



Matrix Metalloproteinase-2 predicts mortality in patients with acute coronary syndrome.

Onkar S Dhillon, Sohail Q Khan, Hafid K Narayan, Kelvin H Ng, Noor Mohammed, Paulene A Quinn, Iain B Squire, Joan E Davies, L L Ng

► To cite this version:

Onkar S Dhillon, Sohail Q Khan, Hafid K Narayan, Kelvin H Ng, Noor Mohammed, et al.. Matrix Metalloproteinase-2 predicts mortality in patients with acute coronary syndrome.. Clinical Science, 2009, 118 (4), pp.249-257. <10.1042/CS20090226>. <hal-00534165>

HAL Id: hal-00534165

<https://hal.science/hal-00534165v1>

Submitted on 9 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Matrix Metalloproteinase-2 predicts mortality in patients with acute coronary syndrome.

Dhillon OS, Khan SQ, Narayan H, Ng KH, Mohammed N, Quinn PA, Squire IB, Davies JE, Ng LL

Department of Cardiovascular Sciences, Clinical Sciences Building, Leicester Royal Infirmary, Leicester LE2 7LX, UK

Corresponding author: Dr Onkar S Dhillon or Professor Leong L. Ng
Department of Cardiovascular Sciences
Clinical Sciences Building
Leicester Royal Infirmary
Leicester LE2 7LX, UK
Phone:+1162523132 ; fax:+1162523108;
e-mail:dhillonos@hotmail.com or llnl@le.ac.uk

Total Word count : Manuscript 3477 (6075 including references)

Keywords acute coronary syndrome; MMP-9; MMP-3; MMP-2; N-terminal B type natriuretic peptide; prognosis

ABSTRACT (Words:250)

Aims

To investigate the predictive value of MMP-2, -3 and -9 levels in patients with acute coronary syndrome for death, readmission with heart failure (HF) or recurrent myocardial infarction (MI) and compare them to established markers N-terminal pro-B-type natriuretic peptide (NTproBNP) and GRACE score.

Methods and Results

A single blood test was taken 4 days after admission in 1024 consecutive patients with AMI with end points observed over 519[134-1059](median[range]) days.

MMP-2 and MMP-3 were raised in patients who died (n=111) compared to survivors (p<0.006 and p=0.01 respectively) but similar in patients with HF (n=106) or MI (n=138). MMP-9 levels were similar across study end points. Using Cox proportional hazards modelling MMP-2 demonstrated independent prediction of death (HR 6.60 p=0.001) along with NTproBNP (HR 4.62 p<0.001) and GRACE score (HR 1.03 p<0.001) but MMP-3 or -9 or log₁₀Troponin I did not. For 1 year mortality the ROC-AUC's were 0.60 and 0.58 for MMP-2 and MMP-3 respectively compared to 0.82 for NT-proBNP and 0.84 for GRACE score (all p<0.001). Kaplan-Meier analysis revealed MMP-2 levels in the top quartile were associated with higher mortality rates (log rank 12.49 p=0.006). On univariate analysis MMP-2 and -3 showed a weak association with HF readmission which was lost after adjustment for clinical factors. None of the MMPs tested predicted MI.

Conclusions

This is the first single centre study that identifies MMP2 as an independent predictor of all cause mortality post ACS however, NT proBNP and the GRACE Score are superior for risk stratification in this cohort.

INTRODUCTION

Matrix Metalloproteinases (MMPs) are a family of zinc containing endoproteinases, which along with their inhibitors (tissue inhibitors of metalloproteinases) are vital in ensuring a balanced turnover of the cardiac extracellular matrix (ECM). MMPs have been classified into 4 groups based on their substrate specificity. MMP-2 and 9 are known as the gelatinases and are also able to degrade type IV collagen found in basement membranes. MMP-3 is one of a group called the stromelysins and is active against a broad spectrum of ECM components [1]. Discovery of elevated levels of MMPs 2,3 and 9 both in patients with an acute coronary syndrome (ACS) [2] and atherosclerosis [3] have stimulated interest in clarifying their precise cardiovascular role.

During the first few days post acute myocardial infarction (AMI) the left ventricle dilates with thinning of the infarct zone, which leads to infarct expansion. MMP levels increase early post AMI and have therefore been implicated in this process, which is known as remodelling [4]. These changes in geometry contribute to the development of congestive heart failure and predict prognosis [5]. Rhode and colleagues were the first to show that administration of an MMP inhibitor attenuated early LV dilation 4 days after MI in mice [6]. Subsequent studies on genetically modified mice with targeted deletion of MMP genes have shown improved survival rates over wild type mice mainly due to reduced cardiac rupture rates [7-9] with corroboratory results recently found in humans with cardiac rupture [10].

In humans post AMI there are multiple reports of MMP levels correlating with echocardiographic parameters of LV function indicating they possibly contribute towards remodelling [11-14]. This is supported by evidence that levels correlate with other neurohormonal markers such as atrial and brain natriuretic peptides [15, 16]. They have also been implicated in atherosclerotic plaque rupture, the underlying cause of ACS [17], with higher levels of MMP-9 found in plasma of the infarct related artery [18]. MMP levels also contribute to the inter-individual susceptibility to cardiovascular disease and the outcome from ACS as shown in studies of MMP gene polymorphisms [19]. This central role of MMPs -2, -3 and -9 in patients with cardiovascular disease is further highlighted by studies demonstrating the prognostic power of MMP levels in predicting adverse events [20-23]. However, their utility as prognostic markers has been hampered by inconsistent results.

B type natriuretic peptide (BNP) and the N terminal of its pro hormone (NTproBNP) have a strong association with extent of LV dysfunction and mortality [24,25]. We aimed to assess the prognostic role of MMPs -2, -3 and -9 in both ST- and non ST-elevation myocardial infarction using NTproBNP as a benchmark comparator.

METHODS

Study population

We studied 1024 consecutive AMI patients admitted to University Hospitals of Leicester NHS trust. The study complied with the Declaration of Helsinki and was approved by the local ethics committee; written informed consent was obtained from patients. Myocardial infarction (MI) was diagnosed if a patient had a plasma creatine kinase-MB elevation greater than twice normal or cardiac troponin I (Advia Centaur Troponin-I assay Siemens, Deerfield, IL, USA) level >0.1 ng/mL taken up to 30 hours after symptom onset with at least one of the following, chest pain lasting >20 minutes or diagnostic serial electrocardiographic (ECG) changes consisting of new pathological Q waves or ST-segment and T-wave changes [26]. AMI was sub categorised into ST segment elevation myocardial infarction (STEMI) or non-ST segment myocardial infarction (NSTEMI). Exclusion criteria were known malignancy, renal replacement therapy, recent/pending blood transfusion or surgery within the previous month. Demographical, clinical, biochemical and echocardiographic data were obtained. All patients received standard medical treatment and revascularisation was at the discretion of the attending physician. Plasma samples were drawn 4 days after the onset of symptoms and after 15 minutes bed rest into tubes containing EDTA and aprotinin. Plasma was stored at minus 80°C until assayed in a blinded fashion in a single batch for determination of plasma MMP-2, MMP-3, MMP-9 and NTproBNP. The estimated GFR (eGFR) was calculated from the simplified formula derived from the Modification of Diet in Renal Disease (MDRD) study [27]. Median length of follow-up was 519 days with a range of 134–1059 days with no patients lost to follow-up.

Echocardiography

Transthoracic echocardiography was performed in 826 (80.7%) patients during the index admission but after day 3 using either a Sonos 5500 or IE 33 instrument (Philips Medical Systems, Reigate, UK). A 16-segment left ventricular wall motion index (LVWMI) was performed based on the American Society of Echocardiography method. In suitable patients left ventricular ejection fraction (LVEF) was calculated using the biplane method of discs formula. Impaired LV systolic function was defined as either an LVEF $<40\%$ or a LVWMI >1.8 .

Global Registry of Acute Coronary Events (GRACE) Scoring

Based on an international observational database of ACS patients GRACE scores can be calculated on initial presentation and on discharge to predict prognosis in ACS patients. The discharge GRACE score includes age, heart rate, systolic blood pressure, serum creatinine, congestive heart failure, ST depression, raised cardiac enzymes, history of previous MI or CABG and whether in-hospital revascularisation was performed. Discharge scores are designed to predict death and re-infarction rates up to 6 months [28] are therefore used in further analyses. Admission scores are mainly designed to predict in hospital mortality and do not utilise knowledge of in hospital revascularisation strategies and consequently is not as strong a predictor of 6 month mortality [29]. Data was available for GRACE scores calculation in 886 (86.5%) patients.

NTproBNP assay

Our NTproBNP assay was based on a non-competitive assay as previously published [30]. Sheep antibodies were raised to the N-terminal of human NTproBNP and monoclonal mouse antibodies were raised to the C-terminal. Samples or NTproBNP standards were incubated in C-terminal IgG-coated wells with the biotinylated N-terminal antibody for 24 hours at 4°C. Detection was with methyl-acridinium ester (MAE)-labelled streptavidin on a MLX plate luminometer (Dynex Technologies Ltd., Worthing, UK). The lower limit of detection was 0.3 pmol/L. There was no cross reactivity with atrial natriuretic peptide, brain natriuretic peptide or C-type natriuretic peptide. Inter and intra coefficients of variation were 2.3% and 4.8% respectively. The results from this in-house assay are highly correlated ($r=0.90$, $P<0.0001$, $n=86$) to those obtained on the NTproBNP assay marketed by Roche Diagnostics Ltd. (Lewes, East Sussex, UK).

Matrix Metalloproteinase Assays

All MMP specific antibodies were obtained from R & D systems Ltd., Abingdon, UK. Mouse monoclonal antibodies (200ng/100µL) specific for MMP-2, MMP-3 and MMP-9 were coated onto ELISA plates overnight at room temperature. Plates were then blocked using 10% foetal calf serum. Samples and standards were incubated in the ELISA plates overnight at room temperature. Following washes, the tracer antibody (5ng/100µL biotinylated goat antibody specific for MMP-2, MMP-3 or MMP-9) was added to the wells and incubated for 2 hours. After washes, detection was with MAE-labelled streptavidin, as for the NTproBNP assay. There was no cross reactivity between the different MMP assays. The intra- and inter-assay coefficients of variation for MMP-2, 3 and 9 were as follows:- MMP-2 1.2% and 8.4%; MMP-3 1.6% and 2.9%; MMP-9 1.4% and 6.7%. The lowest detectable concentration defined as 2 standard deviations above the zero point were 0.26, 0.02 and 0.14 µg/L respectively.

End points

We assessed the value of MMP-2, MMP-3, MMP-9 and NTproBNP for the prediction of major cardiac events (MCE). The primary endpoint was the occurrence of all-cause mortality; heart failure (HF) hospitalisation and recurrent MI were considered as secondary end points. Hospitalization for HF was defined as a hospital readmission for which HF was the primary reason requiring treatment with high dose diuretics, inotropes or intravenous nitrate. AMI was diagnosed on established criteria as above [26]. Endpoints were obtained by reviewing the local hospital databases and the Office of National Statistics Registry and phone calls to patients. We achieved 100% follow-up.

Statistical analysis

Statistical analyses were performed on SPSS Version 14 (SPSS Inc, Chicago, Illinois). MMP-2, MMP-3, MMP-9 and NTproBNP levels were log₁₀ transformed. Proportions between two categorical groups were compared using Chi square tests. Non-parametric tests were employed against non-Gaussian data with Mann Whitney U-test used to compare continuous variables across two conditions and Spearman (r_s) correlations to assess the relationship between two continuous variables. Variation in MMP levels between the several independent end point groups was assessed using the Kruskal Wallis test. Correction for multiple comparisons was done with Hochberg's GT2 test and all significance levels quoted are two tailed. Assuming an event rate of 10% and that the covariates predict up to 30% of the variance of the biomarker, a

sample size of 934 patients would be powered (80% at $P < 0.05$) to detect a hazard ratio of the biomarker of 2. We recruited more than 1000 cases to cover for loss to follow up. In order to test the independent predictive power of the peptides for MCE, survival analysis using Cox proportional hazard modelling was conducted. Due to \log_{10} transformation of peptide levels hazard ratios refer to a tenfold rise in the levels of these markers. Cox models included important clinical variables and any other variables that were statistically significant (at $p < 0.20$) in univariate analyses. These included age, gender, previous history of MI, hypertension, diabetes, type of AMI (STEMI or NSTEMI), Killip class greater than 1 and eGFR with addition of \log_{10} troponin I and echocardiographic data in a subset of patients. MMP-2, -3 and -9 were first added individually to this 'base' model and then entered simultaneously along with NTproBNP. A further Cox model was used to assess the relative prognostic power of MMP-2 with GRACE score (and NTproBNP) which excluded age and eGFR as both are involved in calculating GRACE scores and may lead to collinearity ($r_s = 0.87$ and 0.57 respectively). Receiver operator characteristic curves with areas under the curve (AUC) were calculated. Kaplan-Meier survival analysis and Mantel-Cox log rank tests were performed. A p-value of less than 0.05 was interpreted to be statistically significant. All authors had full access to the data and take responsibility for its integrity and accuracy of the analysis. All authors have read and agree to the manuscript as written.

RESULTS

Patient characteristics

Patient details are recorded in Table 1. We recruited 1024 patients, 478 (46.7%) were STEMI for whom thrombolysis was primary method of revascularisation performed in 310 (64.9%) patients. During follow-up, 111 (10.8%) patients died, 106 (10.3%) were readmitted with HF and 138 (13.5%) suffered a MI.

Plasma Matrix Metalloproteinase levels (see Table 2)

Matrix metalloproteinase-2; Plasma levels ranged from 0.3-371.1 $\mu\text{g/L}$ with a median of 22.8 $\mu\text{g/L}$. MMP-2 was elevated in patients who died compared to event free survivors (median[range] 26.8 [0.7–366.6] vs. 21.8 [0.3-371.1] $\mu\text{g/L}$, $p=0.006$). Patients with HF tended towards higher levels but this was not significant (25.4 [0.6–124.5] $\mu\text{g/L}$, $p=0.55$) and similar levels were found in the recurrent MI group when compared to event free survivors. MMP-2 was also higher in patients with STEMI compared with NSTEMI ($p=0.001$) and patients with Killip class greater than 1 on admission ($p=0.007$). There was no significant gender difference in plasma levels or between those with or without prior history of MI, hypertension, angina or diabetes mellitus. Plasma MMP-2 weakly correlated with eGFR, \log_{10} serum glucose and NTproBNP, but not with age, \log_{10} troponin I or GRACE score.

Matrix Metalloproteinase-3; Median level was 6.0 $\mu\text{g/L}$ in AMI patients and ranged from 0.02-43.7 $\mu\text{g/L}$. MMP-3 was significantly elevated in patients who died over event free survivors (6.9 [1.9–36.8] $\mu\text{g/L}$ vs. 5.8 [0.1-43.7] $\mu\text{g/L}$, $p=0.010$) with similar levels between event free survivors and those who experienced HF (7.6 [0.02–35.5] $\mu\text{g/L}$, $p=0.714$) or MI. Men had higher levels ($p<0.001$) along with patients Killip class above 1 ($p=0.018$) and those with prior MI ($p=0.036$) but was similar across other groups. MMP-3 levels correlated weakly with age, eGFR, \log_{10} glucose, \log_{10} troponin I, discharge GRACE scores and NTproBNP.

Matrix metalloproteinase-9; levels ranged from 0.1-893.5 $\mu\text{g/L}$ with a median level of 74.2 $\mu\text{g/L}$. Levels were higher in patients with NSTEMI compared with STEMI and lower in patients with Killip class >1 (both $p<0.001$). MMP-9 levels were similar between patients that suffered a major cardiac event (MCE) compared with event free survivors and across most other groups. MMP-9 correlated weakly with GRACE score, \log_{10} glucose, \log_{10} troponin I, and NTproBNP but not with eGFR or age.

Relationship between matrix metalloproteinases and echocardiographic parameters; 331 (40.1%) patients were classified as having impaired LV function (see above) and these patients had significantly increased levels of MMP-3 and NT-proBNP but no significant difference in MMP-2 and 9 levels (Table 2).

Matrix metalloproteinases as predictors of Major Cardiac Events (MCE)

Primary end point all cause mortality; As shown above plasma levels of MMP-2 and MMP-3 were significantly higher in patients who died and demonstrated an association with this end point on univariate analysis (Table 3). For MMP-2 this association remained on multivariate analysis (HR 2.45 $p=0.009$) along with NTproBNP (HR 2.87 $p<0.001$), eGFR, age and prior history of MI. In a separate model MMP-2 continued to add independent information (HR [95%CI] 6.60 [2.25-19.36] $p=0.001$) after adjustment for GRACE scores which were also associated with poor outcome (HR 1.03 [1.01-1.04] $p<0.001$) along with NTproBNP (HR 4.62 [2.06-10.36] $p<0.001$) but no other variables. Kaplan-Meier analysis revealed that MMP-2 levels in the top quartile were associated with higher mortality rates than those in the lower three quartiles (pooled comparison log rank test 12.49 $p=0.006$, figure 1). MMP-3 levels did not predict death after adjustment for clinical variables

Of note we found that Killip class >1 and impaired LV function were independent predictors of mortality in the base model ($p=0.043$ and $p=0.033$ respectively) but were displaced when biomarkers were added ($p=0.269$ and $p=0.137$ respectively). Log₁₀ troponin I was not predictive of death in this cohort with likely reasons for this discrepancy being that levels were unlikely to be peak, the cohort are particularly high risk and also possibly not large enough to illustrate this relationship. Type of MI, STEMI or nSTEMI, did not predict mortality.

The areas under the receiver operating characteristic curve (AUC) for MMP-2 and MMP-3 as a predictors of 1 year mortality were 0.60 and 0.58 respectively compared to NTproBNP 0.82 and GRACE score 0.84 (all at $p<0.001$). Models that combined MMP-2 levels with NTproBNP or GRACE score did not reveal a significant improvement in AUC over either marker alone.

Secondary end points; MMP-2 levels tended to be higher in patients readmitted with HF and was associated with this outcome on univariate Cox analysis (HR 2.18 $p=0.015$ along with age, gender, previous MI, hypertension, diabetes, Killip class >1 , eGFR, impaired LV function, MMP-2, and NTproBNP). This weak relationship was lost ($p=0.29$) after adjustment for baseline variables on multivariate analysis with only Killip class >1 (HR 4.01 $p<0.001$), NTproBNP (HR 1.62, $p=0.027$) and age (HR 1.03, $p=0.036$) remaining. Impaired LV function predicted HF readmission in a base model ($p=0.019$) until biomarkers were added ($p=0.071$). Elevated MMP-3 showed a weak association with this outcome (HR 1.70 $p=0.11$) that was lost on multivariate analysis and MMP-9 levels did not show a relationship to HF.

Variables that predicted reinfarction on univariate analysis included previous history of hypertension, diabetes, Killip class greater than 1, eGFR, impaired LV function, NTproBNP levels and GRACE score with none of the MMPs achieving statistical significance. On multivariable analysis only GRACE Score (HR 1.01 $p=0.018$) and presence of diabetes (HR 1.59 $p=0.019$) retained independent association with reinfarction.

DISCUSSION

This single centre study performed in a substantial cohort of patients with AMI revealed that MMP-2 level predicted the primary end point of all cause mortality independent of clinical characteristics, NTproBNP and GRACE clinical risk score. These are novel results post AMI with MMP-2 since previous work has demonstrated prognostic utility in patients with congestive heart failure [21] but not in a smaller population with stable coronary disease [20]. The positive correlation of MMP2 with NT-proBNP, albeit weak, has been previously identified [15] and provides a degree of internal consistency. However, NTproBNP or the GRACE score are superior in risk stratification in this cohort and the results do not support utilising MMP-2 as a prognostic tool over these markers.

A possible explanation for this interesting link between highly elevated MMP-2 levels (top quartile) and mortality is offered by 2 studies [8, 9] that compare the effect of experimental myocardial infarction in wild type (WT) with genetically modified MMP-2 knockout (KO) mice. They found significantly improved survival in MMP-2 KO mice that was driven by a marked reduction of myocardial rupture and less adverse remodelling, due to reduced ECM degradation and inflammatory cell infiltration, resulting in the maintenance of the normal collagen framework that is often absent in ruptured myocardium [31]. Similar mortality reductions were seen in mice treated with a selective MMP-2 inhibitor [9]. However, a recent study in humans did not show a significant difference in MMP-2 levels in post AMI patients with/without cardiac rupture at autopsy [10]. Consequently, one could postulate that it is more likely that the relationship between mortality and MMP-2 levels, as demonstrated here in humans, is secondary to the long term pro-fibrotic effects of MMP-2 on ECM [32] and remodelling or through atherosclerotic plaque instability [17] rather than entirely due to an increase in a cardiac rupture rates. This association of MMP-2 levels with mortality raises the question of transient, timely and possibly most importantly selective MMP-2 inhibition aimed at those with the highest MMP-2 levels. MMP-2 inhibition has been effective in dogs [32] and may herald a potential therapy post AMI requiring further investigation in humans.

We also found elevated levels of MMP-2 in patients with STEMI over those with nSTEMI, which is in line with previous research demonstrating higher levels of MMP-2 in patients with AMI over stable angina over controls [33]. Post infarct remodelling is greater after STEMI and higher MMP-2 in these patients, and those with elevated Killip Class, supports the hypothesis that this enzyme may contribute towards this process. MMP-2 levels were predictive of readmission with HF on univariate but not multivariate analysis and neither MMP-2, -3 nor -9 levels were predictive of re-infarction.

With regard to MMP-3 we found elevated levels in patients with echocardiographic evidence of LV dysfunction, a weak correlation with NTproBNP levels and a univariate association with the primary end point which is all consistent with a previous report from our centre performed on predominantly STEMI patients [12]. Although a prognostic role for MMP-3 has been shown in patients with stable coronary heart disease [20] the initial association with mortality seen here was lost after adjustment for clinical factors and the results do not support using MMP-3 levels as a prognostic tool.

In contrast to several previous reports we did not find an association with echocardiographic parameters of LV function [11, 13, 14, 34] and this may be because our study design was restricted by a single blood sample taken on day 4. MMP-9 is recognised to be highest early after the onset of an AMI, coinciding with the inflammatory response and possible release from neutrophils [4, 35]. As a marker of prognosis MMP-9 is useful in patients with stable coronary disease [21] but its utility post MI is less clear [11, 23, 36] and we did not identify a relationship between MMP-9 levels taken on day 4 post AMI and adverse events. MMP-9 levels were lower in patients with STEMI and it has been reported that a larger drop in MMP-9 relates to more severe left ventricular impairment [11]. Therefore, the lower levels seen in patients with STEMI in this study may be due to MMP-9 falling further by day 4 than in patients with nSTEMI. Correlation between MMP-9 and NT proBNP levels seen here has been previously recognised [11, 16].

LIMITATIONS

Despite the cohort being large this is a single centre study and the results require replication by other groups. Furthermore, this study has established an association between MMP-2 levels and mortality but causation cannot be inferred. The cohort did not include patients with troponin negative ACS. We sampled blood at one time point during the recovery phase and although post ACS studies suggest this is appropriate for MMP-2 and -3 however, as recognised above, MMP-9 peaks early and either admission levels or the change in concentration between serial samples may have shown a stronger relationship with outcome [2, 4, 11, 12, 13, 35]. We did not perform separate analyses to differentiate between active and latent forms of the MMPs; however the ELISA method is in keeping with previous studies.

CONCLUSION

This is the first single centre study that identifies MMP-2 levels as an independent predictor of all cause mortality in a substantial cohort of post ACS patients. However, NTproBNP and the GRACE Score retain prognostic superiority. MMP-3 and -9 were not predictive of adverse events.

FUNDING

This work was supported by The British Heart Foundation.

ACKNOWLEDGEMENTS

None

CONFLICT OF INTEREST

None declared

Table 1: Characteristics of the study cohort as a whole and sub divided according to diagnosis of ST- or non ST- elevation myocardial infarction.

	All	STEMI	nSTEMI	p-value
Number	1024	478	546	-
Demographics				
Age (years)	66.7 ± 12.5	64.0 ± 12.0	71.0 ± 12.5	<0.001
Male Sex	731 (71.4)	357 (74.7)	374 (68.5)	0.03
Previous Cardiovascular Disease				
Myocardial Infarction	233 (22.8)	56 (11.7)	177 (32.4)	<0.001
Angina Pectoris	208 (20.3)	59 (12.3)	149 (27.3)	<0.001
Heart Failure	40 (3.9)	27 (5.6)	13 (2.4)	0.009
Cardiovascular Risk Factors				
Hypertension	542 (52.9)	210 (43.9)	332 (60.8)	<0.001
Diabetes Mellitus	242 (23.6)	84 (17.6)	158 (28.9)	<0.001
Current/Ex Smoker	427 (41.7)	281 (58.8)	320 (58.6)	0.99
Risk Markers on Admission				
Anterior Territory	368 (35.9)	184 (38.5)	184 (33.7)	0.10
Killip Class> I [n=945]	472 (46.1)	184 (46.0)	209 (38.3)	0.02
Glucose (mmol/L) [n=843]	7.6 ± 4.3	8.0 ± 4.3	7.2 ± 4.2	<0.001
Troponin I* (NR<0.06) [n=858]	3.56 ± 26.51	11.87 ± 37.96	2.09 ± 7.83	<0.001
eGFR (ml/min/1.73m ²)	65.3 ± 19.4	68.5 ± 18.7	61.9 ± 19.3	<0.001
Risk Markers on Discharge				
Impaired LV function [n=826]	331 (32.3)	135 (28.2)	196 (35.9)	<0.001
GRACE score [n=886]	117 ± 32	114 ± 29	123 ± 38	0.05
Medications on Discharge				
Aspirin	861 (84.1)	422 (88.3)	439 (80.4)	0.001
Beta-blocker	827 (80.8)	414 (86.6)	413 (75.6)	<0.001
ACE inhibitor or ARB	851 (83.1)	428 (89.5)	423 (77.5)	<0.001
Statin	903 (88.2)	443 (92.7)	460 (84.2)	<0.001
Biomarker Levels				
MMP-2 (µg/L)	22.8 ± 28.1	25.1 ± 15.7	20.8 ± 35.5	0.001
MMP-3 (µg/L)	6.02 ± 6.15	6.18 ± 6.23	5.92 ± 6.09	0.20
MMP-9 (µg/L)	74.2 ± 104.7	59.2 ± 104.8	83.0 ± 104.0	<0.001
NTproBNP (pmol/L)	836 ± 1964	1179 ± 2248	588 ± 1581	<0.001
Major Cardiac Event Rate at 1 yr				
Death	102 (10.0)	43 (9.0)	59 (10.8)	0.35
Heart Failure	97 (9.5)	50 (10.5)	47 (8.6)	0.34
MI	117 (11.4)	60 (12.6)	57 (10.4)	0.33

Data presented as numbers (%) for dichotomous variables or median ± SD for continuous variables.

[n=] indicates number of patients with data available on that variable if less than 1024

*Troponin I level taken up to 30 hours after symptom onset

eGFR estimated glomerular filtration rate, NTproBNP = N-terminal pro-B-type natriuretic peptide, ACE = Angiotensin converting enzyme, ARB = Angiotensin 2 receptor blocker

Table 2: MMP-2, -3, -9 and NTproBNP levels across different patient groups.

	MMP-2 (µg/L)		p-value	MMP-3 (µg/L)		p-value	MMP-9 (µg/L)		p-value	NT pro BNP (pmol/L)		p-value
	Median*			Median			Median			Median		
Sex M (n=731) Vs F	23.6	22.5	0.49	6.4	4.8	<0.001	74.2	73.5	0.78	679.4	1211.5	<0.001
Previous History												
MI (n=233) Vs No MI*	23.2	22.4	0.12	6.4	5.9	0.04	74.6	73.4	0.32	1092.7	742.6	0.002
Angina (n=208)	22.9	22.7	0.32	6.1	6.0	0.82	71.0	74.7	0.62	1105.1	745.0	<0.001
Diabetes (n=242)	24.6	21.9	0.07	6.2	5.9	0.60	75.5	73.0	0.25	1119.2	753.8	0.02
Hypertension (n=542)	22.9	22.4	0.30	6.3	5.8	0.26	71.0	76.9	0.27	1053.4	62.6	<0.001
Clinical Factors												
STEMI (n=478) Vs nSTEMI	25.1	20.8	0.001	6.2	5.9	0.20	58.4	83.0	<0.001	1178.6	585.5	<0.001
Killip Class > 1 (n=472)	23.1	20.1	0.007	6.5	5.6	0.02	65.1	79.0	<0.001	1750.3	522.8	<0.001
Echocardiography [n=826]												
Impaired LV (n=331)	23.9	22.5	0.15	6.4	5.6	0.002	75.3	71.0	0.06	1694.9	508.3	<0.001
Major Cardiac Event												
Death (n=111)	26.8	21.8	0.006	6.9	5.8	0.01	72.7	75.8	1.00	3171.3	616.5	<0.001
HF (n=106)	25.4	21.8	0.55	7.6	5.8	0.66	69.3	75.8	0.73	2009.2	616.5	<0.001
MI (n=138)	22.0	21.8	0.97	5.8	5.8	1.00	69.4	75.8	0.35	685.7	616.5	0.96
Correlations	r_s			r_s			r_s			r_s		
Age [n=1024]	.035		0.26	.149		<0.001	-.036		0.25	.452		<0.001
eGFR [n=1024]	-.087		0.005	-.175		<0.001	-.007		0.82	-.361		<0.001
logGlucose [n=843]	.107		0.002	.081		0.02	-.071		0.04	.236		<0.001
logTroponin [n=854]	.043		0.21	.141		<0.001	-.177		<0.001	.197		<0.001
Discharge GRACE [n=886]	.064		0.05	.227		<0.001	-.074		0.03	.460		<0.001
NT pro BNP [n=1024]	.073		0.02	.229		<0.001	-.184		<0.001	-		-

*Note median values are all presented in the same order with the value for patients with the variable in question present in the first column and those with variable absent in the second.

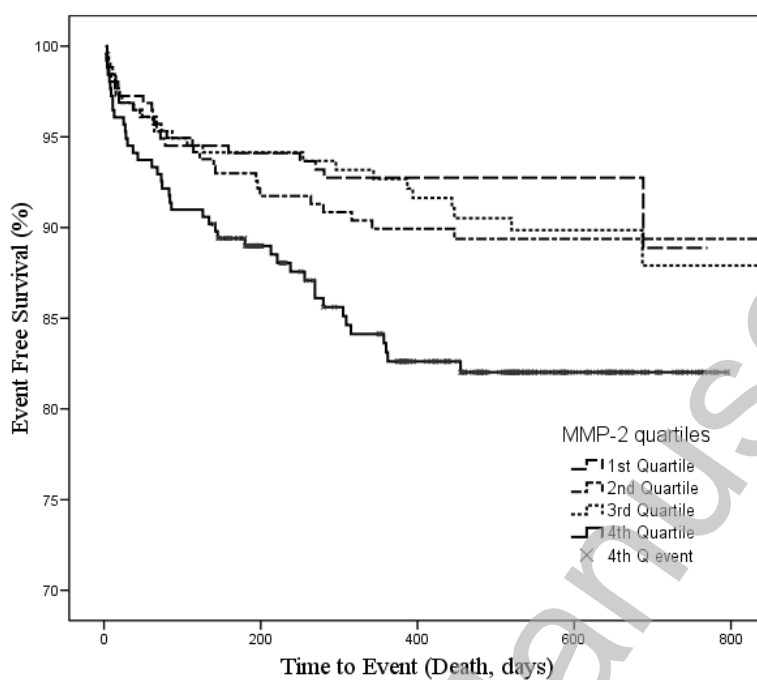
(n=) indicates number of patients with that variable present

[n=] indicates number of patients with data available on that variable

Table 3: Association between MMP-2, -3 and -9 and all cause mortality by univariate and multivariate Cox proportional hazards analysis.

Variable	Hazard Ratio (95% Confidence Interval)				
	Univariable	p-value		Multivariable	p-value
Age	1.08 (1.06-1.10)	<0.001		1.04 (1.01-1.06)	0.004
Male Gender	1.86 (1.28-2.71)	0.001		1.10 (0.70-1.75)	0.68
Hypertension	1.74 (1.18-2.58)	0.006		1.12 (0.72-1.74)	0.61
Diabetes	1.57 (1.06-2.34)	0.03		1.16 (0.75-1.81)	0.50
Previous MI	2.51 (1.72-3.66)	0.001		1.69 (1.10-2.58)	0.016
STEMI	0.83 (0.57-1.21)	0.34		1.25 (0.76-2.06)	0.39
Killip Class > 1	2.90 (1.93-4.36)	< 0.001		1.29 (0.83-1.99)	0.25
eGFR	0.95 (0.94-0.96)	< 0.001		0.97 (0.96-0.99)	<0.001
Impaired LV Function	2.88 (1.84-4.51)	< 0.001		1.44 (0.88-2.36)	0.15
Troponin I	1.04 (0.80-1.37)	0.440		1.18 (0.84-1.67)	0.34
MMP-2	2.78 (1.49-5.17)	0.001		2.45 (1.26-4.74)	0.009
MMP-3	2.83 (1.50-5.33)	0.001		0.79 (0.42-1.46)	0.45
MMP-9	1.02 (0.68-1.51)	0.407		1.18 (0.76-1.82)	0.47
NTproBNP	5.55 (3.67-8.41)	< 0.001		2.87 (1.72-4.78)	< 0.001

Figure 1: Kaplan Meier curve illustrating the difference in mortality in patients with MMP-2 levels in the top quartile compared to first 3 quartiles $p=0.006$.



REFERENCES

- 1 Creemers, E.E., Cleutjens J.P., Smits J.F. and Daemen M.J. (2001) Matrix metalloproteinase inhibition after myocardial infarction: a new approach to prevent heart failure? *Circ. Res.* **89**, 201-210
- 2 Kai, H., Ikeda, H., Yasukawa, H., Kai, M., Seki, Y., Kuwahara, F., Ueno, T., Sugi, K. and Imaizumi, T. (1998) Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes. *J. Am. Coll. Cardiol.* **32**, 368-372
- 3 Dollery, C.M. and Libby, P. (2006) Atherosclerosis and proteinase activation. *Cardiovasc. Res.* **69**, 625-635
- 4 Tao, Z., Cavin, M.A., Yang, F., Liu, Y. and Yang, X. (2004) Temporal changes in matrix metalloproteinase expression and inflammatory response associated with cardiac rupture after myocardial infarction in mice. *Life Sci.* **74**, 1561-1572.
- 5 Pfeffer, M.A. and Braunwald E. (1990) Ventricular remodelling after myocardial infarction. Experimental observations and clinical implications. *Circulation* **81**, 1161-1172
- 6 Rohde, L.E., Ducharme, A., Arroyo, L.H., Aikawa, M., Sukhova, G.H., Lopez-Anaya, A., McLure, K.F., Mitchell, P.G., Libby, P. and Lee, R.T. (1999) Matrix metalloproteinase inhibition attenuates early left ventricular enlargement after experimental myocardial infarction in mice. *Circulation* **99**, 3063-3070
- 7 Heymans, S., Luttan, A., Nuyens, D., Theilmeier, G., Creemers, E., Moons, L., Dyspersin, G.D., Cleutjens, J.P.M., Shipley, M., Angellilo, A., Levi, M., Nube, O., Baker, A., Keshet, F., Lupu, F., Herbert, J.M., Smits, J.F., Shapiro, S.D., Baes M., Borgers, M., Collen, D., Daemen, M.J. and Carmeliet, P. (1999) Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure. *Nat. Med.* **5**, 1135-1142
- 8 Hayashidani, S., Tsutsui, H., Ikeuchi, M., Shiomi, T., Matsusaka, H., Kubota, T., Imanaka-Yoshida, K., Itoh, T. and Takeshita, A. (2003) Targeted deletion of MMP-2 attenuates early LV rupture and late remodeling after experimental myocardial infarction. *Am. J. Physiol. Heart Circ. Physiol.* **285**, H1229-1235
- 9 Matsumura, S., Iwanaga, S., Mochizuki, S., Okamoto, H., Ogawa, S., Okada, Y. (2005) Targeted deletion or pharmacological inhibition of MMP-2 prevents cardiac rupture after myocardial infarction in mice. *J. Clin. Invest.* **115**, 599-609
- 10 van den Borne, S.W., Cleutjens, J.P., Hanemaaijer, R., Creemers, E.E., Smits, J.F., Daemen, M.J. and Blankesteijn, W.M. (2009) Increased matrix metalloproteinase-8 and -9 activity in patients with infarct rupture after myocardial infarction. *Cardiovasc. Pathol.* **18**, 37-43
- 11 Kelly, D., Cockerill, G., Ng, L.L., Thompson, M., Khan, S., Samani, N.J. and Squire, I.B. (2007) Plasma matrix metalloproteinase-9 and left ventricular remodelling after acute myocardial infarction in man: a prospective cohort study. *Eur. Heart J.* **28**, 711-718
- 12 Kelly, D., Khan, S., Cockerill, G., Ng, L.L., Thompson, M., Samani, N.J. and Squire, I.B. (2008) Circulating stromelysin-1 (MMP-3): a novel predictor of LV dysfunction, remodelling and all-cause mortality after acute myocardial infarction. *Eur. J. Heart Fail.* **10**, 133-139
- 13 Squire, I.B., Evans, J., Ng, L.L., Loftus, I.M. and Thompson, M.M. (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**, 328-333
- 14 Yan, A.T., Yan, R.T., Spinale, F.G., Afzal, R., Gunasinghe, H.R., Arnold, M., Demers, C., McKelvie, R.S. and Liu, P.P. (2006) Plasma matrix metalloproteinase-9 level is correlated with left ventricular volumes and ejection fraction in patients with heart failure. *J. Card. Fail.* **12**, 514-519
 - 15 Yan, A.T., Yan, R.T., Spinale, F.G., Afzal, R., Gunasinghe, H.R., Stroud, R., McKelvie, R.S. and Liu, P.P. (2008) Relationships between plasma levels of matrix metalloproteinases and neurohormonal profile in patients with heart failure. *Eur. J. Heart Fail.* **10**, 125-128
 - 16 Tziakas, D.N., Chalikias, G.K., Hatzinikolaou, E.I., Stakos, D.A., Tentas, I.K., Kortsaris, A., Hatseras, D.I. and Kaski, J.C. (2005) N-terminal Pro-B Type Natriuretic Peptide and matrix metalloproteinases in early and late left ventricular remodelling after acute myocardial infarction. *Am. J. Cardiol.* **96**, 31-34
 - 17 Newby, A.C. (2006) Do metalloproteinases destabilize vulnerable atherosclerotic plaques? *Curr. Opin. Lipidol.* **17**, 556-561
 - 18 Higo, S., Uematsu, M., Yamagishi, M., Ishibashi-Ueda, H., Awata, M., Morozumi, T., Ohara, T., Nanto, S. and Nagata, S. (2005) Elevation of plasma matrix metalloproteinase-9 in the culprit coronary artery in patients with acute myocardial infarction: clinical evidence from distal protection. *Circ. J.* **69**, 1180-1185
 - 19 Ye, S. (2006) Influence of matrix metalloproteinase genotype on cardiovascular disease susceptibility and outcome. *Cardiovasc. Res.* **69**, 636-645
 - 20 Wu, T.C., Leu, H.B., Lin, W.T., Lin, C.P., Lin, S.J. and Chen, J.W. (2005) Plasma matrix metalloproteinase-3 level is an independent prognostic factor in stable coronary artery disease. *Eur. J. Clin. Invest.* **35**, 537-545
 - 21 Blankenberg, S., Rupprecht, H.J., Poirier, O., Bickel, C., Smieja, M., Hafner, G., Meyer, J., Cambien, F. and Tiret, L.; AtheroGene Investigators. (2003) Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. *Circulation* **107**, 1579-1585
 - 22 George, J., Patal, S., Wexler, D., Roth, A., Sheps, D. and Keren, G. (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**, 484-487
 - 23 Wagner, D.R., Delagardelle, C., Ernens, I., Rouy, D., Vaillant, M. and Beissel, J. (2006) Matrix metalloproteinase-9 is a marker of heart failure after acute myocardial infarction. *J. Card. Fail.* **12**, 66-71
 - 24 Richards, A.M., Nicholls, M.G., Yandle, T.G., Frampton, C., Espiner, E.A., Turner, J.G., Buttimore, R.C., Lainchbury, J.G., Elliott, J.M., Ikram, H., Crozier, I.G. and Smyth, D.W. (1998) Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: new neurohormonal predictors of left ventricular function and prognosis after myocardial infarction. *Circulation* **97**, 1921-1929
 - 25 Talwar, S., Squire, I.B., Downie, P.F., McCullough, A.M., Campton, M.C., Davies, J.E., Barnett, D.B. and Ng, L.L. (2000) Profile of plasma N-terminal proBNP following acute myocardial infarction; correlation with left ventricular systolic dysfunction. *Eur. Heart J.* **21**, 1514-1521
 - 26 Thygesen, K., Alpert, J.S. and White, H.D.; Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. (2007) Universal definition of myocardial infarction. *Circulation.* **116**, 2634-53

- 27 Smilde, T.D., van Veldhuisen, D.J., Navis, G., Voors, A.A. and Hillege, H.L. (2006) Drawbacks and prognostic value of formulas estimating renal function in patients with chronic heart failure and systolic dysfunction. *Circulation* **114**, 1572-80
- 28 Eagle, K.A., Lim, M.J., Dabbous, O.H., Pieper, K.S., Goldberg, R.J., Van de Werf, F., Goodman, S.G., Granger, C.B., Steg, P.G., Gore, J.M., Budaj, A., Avezum, A., Flather, M.D. and Fox, K.A. (2004) A validated prediction model for all forms of acute coronary syndrome: estimating the risk of 6-month post discharge death in an international registry. *J.A.M.A.* **291**, 2727-2733
- 29 Granger, C.B., Goldberg, R.J., Dabbous, O., Pieper, K.S., Eagle, K.A., Cannon, C.P., Van de Werf, F., Avezum, A., Goodman, S.G., Flather, M.D. and Fox, K.A., for the Global Registry of Acute Coronary Events Investigators. (2003) Predictors of Hospital Mortality in the Global Registry of Acute Coronary Events. *Arch. Intern. Med.* **163**, 2345-2353
- 30 Omland, T., Persson, A., Ng, L., O'Brien, R., Karlsson, T., Herlitz, J., Hartford, M. and Caidahl, K. (2002) N-terminal pro-B-type natriuretic peptide and long-term mortality in acute coronary syndromes. *Circulation* **106**, 2913-2918
- 31 Factor, S.M., Robinson, T.F., Dominitz, R. and Cho, S.H. (1987) Alterations of the myocardial skeletal framework in acute myocardial infarction with and without ventricular rupture. A preliminary report. *Am. J. Cardiovasc. Pathol.* **1**, 91-97
- 32 Morita, H., Khanal, S., Rastogi, S., Suzuki, G., Imai, M., Todor, A., Sharov, V.G., Goldstein, S., O'Neill, T.P. and Sabbah, H.N. (2006) Selective matrix metalloproteinase inhibition attenuates progression of left ventricular dysfunction and remodelling in dogs with chronic heart failure. *Am. J. Physiol. Heart Circ. Physiol.* **290**, H2522-H2527
- 33 Hlatky, M.A., Ashley, E., Quertermous, T., Boothroyd, D.B., Ridker, P., Southwick, A., Myers, R.M., Iribarren, C., Fortmann, S.P. and Go, A.S. Atherosclerotic Disease, Vascular Function and Genetic Epidemiology (ADVANCE) Study. (2007) Matrix metalloproteinase circulating levels, genetic polymorphism, and susceptibility to acute myocardial infarction among patients with coronary artery disease. *Am. Heart J.* **154**, 1043-1051
- 34 Levy, D., Larson, M.G., Sawyer, D.B., Siwik, D.A., Colucci, W.S., Sutherland, P., Wilson, P.W. and Vasan, R.S. (2004) Relations of plasma matrix metalloproteinase-9 to clinical cardiovascular risk factors and echocardiographic left ventricular measures: the Framingham Heart Study. *Circulation* **109**, 2850-2856
- 35 Tayebjee, M.H., Lip, G.Y., Tan, K.T., Patel, J.V., Hughes, E.A. and MacFadyen, R.J. (2005) Plasma matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-2, and CD40 ligand levels in patients with stable coronary artery disease. *Am. J. Cardiol.* **96**, 339-345
- 36 Cavusoglu, E., Ruwende, C., Chopra, V., Yanamadala, S., Eng, C., Clark, L.T., Pinsky, D.J. and Marmur, J.D. (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**, 1101.e1-1101.e8