2005). "N-terminal pro-B-type natriuretic peptide and matrix metalloproteinases in early and late left ventricular remodeling after acute myocardial infarction." Am J Cardiol **96**(1): 31-4.

Tziakas, D. N., G. K. Chalikias, et al. (2005). "Comparison of levels of matrix metalloproteinase-2 and -3 in patients with ischemic cardiomyopathy versus nonischemic cardiomyopathy." Am J Cardiol **96**(10): 1449-51.

Unal, B., J. A. Critchley, et al. (2004). "Explaining the decline in coronary heart disease mortality in England and Wales between 1981 and 2000." Circulation **109**(9): 1101-7.

Vaccarino, V., L. Parsons, et al. (2000). "Impact of history of diabetes mellitus on hospital mortality in men and women with first acute myocardial infarction. The National Registry of Myocardial Infarction 2 Participants." Am J Cardiol **85**(12): 1486-9; A7.

Van de Werf, F., C. P. Cannon, et al. (1999). "Safety assessment of single-bolus administration of TNK tissue-plasminogen activator in acute myocardial infarction: the ASSENT-1 trial. The ASSENT-1 Investigators." Am Heart J **137**(5): 786-91.

Van Wart, H. E. and H. Birkedal-Hansen (1990). "The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family." Proc Natl Acad Sci U S A **87**(14): 5578-82.

Vasku, A., M. Goldbergova, et al. (2003). "Two MMP-2 promoter polymorphisms (-790T/G and -735C/T) in chronic heart failure." Clin Chem Lab Med **41**(10): 1299-303.

Volmink, J. A., J. N. Newton, et al. (1998). "Coronary event and case fatality rates in an English population: results of the Oxford myocardial infarction incidence study. The Oxford Myocardial Infarction Incidence Study Group." Heart **80**(1): 40-4.

Volpi, A., C. De Vita, et al. (1993). "Determinants of 6-month mortality in survivors of myocardial infarction after thrombolysis. Results of the GISSI-2 data base. The Ad hoc Working Group of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-2 Data Base." Circulation **88**(2): 416-29.

Wackenfors, A., M. Emilson, et al. (2004). "Ischemic heart disease induces upregulation of endothelin receptor mRNA in human coronary arteries." Eur J Pharmacol **484**(1): 103-9.

Wagner, D. R., C. Delagardelle, et al. (2006). "Matrix metalloproteinase-9 is a marker of heart failure after acute myocardial infarction." J Card Fail **12**(1): 66-72.

Wahab, N. N., E. A. Cowden, et al. (2002). "Is blood glucose an independent predictor of mortality in acute myocardial infarction in the thrombolytic era?" J Am Coll Cardiol **40**(10): 1748-54.

Walther, T., A. Schubert, et al. (2001). "Regression of left ventricular hypertrophy after surgical therapy for aortic stenosis is associated with changes in extracellular matrix gene expression." Circulation **104**(12 Suppl 1): I54-8.

Wang, J., D. Warzecha, et al. (2001). "Polymorphism in the gelatinase B gene and the severity of coronary arterial stenosis." Clin Sci (Lond) **101**(1): 87-92.

Wang, S., S. R. Lee, et al. (2006). "Reduction of tissue plasminogen activator-induced matrix metalloproteinase-9 by simvastatin in astrocytes." Stroke **37**(7): 1910-2.

Warren, S. E., H. D. Royal, et al. (1988). "Time course of left ventricular dilation after myocardial infarction: influence of infarct-related artery and success of coronary thrombolysis." J Am Coll Cardiol **11**(1): 12-9.

Webb, C. S., D. D. Bonnema, et al. (2006). "Specific temporal profile of matrix metalloproteinase release occurs in patients after myocardial infarction: relation to left ventricular remodeling." Circulation **114**(10): 1020-7.

Webb, K. E., A. M. Henney, et al. (1997). "Expression of matrix metalloproteinases and their inhibitor TIMP-1 in the rat carotid artery after balloon injury." Arterioscler Thromb Vasc Biol **17**(9): 1837-44.

Welgus, H. G., J. J. Jeffrey, et al. (1981). "The collagen substrate specificity of human skin fibroblast collagenase." J Biol Chem **256**(18): 9511-5.

Werb, Z. and S. Gordon (1975). "Elastase secretion by stimulated macrophages. Characterization and regulation." J Exp Med **142**(2): 361-77.

Westermann, D., S. Rutschow, et al. (2007). "Contributions of inflammation and cardiac matrix metalloproteinase activity to cardiac failure in diabetic cardiomyopathy: the role of Angiotensin type 1 receptor antagonism." Diabetes **56**(3): 641-6.

White, H. D., R. M. Norris, et al. (1987). "Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction." Circulation **76**(1): 44-51.

Wilson, W. R., M. Anderton, et al. (2006). "Matrix metalloproteinase-8 and -9 are increased at the site of abdominal aortic aneurysm rupture." Circulation **113**(3): 438-45.

Woessner, J. F., Jr. (1991). "Matrix metalloproteinases and their inhibitors in connective tissue remodeling." Faseb J **5**(8): 2145-54.

Wu, T. C., H. B. Leu, et al. (2005). "Plasma matrix metalloproteinase-3 level is an independent prognostic factor in stable coronary artery disease." Eur J Clin Invest **35**(9): 537-45.

Xu, X. P., S. R. Meisel, et al. (1999). "Oxidized low-density lipoprotein regulates matrix metalloproteinase-9 and its tissue inhibitor in human monocyte-derived macrophages." Circulation **99**(8): 993-8.

Yamazaki, T., I. Komuro, et al. (1995). "Angiotensin II partly mediates mechanical stress-induced cardiac hypertrophy." Circ Res **77**(2): 258-65.

Yarbrough, W. M., R. Mukherjee, et al. (2003). "Selective targeting and timing of matrix metalloproteinase inhibition in post-myocardial infarction remodeling." Circulation **108**(14): 1753-9.

Yasuda, S., S. Miyazaki, et al. (2007). "Enhanced cardiac production of matrix metalloproteinase-2 and -9 and its attenuation associated with pravastatin treatment in patients with acute myocardial infarction." Clin Sci (Lond) **112**(1): 43-9.

Ye, S. (2000). "Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases." Matrix Biol **19**(7): 623-9.

Ye, S., P. Eriksson, et al. (1996). "Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression." J Biol Chem **271**(22): 13055-60.

Ye, S., C. R. Gale, et al. (2003). "Variation in the matrix metalloproteinase-1 gene and risk of coronary heart disease." Eur Heart J **24**(18): 1668-71.

Ye, S., G. F. Watts, et al. (1995). "Preliminary report: genetic variation in the human stromelysin promoter is associated with progression of coronary atherosclerosis." Br Heart J **73**(3): 209-15.

Young, M. E., P. McNulty, et al. (2002). "Adaptation and maladaptation of the heart in diabetes: Part II: potential mechanisms." Circulation **105**(15): 1861-70.

Yu, W. H., S. Yu, et al. (2000). "TIMP-3 binds to sulfated glycosaminoglycans of the extracellular matrix." J Biol Chem **275**(40): 31226-32.

Yusuf, S., F. Zhao, et al. (2001). "Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation." N Engl J Med **345**(7): 494-502.

Zahn, R. and J. Senges (2000). "The volume of primary angioplasty procedures and survival after acute myocardial infarction." N Engl J Med **343**(14): 1045.

Zeller, M., B. Verges, et al. (2006). "Glycemia in acute coronary syndromes." Diabetes Metab **32 Spec No2**: 2S42-7.

Zhang, B., S. Ye, et al. (1999). "Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis." Circulation **99**(14): 1788-94.

Zhang, Y. and L. Wang (2006). "B-type natriuretic peptide is an independent predictor for cardiovascular death in patients with no clinical signs of ventricular dysfunction after acute myocardial infarction." Med Hypotheses **67**(5): 1076-8.

Chapter 11

Appendix

Appendix

Table 11.1 MMP-1 and patient demographics. Pearsons correlation coefficients (r-above) with significance values (p-below) between continuous variables and MMP-1 at the various time periods

Time period	0-12hrs	12-24	24-48	48-72	72-96	96+	Mean
Age	*0.153* *0.194*	*0.046* *0.692*	*0.075* *0.510*	*-0.046* *0.683*	*0.019* *0.888*	*-0.257* *0.623*	*0.052* *0.635*
Troponin	*-0.219* *0.102*	*-0.341* ***0.008***	*-0.205* *0.117*	*-0.221* *0.085*	*-0.151* *0.333*	*-0.60* ***0.040***	*-0.217* *0.086*
CK	*-0.068* *0.573*	*-0.015* *0.899*	*0.087* *0.460*	*0.100* *0.0395*	*0.042* *0.767*	*0.086* *0.872*	*0.069* *0.538*
Glucose	*0.194* *0.103*	*0.099* *0.394*	*0.177* *0.123*	*0.148* *0.195*	*0.083* *0.549*	*0.543* *0.266*	*0.199* *0.069*
Creatinine	*0.186* *0.113*	*0.125* *0.274*	*0.085* *0.454*	*0.132* *0.244*	*0.205* *0.130*	*0.60* *0.208*	*0.160* *0.141*
WCC	*0.013* *0.916*	*0.019* *0.867*	*0.091* *0.423*	*0.006* *0.956*	*0.166* *0.220*	*-0.771* *0.072*	*0.214* ***0.048***
Neurtophils	-0.066 0.575	-0.015 0.894	0.045 0.692	0.000 0.997	*0.128* *0.348*	-0.771 0.072	0.177 0.103

Table 11.2. Difference in MMP-1 levels between categorical variables in patient demographics.

Time	0-12hrs	12-24	24-48	48-72	72-96	96+	Mean
Sex (Male/Female)	285.3 / 212.8	293.1 / 253.3	245.5 / 388.9	334.6 / 243.8	365.0 / 376.9	91.5 / 215.3	371.4/271.3
Sig. (*p*)	0.27	0.689	0.235	0.581	0.573	0.643	0.961
Type of infarct (STEMI/NSTEMI)	222.7 / 463.9	253.3 / 341.4	271.8 / 383.4	315.6 / 467.6	392.1 / 315.5	83.6 / 257.2	320.9/451.1
Sig (p)	0.087	0.280	0.405	0.201	0.826	0.165	0.382
Territory (Anterior/Inferior)	176.1 / 352.0	163.7 / 344.8	219.3 / 362.7	249.7 / 385.8	265.9 / 407.4	116.6 / 100.8	252.8/426.5
Sig (p)	0.099	0.076	**0.036**	0.242	0.175	0.827	0.037
Thrombolysis (yes/no)	236.0 / 330.0	191.1 / 293.1	222.7 /352.6	315.6 / 385.6	392.1 / 310.1	83.6 / 223.2	320.9/349.7
Sig (p)	0.609	0.387	0.057	0.467	0.755	0.355	0.406
Current Smoker (yes/no)	261.5 / 291.9	327.5 / 233.6	341.9 / 266.7	388.5 / 328.1	457.1 / 339.6	116.6 / 66.4	415.3/319.7
Sig (p)	0.987	0.319	0.640	0.479	0.758	0.275	0.746
History of:							
Diabetes (yes/no)	534.5 / 220.6	391.7 / 243.5	309.9 / 263.1	342.7/ 328.3	390.1 / 363.9	232.1 / 108.7	516.8/315.8
Sig (p)	**0.05**	0.115	0.438	0.159	0.781	0.643	0.202
Angina (yes/no)	368.1 / 222.7	376.8 / 243.4	256.0 / 276.3	361.9 / 315.6	559.2 / 350.9	363.8 / 83.5	460.9/320.9
Sig (p)	0.303	0.286	0.918	0.613	0.557	0.064	0.390
Hypertension (yes/no)	278.7 / 249.4	191.1 / 278.7	266.7 / 282.1	240.6 / 335.4	462.2 / 365.0	65.0 / 223.2	330.8/347.1
Sig (p)	0.603	0.517	0.960	0.486	0.493	0.165	0.940
Previous MI (yes/no)	380.8 / 249.4	289.9 / 262.5	222.7 / 289.9	228.6 / 344.6	510.7 / 339.6	190.8 / 100.7	460.9/320.9
Sig (p)	0.359	0.869	0.431	0.255	0.623	0.143	0.925

Time	0-12hrs	12-24	24-48	48-72	72-96	96+	Mean
Antiplatelet Agent (yes/no)	434.1 / 236.0	396.6 / 243.4	383.4 / 263.5	388.5 / 302.8	684.5 / 350.9	190.8 / 116.6	371.4/322.8
Sig (p)	0.184	0.557	0.346	0.696	0.205	0.143	0.462
Beta Blocker (yes/no)	305.5 / 222.7	379.3 / 254.4	251.7 / 289.3	328.1 / 332.7	365.0 / 363.9	190.8 / 100.8	421.3/319.7
Sig (p)	0.522	0.892	0.758	0.872	0.880	0.143	0.917
ACE-I (yes/no)	342.6 / 261.6	786.9 / 253.3	496.2 / 259.9	440.0 / 328.3	861.4 / 328.4	190.8 / 100.8	451.1/320.9
Sig (p)	0.206	0.251	0.095	0.389	**0.05**	0.143	0.234
Statin (yes/no)	361.7 / 202.8	420.1 / 233.4	267.2 / 276.3	328.1 / 329.6	621.8 / 321.9	190.8 / 122.9	441.1/315.8
Sig (p)	0.081	0.063	0.380	0.924	0.097	0.143	0.353

Table 11.3. Correlation between MMP-1 and echocardiographic markers during admission[1] (above) and follow up[2] (below) for each time period

	0-12	12-24	24-48	48-72	72-96	96+	Mean
WMIS	.031 .799	-.031 .798	.024 .843	.074 .529	.076 .591	-.206 .695	.061 .592
LVEF	-.164 .189	-.030 .808	-.016 .896	-.117 .331	-.151 .305	-.498 .315	-.110 .342
LVIDd	-.100 .402	-.144 .216	-.126 .275	-.072 .530	.025 .858	.125 .814	-.116 .293
LVIDs	.111 .351	.034 .773	-.038 .745	-.018 .877	-.004 .975	-.217 .679	.034 .759
EDV	-.067 .593	-.180 .135	-.195 .103	-.146 .222	.115 .431	-.397 .436	-.126 .272
ESV	.015 .904	-.186 .127	-.106 .381	.007 .951	.182 .215	-.118 .823	-.015 .846
$WMIS^2$	.004 .976	-.024 .854	-.070 .587	.002 .985	.048 .761	.196 .804	-.043 .728
$LVEF^2$	-.129 .350	-.161 .219	-.116 .372	-.105 .416	-.021 .898	-.117 .883	-.089 .475
$LVIDd^2$	-.150 .275	-.132 .310	-.169 .190	-.105 .415	.027 .868	-.053 .947	-.117 .346
$LVIDs^2$	-.090 .512	-.096 .461	-.106 .412	-.093 .466	.009 .955	.117 .883	-.089 .476
EDV^2	-.099 .475	-.280 .032	-.131 .317	-.154 .236	.022 .896	-.763 .237	-.147 .243
ESV^2	.008 .956	-.109 .411	-.036 .786	-.030 .817	.027 .871	-.570 .430	-.056 .659
ΔEDV	.064 .659	-.094 .495	.086 .529	-.070 .605	-.087 .614	-.987 .013	-.016 .904
ΔESV	.109 .452	.042 .760	.108 .426	-.057 .674	-.113 .511	-.689 .311	-.011 .935

Table 11.4. MMP-2 and patient demographics. Pearsons correlation coefficients (r-above) with significance values (p-below) between continuous variables and MMP-2 at the various time periods

Time period	0-12hrs	12-24	24-48	48-72	72-96	96+	Mean
Age	**0.335** **0.006**	0.091 0.518	**0.309** **0.003**	**0.271** **0.009**	**0.332** **0.002**	-0.01 0.954	.211 **.044**
Troponin	0.196 0.177	-0.037 0.820	-0.123 0.316	-0.044 0.719	-0.013 0.919	0.022 0.907	-.012 .921
CK	**-0.318** **0.012**	-0.319 0.021	**-0.325** **0.002**	**-0.245** **0.023**	-0.212 0.057	-0.006 0.975	-.280 **.009**
Glucose	-0.057 0.651	-0.137 0.334	0.001 0.995	-0.031 0.770	-0.095 0.388	0.067 0.696	-.080 .457
Creatinine	-0.045 0.718	0.018 0.899	-0.060 0.569	-0.031 0.774	-0.061 0.577	0.021 0.897	-.089 .400
WCC	**-0.245** **0.048**	-0.087 0.536	**-0.340** **0.001**	**-0.261** **0.013**	-0.184 0.089	-0.024 0.883	-.172 .102
Neutrophils	-0.201 0.106	-0.064 0.648	**-0.272** **0.009**	-0.199 0.059	-0.110 0.315	0.019 0.908	-.104 .325

Table11.5. Difference in MMP-2 levels between categorical variables in patient demographics

Time	0-12hrs	12-24	24-48	48-72	72-96	96+	Mean
Sex (Male/Female)	19.6 / 28.7	20.5 / 21.7	17.7 / 20.1	18.6 / 20.4	20.0 / 21.0	21.0 / 24.3	19.8/21.9
Sig. (*p*)	**0.032**	0.906	0.199	0.233	0.433	0.364	0.197
Type of infarct (STEMI/NSTEMI)	21.9 / 20.3	20.8 / 21.5	17.7 / 20.1	18.1 / 23.5	19.3 / 25.3	20.5 / 23.8	19.8/23.3
Sig (p)	0.583	0.962	**0.034**	**0.001**	**0.002**	0.380	**0.04**
Territory (Anterior/Inferior)	24.3 / 19.0	25.1 / 17.6	21.1 / 17.1	21.7 / 17.4	24.0 / 19.0	25.0 / 20.1	24.1/18.5
Sig (p)	**0.02**	0.176	**0.007**	**0.008**	**0.07**	0.381	**0.006**
Thrombolysis (yes/no)	22.5 / 18.6	21.9 / 18.0	18.7 / 18.3	18.7 / 20.2	19.1 / 21.2	20.8 / 22.1	20.9/19.1
Sig (p)	0.127	0.199	0.631	0.240	0.128	0.324	0.872
Current Smoker (yes/no)	19.6 / 22.6	20.7 / 21.1	17.6 / 19.1	18.8 / 19.5	20.2 / 20.1	22.1 / 21.0	20.5/20.7
Sig (p)	0.072	0.493	0.351	0.455	0.327	0.366	0.594
History of:							
Diabetes (yes/no)	18.2 / 21.9	15.6 / 21.8	17.6 / 18.8	18.0 / 19.6	18.5 / 20.6	22.8 / 21.0	17.9/21.1
Sig (p)	0.399	0.148	0.770	0.349	0.274	0.651	0.160
Angina (yes/no)	19.0 / 21.7	17.1/ 21.3	18.0 / 18.6	22.3 / 18.7	20.4 / 20.0	17.9 / 22.8	19.1/20.9
Sig (p)	0.744	0.343	0.797	0.333	0.711	0.072	0.521
Hypertension (yes/no)	21.4 / 22.5	21.1 / 20.7	18.4 / 18.6	18.6 / 19.6	19.8 / 20.4	25.9 / 19.2	20.8/20.5
Sig (p)	0.469	0.591	0.996	0.745	0.933	**0.07**	0.890
Previous MI (yes/no)	19.0 / 22.1	20.0 / 21.1	18.3 / 18.6	21.1 / 18.8	20.9 / 20.1	17.7 / 22.6	20.1/20.6
Sig (p)	0.365	0.633	0.621	0.428	1.0	0.228	0.695

Time	0-12hrs	12-24	24-48	48-72	72-96	96+	Mean
Antiplatelet Agent (yes/no)	22.1 / 21.2	26.2 / 19.9	18.6 / 18.4	19.6 / 19.0	20.4 / 20.0	23.6 / 21.0	19.8/20.7
Sig (p)	0.858	0.106	0.323	0.483	0.517	0.411	0.305
Beta Blocker (yes/no)	25.2 / 19.6	22.9 / 19.9	20.9 / 17.6	20.5 / 18.1	25.0 / 18.8	29.4 / 19.8	24.4/19.4
Sig (p)	**0.044**	0.364	**0.023**	**0.047**	**0.002**	**0.034**	**0.01**
ACE-I (yes/no)	15.7 / 22.1	15.4 / 21.5	17.2 / 18.9	16.6 / 20.0	19.2 / 20.5	23.1 / 21.0	17.7/21.7
Sig (p)	0.194	0.471	0.312	**0.045**	0.217	0.798	0.1
Statin (yes/no)	19.0	24.8	15.3 / 19.0	18.8 / 19.6	19.2 / 20.8	17.9 / 22.6	18.6/21.5
Sig (p)	0.431	0.516	0.123	0.668	0.338	0.151	0.323

Table 11.6. Correlation between MMP-2 and echocardiographic markers during admission[1] (above) and follow up[2] (below) for each time period – parametric data

	0-12	12-24	24-48	48-72	72-96	96+	Mean
WMIS	-.046 .724	.077 .603	.012 .917	.021 .851	.056 .626	-.042 .807	.041 .714
LVEF	.009 .947	.048 .755	.105 .350	.056 .615	.057 .621	.141 .400	.058 .602
LVIDd	.113 .375	.069 .628	.080 .457	.059 .580	.090 .417	-.121 .463	.086 .421
LVIDs	-.107 .398	-.120 .400	-.119 .266	-.169 .113	-.217 .048	-.122 .460	-.173 .105
EDV	-.031 .813	-.059 .696	-.042 .704	-.076 .494	-.057 .617	.143 .393	-.039 .725
ESV	-.064 .634	-.124 .411	-.103 .357	-.113 .311	-.097 .403	.090 .598	-.088 .430
$WMIS^2$	.247 .084	.148 .343	.094 .433	.186 .117	.196 .106	-.282 .139	.192 .106
$LVEF^2$	-.185 .208	-.178 .252	.018 .885	-.060 .619	-.047 .705	.370 .058	-.084 .487
$LVIDd^2$	.121 .409	.113 .471	-.025 .837	-.047 .700	-.069 .577	-.514 .005	-.033 .787
$LVIDs^2$	.135 .356	.122 .436	-.089 .458	-.070 .564	-.086 .486	-.493 .008	-.048 .693
EDV^2	.001 .993	.171 .274	-.068 .577	-.117 .338	-.113 .368	-.180 .378	-.049 .687
ESV^2	.035 .813	.217 .162	-.031 .800	-.072 .558	-.079 .530	-.326 .104	-.009 .941
ΔEDV	-.024 .878	.348 .030	-.069 .583	-.110 .382	-.060 641	-.192 .347	-.002 .984
ΔESV	.101 .512	.428 .007	.039 .756	-.021 .866	.014 913	-.296 .143	.097 .443

Table 11.7 MMP-9 and patient demographics. Pearsons correlation coefficients (r-above) with significance values (p-below) between continuous variables and MMP-9 at the various time periods

Time period	Peak	0-12hrs	12-24	24-48	48-72	72-96	96+	Trough
Age	-.100 .347	-.294 **.014**	-.017 .902	-.106 .318	-.030 .779	-.027 .806	-.089 .597	-.398 **<0.001**
Troponin	.065 .595	.044 .754	.098 .551	-.051 .676	.253 .036	-.149 .224	.021 .912	-.045 .712
CK	.198 .068	.127 .313	.200 .159	.027 .805	.019 .863	-.016 .889	-.095 .598	-.071 .518
Glucose	-.010 .926	.175 .153	-.121 .397	-.137 .202	-.190 .075	-.058 .600	-.046 .792	-.180 .092
Creatinine	-.163 .122	-.342 **.004**	-.028 .844	-.116 .275	.078 .462	-.100 .355	-.015 .930	-.201 .056
WCC	.362 **.001**	.368 **.002**	.434 **.001**	.147 .165	.212 **.044**	.284 .008	.350 **.031**	.332 **.001**
Neut	.362 **.001**	.298 **.013**	.403 **.003**	.145 .170	.215 **.041**	.269 **.012**	.298 .069	.285 **.006**

Table 11.8. Difference in MMP-9 levels between categorical variables in patient demographics

Time	Peak	0-12hrs	12-24	24-48	48-72	72-96	96+	Trough
Sex (Male/Female)	115.2/113.9	67.1 / 87.0	44.3 / 47.3	51.3 / 46.0	51.9/47.6	50.5 / 48.5	51.5 / 39.6	36.5/33.3
Sig. (*p*)	0.379	0.373	0.621	0.551	0.783	0.736	0.171	0.466
Type of infarct (STEMI/NSTEMI)	122.5/104.7	78.5 / 52.0	45.3 / 47.6	46.7 / 56.1	51.6 / 48.7	49.6 / 53.2	46.4 / 49.8	36.1/41.9
Sig (p)	0.422	0.326	0.980	0.741	0.468	0.793	0.440	0.471
Territory (Anterior/Inferior)	134.6/107.0	84.9 / 70.0	44.3 / 47.3	45.4 / 52.1	54.3 / 49.7	50.1 / 49.7	43.9 / 49.8	30.2/37.0
Sig (p)	0.135	0.877	0.524	0.074	0.911	0.819	0.619	0.153
Thrombolysis (yes/no)	111.1/124.9	84.0 / 57.0	45.7 / 45.8	45.9 / 55.3	48.4 / 53.3	50.7 / 49.1	46.7 / 49.5	34.5/37.4
Sig (p)	0.948	0.310	0.857	0.313	0.103	0.776	0.884	0.363
Current Smoker (yes/no)	108.7/117.2	99.0 / 57.0	45.3 / 46.4	53.8 / 46.9	49.0 / 51.4	51.8 / 48.8	70.5 / 47.5	41.3/30.1
Sig (p)	0.704	**0.018**	0.767	0.087	0.765	0.441	0.237	**<0.001**
History of:								
Diabetes (yes/no)	110.0/122.2	80.0 / 70.5	47.3 / 43.1	47.1 / 51.6	48.3 / 51.1	59.8 / 48.5	40.8 / 49.3	36.4/36.3
Sig (p)	0.807	0.806	0.441	0.488	0.423	0.107	0.775	0.632
Angina (yes/no)	133.8/111.1	70.6 / 71.0	47.1 / 45.7	56.8 / 48.7	49.7 / 50.9	58.1 / 49.6	48.7 / 49.8	34.1/36.5
Sig (p)	0.898	0.443	0.952	0.740	0.677	0.762	0.668	0.299
Hypertension (yes/no)	123.7/108.7	58.0 / 81.0	40.0 / 48.0	46.3 / 55.3	51.7 / 49.7	48.2 / 51.8	47.7 / 49.0	30.1/40.3
Sig (p)	0.537	0.206	**0.034**	0.151	0.890	0.409	0.375	**0.002**
Previous MI (yes/no)	99.1/120.4	77.0 / 70.5	49.5 / 45.3	57.3 / 48.4	38.9 / 51.6	43.4 / 50.3	59.3 / 48.2	34.4/36.6
Sig (p)	0.960	0.874	0.475	0.839	0.316	0.585	0.356	0.335

Time	Peak	0-12hrs	12-24	24-48	48-72	72-96	96+	Trough
Antiplatelet Agent (yes/no)	154.3/108.8	61.0 / 78.5	45.6 / 45.8	56.8 / 47.8	53.7 / 48.5	53.7 / 48.5	59.5 / 42.4	36.7/34.7
Sig (p)	0.498	0.427	0.939	0.486	0.215	0.215	0.109	0.477
Beta Blocker (yes/no)	117.5/117.2	60.6 / 74.0	40.3 / 46.4	33.7 / 54.4	45.5 / 50.9	47.5 / 50.7	50.1 / 48.2	28.5/38.4
Sig (p)	0.574	0.160	0.190	**0.008**	0.317	0.326	0.947	**0.002**
ACE-I (yes/no)	133.7/112.1	60.0 / 80.0	49.0 / 44.6	39.5 / 52.2	55.3 / 49.7	58.1 / 48.9	43.9 / 49.2	34.1/36.4
Sig (p)	0.868	0.516	0.594	0.169	0.876	0.334	0.865	0.407
Statin (yes/no)	110.0/120.4	80.0 / 67.1	64.5 / 43.9	57.4 / 48.4	49.7 / 51.2	57.2 / 50.1	43.9 / 49.8	34.7/36.4
Sig (p)	0.742	0.867	0.033	0.657	0.420	0.605	0.961	0.746

Table 11.9 Correlation between MMP-9 and echocardiographic markers during admission[1] (above) and follow up[2] (below) for each time period

	Peak	0-12	12-24	24.48	48-72	72-96	96+	Trough
WMIS	.217 .048	.058 .647	.251 .089	-.033 .765	.127 .251	-.028 .806	.146 .402	-.122 .158
LVEF	-.316 .004	-.196 .130	-.237 .121	-.112 .316	-.077 .491	-.051 .656	.099 .558	.002 .984
LVIDd	.148 .166	-.052 .679	.084 .562	.134 .211	.127 .236	.022 .841	-.043 .797	.040 .711
LVIDs	.155 .148	.019 .877	.060 .681	.029 .785	.197 .064	.178 .104	.088 .601	.040 .711
EDV	-.051 .648	-.060 .645	-.037 .809	.067 .549	.129 .246	.098 .389	.202 .230	.106 .341
ESV	.087 .438	-.011 .935	.077 .617	.002 .988	.222 .045	.065 .572	.116 .501	.116 .298
WMIS2	.186 .117	-.061 .666	.179 .251	-.041 .733	-.058 .631	.099 .414	-.023 .906	-.230 .032
LVEF2	-.131 .281	.049 .736	-.189 .231	.044 .721	.030 .806	.036 .772	.177 .376	.290 .015
LVIDd2	.067 .577	-.234 .098	.140 .375	.043 .724	.039 .746	.110 .370	.178 .366	-.109 .365
LVIDs2	.056 .640	-.101 .482	.229 .145	-.046 .705	-.010 .936	.071 .560	.022 .911	-.146 .224
EDV	.149 .221	.022 .879	.117 .460	.022 .860	.205 .091	.085 .493	-.069 .737	.158 .195
ESV	.137 .260	-.057 .695	.101 .525	.005 .966	.096 .434	.022 .857	-.162 .428	-.38 .008
ΔEDV	.30 .016	.295 .047	.106 .528	.041 .746	.014 .911	-.063 .623	-.176 .388	-.280 .024
ΔESV	.123 .331	.078 .608	-.005 .978	-.012 .927	-.094 .454	-.138 .280	-.267 .187	-.40 .001

Table 11.10 MMP-3 and patient demographics. Pearsons correlation coefficients (r-above) with significance values (p-below) between continuous variables and MMP-3 at the various time periods

Time period	0-12hrs	12-24	24-48	48-72	72-96	96+	Mean
Age	.220 .049	.159 .151	.186 .103	.232 .052	.160 .210	.149 .750	.220 .043
Troponin	-.180 .165	-.157 .223	-.175 .186	-.046 .747	.074 .617	-.526 .362	-.010 .937
CK	.108 .354	.093 .416	.123 .297	.208 .089	.260 .049	-.575 .177	.196 .082
Glucose	.114 .318	.154 .171	.175 .130	.104 .396	.028 .833	.445 .317	.164 .139
Creatinine	.441 .000	.401 .000	.422 .000	.498 .000	.373 .003	.181 .698	.498 .000
WCC	.012 .916	.046 .682	.071 .537	.111 .355	.235 .064	-.006 .989	.067 .540
Neutrophils	.026 .820	.081 .466	.143 .212	.183 .126	.251 .047	-.012 .979	.098 .371

Table 11.11. Difference in MMP-3 levels between categorical variables in patient demographics

Time	0-12hrs	12-24	24-48	48-72	72-96	96+	Mean
Sex (Male/Female)	3086.0 / 2567.4	2965.1 / 3144.7	3826.9 / 3855.3	4033.1 / 3254.3	4294.9 / 4985.8	2792.0 / 3867.5	4256.1/3234.8
Sig. (*p*)	0.477	0.910	0.798	0.227	0.891	0.699	0.311
Type of infarct (STEMI/NSTEMI)	2639.5 / 5204.1	2696.9 / 5683.9	3548.8 / 8037.1	3174.6 / 6868.3	4294.9 / 4944.3	1948.9 / 3905.8	3731.0/6145.9
Sig (p)	0.057	0.157	0.41	0.098	0.421	0.157	0.150
Territory (Anterior/Inferior)	2582.3 / 3084.2	2497.1 / 3160.9	3595.2 / 3974.9	3882.5 / 3094.9	4501.6 / 4294.9	10640.2 / 2260.5	4187.4/3818.9
Sig (p)	0.222	0.222	0.855	0.769	0.953	0.053	0.700
Thrombolysis (yes/no)	2608.7 / 3372.6	2431.7 / 3848.2	3361.5 / 4219.8	3407.4 / 3553.5	4228.1 / 4744.2	2260.5 / 5063.1	4179.7/4380.1
Sig (p)	0.370	0.175	0.171	0.406	0.517	0.157	0.391
Current Smoker (yes/no)	2547.0 / 3295.9	2448.6 / 3848.2	2616.4 / 4339.0	2398.8 / 4646.9	2614.7 / 5227.3	3348.9 / 226-.5	2609.9/4904.1
Sig (p)	0.299	0.134	0.133	0.064	0.149	0.724	**0.019**
History of:							
Diabetes (yes/no)	3086.0 / 3726.7	3444.0/ 2696.9	3891.3 / 3826.9	3801.0 / 3424.3	2363.8 / 4744.2	2526.3 / 3905.8	4540.0/4183.5
Sig (p)	0.403	0.523	0.775	0.840	0.172	0.699	0.544
Angina (yes/no)	4388.9 / 2738.2	4091.8 / 2859.3	4666.7 / 3641.5	4560.6 / 3390.5	4893.2 / 4294.9	4506.2 / 2260.5	6332.9/4040.9
Sig (p)	0.431	0.256	0.194	0.394	0.540	0.439	0.226
Hypertension (yes/no)	4641.9 / 2412.6	4457.9 / 2451.4	4946.8 / 3020.7	4703.4 / 2729	5379.9 / 3905.8	1637.3 / 5063.1	6054.9/2703.9
Sig (p)	**0.05**	**0.015**	**0.049**	0.174	0.671	0.034	**0.017**
Previous MI (yes/no)	6537.4 / 2738.2	6521.2 / 4082.7	5714.4 / 4955.1	4367.4 / 5254.7	5532.5 / 5987.2	N/A	5937.2/4176.0
Sig (p)	0.373	0.378	0.492	0.876	0.517		0.576

Time	0-12hrs	12-24	24-48	48-72	72-96	96+	Mean
Antiplatelet Agent (yes/no)	2742.9 / 3081.7	3536.3 / 2984.5	3747.9 / 3905.8	2759.7 / 3553.5	4437.6 / 4296.4	N/A	6970.4/4179.7
Sig (p)	0.291	0.335	0.320	0.527	0.850		0.259
Beta Blocker (yes/no)	4314.3 / 2738.2	3814.0 / 2859.3	4666.7 / 3654.0	4367.4 / 3094.9	4650.5 / 4295.6	N/A	7117.3/3731.0
Sig (p)	0.452	0.382	0.516	0.717	0.936		0.123
ACE-I (yes/no)	3565.5 / 2818.3	4091.8 / 2696.9	4503.3 / 3666.6	4753.8 / 3407.4	2531.6 / 4520.3	N/A	4904.1/3818.9
Sig (p)	0.355	0.256	0.587	0.611	0.316		0.254
Statin (yes/no)	2667.5 / 2818.3	3852.7 / 2753.5	2819.5 / 3826.9	3390.5 / 3424.3	4294.9 / 3390.5	N/A	2856.3/4183.5
Sig (p)	0.328	0.128	0.571	0.686	0.788`		0.511

Table11.12 Correlation between MMP-3 and echocardiographic markers during admission[1] (above) and follow up[2] (below) for each time period

	0-12	12-24	24-48	48-72	72-96	96+	Mean
WMIS	.014 .903	.119 .304	.172 .149	.121 .338	.240 .069	.403 .370	.137 .228
LVEF	.079 .512	-.020 .863	-.068 .575	-.055 .663	-.034 .806	.105 .842	-.088 .450
LVIDd	.034 .766	.137 .224	.125 .283	.038 .757	.161 .216	.559 .192	.080 .474
LVIDs	-.006 .955	.091 .418	.087 .453	.110 .368	.125 .336	.252 .586	.147 .184
EDV	-.035 .771	.056 .630	.054 .654	-.010 .940	.275 .041	.614 .195	.037 .751
ESV	-.110 .358	.028 .815	.059 .625	.046 .719	.139 .313	.274 .599	.098 .398
WMIS2	.113 .768	.164 .189	.172 .177	.112 .412	.179 .213	-.493 .261	.181 .139
LVEF2	-.010 .938	-.137 .282	-.124 .340	-.068 .625	-.087 .557	.747 .054	-.145 .245
LVIDd2	.009 .945	.161 .200	.087 .502	.001 .992	.090 .537	.196 .674	.131 .291
LVIDs2	-.063 .621	.097 .441	-.007 .957	-.058 .676	.024 .871	.126 .788	.101 .417
EDV	-.058 .655	.076 .555	-.027 .839	-.064 .647	.168 .260	.390 .388	.143 .255
ESV	-.050 .703	.093 .471	.000 .998	-.033 .812	.071 .634	-.271 .556	.127 .314
ΔEDV	.026 .848	.113 .392	-.024 .862	-.038 .792	-.008 .960	-.015 .977	.101 .439
ΔESV	-.011 .935	.089 .501	-.053 .695	-.065 .653	-.090 .563	-.669 .147	.010 .938

Appendix 11.13. Patient Information and consent form.
Printed on letter headed paper

Patient information Sheet

Study title: Plasma matrix metalloproteinases as predictors of prognosis and left ventricular remodelling after acute myocardial infarction

Principal Investigator: Dr Iain Squire, Senior Lecturer in Medicine, University of Leicester, and Consultant Physician, Leicester Royal Infirmary

Contact details: (0116) 252 3125

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, called Matrix Metalloproteinases, which are released after a 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 1 day during your hospital stay or daily for 7 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 2 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 and we will ask you to attend for a second echo scan of the heart 6 months later. The results of both echo scans will be sent to the doctors looking after you and will be a part of your medical records. The study will run for a total of 2 years. If you have a hospital admission during this period we would like to know about this and we would ask that you or your family inform us if this happens, no matter the reason for the admission. We may also wish to contact you by telephone to monitor your progress. You will not be expected to attend for extra clinic visits.

As the echo scan at 6 months is not part of standard care, we will offer to pay reasonable travel expenses for your trips to and from the hospital for this appointment. By this we mean the cost of public transport or taxi fare to and from home, or petrol expenses at 30 pence per mile.

5. What are the possible benefits of taking part?

The results of both echo scans will be sent to the doctors looking after you and will be a part of your medical records. This information may help guide your treatment. 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, unless you wish us not to do so.

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

The results of this research are likely to be published in 2007 in one or more medical journals. 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:

Dr Iain Squire
University of Leicester
Clinical Sciences Building
Leicester Royal Infirmary
Leicester
LE2 7LX
Tel: 0116 252 3125

You will be given a copy of the information sheet and a signed consent form to keep

Thank you for reading the above information

CONSENT FORM

Title of Project: Plasma matrix metalloproteinases as predictors of prognosis and left ventricular remodelling after acute myocardial infarction.

Name of Researcher: Dr I Squire please initial boxes

1. I confirm that I have read and understand the information sheet dated February 2004 (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. ☐

1. 3. I understand that sections of any of my medical notes may be looked at by responsible individuals from the Leicester Royal Infirmary or from regulatory authorities where it is relevant to my taking part in research. 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

Plasma matrix metalloproteianses as predictors of prognosis and left ventricular remodelling after acute myocardial infarction

Submitted for the degree of Doctorate of Medicine (MD)

Dr Dominic Kelly. BSC. MBChB. MRCP.

Correspondence:

Dr Dominic Kelly
White Gates

Waterworks Rd
Otterbourne
Hampshire
SO21 2DP

kelly.dominic@talk21.com

Word Count: 33, 398
No. References: 363