Plasma matrix metalloproteinase-9 and left ventricular remodelling after acute myocardial infarction in man: a prospective cohort study

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Abstract

Aims—To describe temporal profiles of plasma matrix metalloproteinases (MMP-2 and MMP-9), and their relationship with echocardiographic (ECG) parameters of left ventricular (LV) function and remodelling, after acute myocardial infarction (AMI) in man.

Methods and results—Plasma MMP-2 and MMP-9 were assayed at intervals (0-12, 12-24, 24-48, 48-72, 72-96, and >96 h) in 91 patients with AMI (ST-elevation/non-ST-elevation 77/24; 73% male; 40% anterior site) and on a single occasion in 172 age- and sex-matched control subjects with stable coronary artery disease. ECG assessment of LV volumes, LV ejection fraction (LVEF), and wall motion index score were assessed before discharge and at follow-up (median 176, range 138-262 days) for patients and on a single occassion in controls. Plasma MMP-2 was similar at all times after AMI, elevated when compared with control ($P = 0.005\text{-}0.001$) and unrelated to LV function or volume during index admission or at follow-up. Maximal MMP-9 was seen at 0-12 h and was elevated when compared with control ($P = 0.002$) followed by fall to a plateau. Both maximal and plateau MMP-9 concentration correlated with white blood cell (WBC, $P = 0.023$ to <0.001) and neutrophil count ($P = 0.014$ to <0.001). Maximal MMP-9 had independent predictive value for lower LVEF ($P = 0.004$) during admission and for greater change in LV end-diastolic volume between admission and follow-up ($R = 0.3$, $P = 0.016$). In contrast, higher plateau levels of MMP-9 were associated with relative preservation of LV function (increasing LVEF, $P = 0.002$; decreasing WMIS, $P = 0.009$) and less change in end-systolic volume and end-diastolic volumes after discharge ($P = 0.001$ and 0.024, respectively).

Conclusion—Both MMP-9 and MMP-2 are elevated following AMI. The biphasic profile of plasma MMP-9 is related to LV remodelling and function following AMI in man. Higher early levels of MMP-9 associate with the extent of LV remodelling and circulating WBC levels. In contrast, higher plateau levels later after AMI are associated with relative preservation of LV function. Temporal profile, rather than absolute magnitude, of MMP-9 activity appears to be important for LV remodelling after AMI.

Keywords

Matrix metalloproteinase; Myocardial infarction; Remodelling

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Introduction

One of the most prognostically significant consequences of acute myocardial infarction (AMI) is the development of adverse left ventricular (LV) remodelling. The degree of LV remodelling, in particular the extent of changes in LV volume, correlates closely with the severity of LV dysfunction and the likelihood of the development of congestive heart failure (CHF). These structural and clinical events are intimately linked to adverse prognosis.[1-3]

Remodelling involves changes in ventricular geometry, structure, and function affecting both infarcted and non-infarcted myocardium. Changes in the extracellular collagen matrix of the myocardium are central to the remodelling process after AMI. In the healthy state, structure and function of the extracellular matrix are maintained by the matrix metalloproteinase (MMP) family of endopeptidases. Alteration in the activity of members of the MMP family is implicated in the process of LV remodelling so crucial to prognosis after AMI. In a variety of animal models of AMI, increased interstitial MMP expression occurs during the development of heart failure[4,5] and non-specific pharmacological MMP inhibition attenuates ventricular remodelling.[6,7]

Importantly, in pathophysiological conditions changes in activity of the MMP enzymes are selective. The gelatinase MMP-9 is specifically implicated in adverse LV remodelling. In animal models of AMI, selective MMP inhibition reduces LV wall thinning and dilatation[8,9] and targeted deletion of the MMP-9 gene is associated with reduced incidence of LV rupture.[10] MMP-9 is also implicated in the progression of cardiovascular disease in man. In a Framingham Study cohort of individuals free of prior AMI or CHF, detectable plasma MMP-9 was associated with greater LV dimensions and wall thickness in males.[11] In patients with cardiovascular disease, plasma MMP-9 levels predict fatal CHD events,[12] and MMP-9 and MMP-2 levels are increased after AMI.[13] Upregulation of the MMP system occurs in human heart failure[14,15] while that of MMP-2 is unchanged.[14] Greater understanding of the influence of the role of the MMP system in LV remodelling may allow better identification of patients at risk of adverse outcome after AMI. Moreover, such knowledge may advance our knowledge of possible therapeutic manipulation of MMP activity in this context.[16,17]

Following AMI, plasma natriuretic peptide levels, in particular those of B-type natriuretic peptide (BNP) and the N-terminal of its prohormone (N-terminal proBNP, N-BNP) have a strong association with the extent of LV dysfunction[18] and with prognosis.[19] In a mouse model of AMI, BNP overexpression increases MMP-9 activity.[20] Few studies have assessed the relationship between plasma MMP levels with either plasma natriuretic peptide levels or with ventricular remodelling after AMI in man.

Given the possible contribution of the MMP system to remodelling, and the relationship between plasma natriuretic peptide levels and LV structure and function, it is reasonable to postulate relationships between the natriuretic peptide and MMP systems. We previously reported a correlation between plasma N-BNP and MMP-9 after anterior AMI in man.[21] Higher MMP-9 levels soon after AMI were associated with more severe LV dysfunction during the index admission and follow-up at 6 weeks.

The current study had three main aims. First, to describe profiles of plasma concentrations of MMP-2 and MMP-9 following AMI in man. Secondly, to investigate the relationships between plasma MMP concentrations and parameters of both LV function (LVEF and WMIS, NBNP) and volumes. Thirdly, to investigate the relationship of MMP concentrations with the extent of remodelling in the weeks following AMI as assessed by the change (Δ) in LV volumes between discharge and follow-up.

Methods

Study population

Patients admitted to our Coronary Care Unit (CCU) with AMI were eligible for inclusion in the study. We excluded patients with known malignancy or with inflammatory or connective tissue disease. In addition, we excluded from analysis recruited patients with poor echocardiographic (ECG) images making valid measurements difficult ($N = 15$). No patients approached refused consent to inclusion, and no patient was 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 and underwent ECG studies during their index admission and at follow-up (median 176 days, range 138-262 days after AMI). 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.

Our control population consisted of 172 age- (median 63, range 36-86) and sex- (77.3% 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.

Laboratory methods

Venous blood samples were drawn at intervals during the index admission (0-12, 12-24, 24-48, and 24 h intervals thereafter) from the onset of chest pain until discharge. Plasma MMP-9 concentrations were measured at each of these time intervals. Based on previous work from our group,[22] we measured N-BNP immediately pre-discharge. In the control population, blood was sampled at one point only, at least 90 days after AMI. Samples were centrifuged within 30 min and plasma stored at −70°C until assayed. Our non-competitive assay for plasma N-BNP has been described previously.[23] The lower limit of detection was 0.3 fmol/mL. Interassay coefficient of variation was <8%.

Plasma MMP-2 and MMP-9 were measured using similarly designed non-competitive immunoluminometric assays. ELISA plates were coated with 100 ng/well of anti-mouse IgG (Sigma Chemical Co., Poole, UK). Following washes, wells were blocked with 5 g/L bovine serum albumin. For the assay, 100 μL of assay buffer containing 20 ng of MMP-9 or 50 ng of MMP-2 mouse monoclonal antibodies (Research Diagnostics, NJ, USA) were pipetted into the wells, followed by 10 μL of plasma samples or standards of recombinant MMP-2 or MMP-9 (Research Diagnostics). ELISA plates were incubated for 24 h, and after washes, the second antibody (biotinylated goat anti-MMP2 or anti-MMP9, 10 ng/100 μL assay buffer in each well) was introduced and incubated for another 4 h. Plates were washed and streptavidin-MAE was used to build up the final component of the sandwich assay. Chemiluminescence was detected as described for N-BNP.

Echocardiographic assessment

Echocardiographic assessment was carried out during the index admission (immediately prior to discharge) and at follow-up, by a single operator (D.K.) using either Sonos 5500 or IE33 scanner (Philips Medical Systems, Reigate, UK). LV end-systolic volume (LVESV), end-diastolic volume (LVEDV), and LVEF were estimated using the bi-planar modified Simpson's rule from apical two- and four-chamber views. LV function was also assessed using the LV wall motion index score (WMIS), using a standard 16-segment model from para-sternal long- and short-axis and apical two- and four-chamber views. Each LV segment

is scored as 0, hyperkinetic; 1, normal; 2, hypokinetic; 3, akinetic; 4, dyskinetic. The total is divided by the number of segments analysed to give an overall score with higher values indicating more impaired LV function. For patients attending ECG at follow-up, the change (Δ) in LVESV (ΔLVESV) and LVEDV (ΔLVEDV) were calculated as percentages of the pre-discharge volume. 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.

Statistical analysis

Sample size—We based our sample size on the estimated change in LVEDV between discharge and follow-up ECG 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 ECG examinations. The observed mean ΔLVEDV between discharge and follow-up was 22% (SD 25%), giving our study over 90% power to detect a mean change of 20% in LVEDV.

The main points of interest were the plasma profiles of MMP-2 and MMP-9, and the relationship of plasma concentrations with ECG parameters. Normality of distribution was assessed for continuous variables via Tukey test. Non-Gaussian distributed variables (N-BNP, MMP-2, MMP-9, CK, TnI, WMIS) were log-transformed prior to all analysis. Data are presented as mean ± SD or median (range) for non-Gaussian distributed data.

Differences among log-transformed MMP-2 and MMP-9 concentrations at individual time periods were analysed by repeated-measures analysis of variance (ANOVA), followed when appropriate by multiple Bonferroni comparisons. 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, hypertension or diabetes, individual pre-admission drug therapy, thrombolysis, and current smoking/not smoking were entered as categorical variables. Age, creatinine, peak CK, and WBC/neutrophils count were entered as continuous variables (Table 1). We performed similar analyses for the relationship between log-transformed MMP concentration and ECG parameters (LVEF, WMIS, LVEDV, LVESV, and ΔLVEDV). For multivariable analysis of the determinants of the variable of interest (MMP concentration or ECG parameter), factors with univariable association at two-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 11 (SPSS Inc., Chicago, USA).

Results

We studied 91 patients admitted to the CCU of our hospital between 1 September 2004 and 31 March 2005 and 172 age- and sex-matched control subjects admitted to the same unit with AMI >90 days previously. The admission demographic features of the study population are shown in Table 1. Approximately 75% of the population were male, ST-elevation was present on the admission ECG in over 80%, and median creatine kinase was >1000 I.U. Sixty five patients (77%) of those with STEMI, received thrombolytic therapy. No patient received primary percutaneous revascularization.

Temporal profiles of MMP-2 and MMP-9

Plasma MMP-2 levels (median, range) were similar at all time periods (0-12 h–21.5 ng/mL, 5.31-50.33; 12-24 h–21.1 ng/mL, 9.97-61.75; 24-48 h–18.6 ng/mL, 3.84-54.01; 48-72 h–19.3 ng/mL, 3.62-40.9; 72-96 h–20.2 ng/mL, 3.72-53.35; >96 h–21.1 ng/mL, 7.3-55.34) and elevated when compared with controls (16.1 ng/mL, 0.19-50.21, all $P < 0.001$). Plasma MMP-2 was consistently higher in anterior when compared with inferior AMI (Figure 1). In contrast, maximal MMP-9 level was observed within 0-12 h after AMI [median 71.0 ng/mL (range 15.0-376.0)] with a fall to a plateau thereafter [12-24 h, 45.8 (20.5-293.2 ng/mL), $P = 0.03$; 24-48 h, 49.1 (22.9-361.4), $P = 0.01$; 48-72 h, 50.6 (18.75-2965), $P = 0.004$; 72-96 h, 50.1 (18.3-395.2), $P < 0.001$; >96 h, 49.0 (23.0-398.1), $P = 0.03$]. All comparisons are with 0-12 h (Figure 1). MMP-9 levels at each time were similar in anterior or inferior AMI.

Only MMP-9 at 0-12 h (median 71.0 ng/mL, range 15.0-376.0) was significantly higher than in our control population (50.8 ng/mL, 18.8-319.5, $P = 0.002$). Plasma MMP-9 at all later times was similar to control values ($P = 0.438$-0.952).

Correlates of plasma metalloproteinase concentration

<u>Univariable analysis:</u> We considered mean MMP-2 (average of all measurements) as a representative measure. Plasma MMP-2 correlated with patient age ($R = 0.304$, $P = 0.003$) and was higher in anterior (median 24.1, range 9.6-42.1) compared with inferior (18.5, range 4.13-40.6, $P = 0.014$) AMI. MMP-2 showed inverse correlation with peak CK ($R = -0.245$, $P = 0.023$), was higher in patients who were prescribed beta-blocker medication prior to admission (24.4 vs. 19.4 ng/mL, $P = 0.007$) and in non-smokers (22.6 ng/mL, 10.2-50.3) when compared with smokers (19.6 ng/mL, 5.3-31.9; $P = 0.044$).

For MMP-9, we considered the observed peak (highest concentration in an individual patient irrespective of the time period), and plateau (the lowest concentration in an individual patient). Peak or plateau MMP-9 concentration showed no relationship with any of the factors associated with MMP-2 but correlated with WBC ($R = 0.363$, $P < 0.001$ and $R = 0.410$, $P < 0.001$, respectively) and neutrophil ($R = 0.363$, $P < 0.001$ and $R = 0.410$, $P < 0.001$, respectively) count. These correlations were maintained at all individual time periods (WBC: $R = 0.212$-0.434, $P = 0.043$ to <0.001; neutrophil count: $R = 0.217$-0.404, $P = 0.039$-0.003).

Other than the association of MMP-2 with beta-blocker, no pre-admission pharmacological therapy, including thrombolysis, influenced plasma levels of either MMP-2 or MMP-9.

<u>Multivariable analyses:</u> In multivariable analyses, we included the prediction of MMP levels (at peak and plateau), WBC/neutrophil count, sex, territory of infarct, ST-elevation/non-ST-elevation MI, age, and creatinine. Mean MMP-2 concentration was associated with age ($P = 0.004$) and territory of infarct ($P = 0.008$). Both peak and trough MMP-9 retained independent association with WBC (each $P < 0.001$) and neutrophil count (each $P < 0.001$).

Echocardiographic assessment

Of the 91 pre-discharge Echocardiographic examinations, WMIS could be assessed in 84 and LVEF and volumes in 82. Of 72 patients attending follow-up assessment, WMIS was measurable in 72 and LVEF in 70. Overall change in LV volumes (ΔEDV and ΔESV) was available in 65 cases.

Increasing WMIS (more impaired LV systolic function) was associated with age ($R = 0.285$, $P = 0.009$), peak CK ($R = 0.258$, $P = 0.022$), history of angina (1.71 vs. 1.31, $P = 0.029$), prior MI (1.94 vs. 1.31, $P = 0.012$), and anterior territory of AMI (1.8 vs. 1.19, $P < 0.001$).

LVEF was associated with many of the same variables and was lower in patients with previous angina (32 vs. 41%, $P = 0.002$), prior MI (32 vs. 41%, $P = 0.005$), and anterior territory of AMI (35 vs. 46%, $P = 0.002$).

Matrix metalloproteinase-2—There was no apparent correlation between MMP-2 and any ECG parameter of LV function either during pre-discharge or at follow-up.

Matrix metalloproteinase-9—Peak MMP-9 correlated with more severe impairment of LV function during admission (Table 2) 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 (113 ng/mL) had lower LVEF (median LVEF 38%, range 22-66) when compared with patients having MMP-9　113 ng/mL (46%, range 19-66, $P = 0.014$) (Figure 2). In contrast, higher plateau levels of MMP-9 during admission were associated with relative preservation of LV function at follow-up (Table 2) and 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 plateau MMP-9 levels above the median (36.4 ng/mL) when compared with those at or below the median (median LVEF 52%, range 18-69 vs. 42%, range 16-62, $P = 0.035$) (Figure 2). On multivariable analyses, only peak MMP-9 concentration ($R = -0.260$, $P = 0.026$) and anterior territory of AMI ($P = 0.003$) retained independent predictive value for lower LVEF during admission. Factors with independent predictive value for higher WMIS were age ($P = 0.014$), previous MI ($P = 0.004$), anterior territory of AMI ($P < 0.001$), and peak CK ($P = 0.001$).

MMPs and LV volume: There was no apparent relationship with LVEDV or LVESV during the index admission for either MMP-2 or MMP-9 (Table 2). However, MMP-9 at 0-12 h 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) ($R = 0.3$, $P = 0.016$) (Figure 3). Similarly, peak MMP-9 was higher in those patients in whom EDV increased (median 157.9 ng/mL, range 45.0-398.1) when compared with those in whom EDV decreased (135 ng/mL, range 35.6-365.5, $P = 0.047$). In contrast, increasing plateau MMP-9 concentrations were associated with relative preservation of LVEF at follow-up (Figure 2) and decrease in LV volumes between discharge and follow-up (Figure 3). On multivariable analyses, peak MMP-9 concentration maintained independent association with ΔEDV ($P = 0.007$) and plateau MMP-9 with ΔESV ($P = 0.005$).

A history of hypertension (hypertension +4.5 mL vs. no hypertension −13.0 mL, $P = 0.027$), and diuretic use at discharge (diuretic +12.0 mL vs. no diuretic −13.0 mL, $P = 0.003$) also had univariable association with ΔEDV. No other pre-discharge medication was associated with the extent of remodelling. There was no univariable or multivariable relationship between neutrophil counts and Δvolumes.

MMP and N-pro-BNP

As expected, higher pre-discharge levels of N-BNP correlated with WMIS measured during admission (WMIS[1], $R = 0.394$, $P < 0.001$), and with WMIS (WMIS[2], $R = 0.379$, $P < 0.001$) and LVEF (LVEF[2], $R = -0.320$, $P = 0.002$) measured at follow-up. Relationships with WMIS were maintained on multivariable analyses (WMIS[1], $P = 0.003$; WMIS$_2$, $P = 0.045$). No correlations were seen between N-BNP and ΔLV volumes between admission and follow-up.

Discussion

Our study has several novel findings. First, plasma MMP-9 after AMI correlated with circulating WBC and neutrophil count. Secondly, higher plasma MMP-9 was associated with more severe LVSD in the immediate post-AMI period. Thirdly, higher MMP-9 levels during index hospitalization predict greater LV remodelling in the weeks after AMI. Finally, following the fall from the early peak level, higher plateau levels of MMP-9 are associated with relative preservation of LV function and less extensive remodelling.

The differing temporal profiles of plasma MMP-9 and MMP-2 levels after AMI are in keeping with previous reports from our own[21] and other[24-27] groups. The early peak in MMP-9 with a later, lower plateau are also in keeping with most[21,24-26] but not all[27] of these and with a recent study utilizing indirect imaging of MMP activity after experimental AMI in mice.[28]

The current study extends, in several important ways, the previous knowledge of the MMP system after AMI in man. Previous clinical studies suggested acute plaque rupture to be the source of high plasma MMP-9 following AMI.[13,29] 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,[8,30,31] in which neutrophils appear to be the predominant source of MMP-9 in the early period of inflammation. Indeed, plaque-derived MMP-9 may facilitate neutrophil infiltration and degranulation, and exacerbate the ischaemic insult.[32]

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. More severe impairment of LV function was associated with higher MMP-9 levels in the early period post-AMI. Moreover, we also found higher MMP-9 in the early post-AMI period to indicate more extensive LV remodelling in the following weeks and months. While these data may simply reflect an association between higher peak MMP-9 and the extent of AMI, they are in keeping with studies of experimental AMI. [10,28,33] 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.[34,35]

Our data are also broadly in keeping with a recent clinical report[27] in approximately 50 patients with AMI, which suggested higher MMP-9 over the first few days after AMI to be associated with greater increase in LV volume. However, the previous study considered changes in LV volume based upon dichotomized 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, extend the results form the previous study and support 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 plateau levels of MMP-9 in the days following AMI were associated with less remodelling, and relative preservation of LV function, thereafter. The nature of these associations indicates that higher peak levels, and lower plateau 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.33 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.32-35 Later in the process, this same proteolytic activity allows infiltration of other cell types which mediate wound healing.36 This observation would be in keeping with the possibility that the early peak and later plateau in plasma MMP-9 have different sources.

Our findings, when considered with previous experimental data, suggest that, in addition to contributing to adverse LV remodelling, MMP-9 activity may also be important for wound healing after AMI. There are potentially important clinical implications for these findings, in particular for the potential therapeutic use of MMP inhibition. In the recent PREMIER (*Pre*vention of *M*yocardial *I*nfarction *E*arly *R*emodelling) trial, the MMP inhibitor PG-116800 failed to attenuate adverse LV remodelling after AMI in man.37 However, PG-116800 was administered for 90 days with the first dose given on average >48 h 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. Although elevated when compared with long-term survivors of AMI, plasma MMP-2 was unrelated to LV function or remodelling, suggesting that this enzyme is unlikely to be a target for therapeutic manipulation after AMI.

We observed strong correlations between plasma N-BNP and the severity of LV dysfunction both during the index admission and at follow-up. These observations, which are as predicted, provide support for our observations regarding the relationship between MMP-9 and LV structure and function. The association of lower N-BNP with higher plateau MMP-9 concentrations is in keeping with the association between higher plateau MMP-9 concentrations and relative preservation of LV function at follow-up, and with less remodelling.

Our study identified other associations between plasma MMP levels and a number of clinical parameters. The observation in current smokers of lower MMP-2, in the early hours after AMI is of note. The MMPs have a role in the pathogenesis of smoking-related lung diseases, mediating smoke-induced inflammatory cell recruitment into lung tissue.38,39 Moreover, both MMP-9 and MMP-2 are increased in a rat model of emphysema.40 Relevant to the pathogenesis of AMI, cigarette smoking reduces nitric oxide production and increases that of reactive oxygen species.41 We suggest that the observed association between smoking and variation in plasma MMP levels in the immediate post-AMI period is worthy of further study.

Limitations

Differences in pharmacological therapy may have influenced our findings. However, a minority of our patients were receiving cardiovascular drug treatment prior to the index AMI, and pharmacological treatment following admission was relatively uniform. Greater increase in LVEDV in association with discharge diuretic treatment is likely to reflect the presence of clinical heart failure in such patients. We recognize the relatively weak statistical association between MMP levels and the assessed ECG parameters (Table 2, Figure 3). We recognize the possibility of inflation of error associated with multiple measures. In this context, we took representative measures of each MMP profile and entered only single values into our analyses. More importantly, our data are internally consistent and indicate the need for further studies. Sensitive imaging methodology, such as cardiac magnetic resonance imaging, may provide more detailed information on the relationship

between plasma MMP concentrations and remodelling. 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.

Our observations pertain to plasma levels of only MMP-2 and MMP-9. We cannot comment regarding plasma levels of other MMP entities, and the physiological regulators of MMP activity, the tissue inhibitors of matrix metalloproteinases (TIMPs). The findings from our observational study, if real, might be expected to be reflected in an association between early MMP-9 concentration after AMI and outcome. We are addressing these important issues in a prospective study.

Conclusions

Following AMI in man, plasma MMP-9 may be derived from circulating WBCs. Peak plasma concentrations of MMP-9 are associated with greater impairment of LV function during the index admission and predicts the degree of LV remodelling in the weeks after discharge. However, higher plateau levels of MMP-9 are associated with less remodelling in the same period. Plasma MMP-9 concentration in the early post-AMI period, and the change thereafter, may usefully predict LV remodelling after AMI.

Acknowledgments

References

1. The Multicenter Postinfarction Research Group. Risk stratification and survival after myocardial infarction. N Engl J Med. 1983; 309:331–336. [PubMed: 6866068]

2. White HD, Norris RM, Brown MA, Brandt PW, Whitlock RM, Wild CJ. Left ventricular end systolic volume as the major determinant of survival after recovery from myocardial infarction. Circulation. 1987; 76:44–51. [PubMed: 3594774]

3. Pfeffer MA, Braunwald E. Ventricular remodelling after myocardial infarction: experimental observations and clinical implications. Circulation. 1990; 81:1161–1172. [PubMed: 2138525]

4. Peterson JT, Li H, Dillon L, Bryant JW. Evolution of matrix metalloproteinase and tissue inhibitor during heart failure progression in the infarcted rat. Cardiovasc Res. 2000; 46:307–315. [PubMed: 10773235]

5. Etoh T, Joffs C, Deschamps AM, Davis J, Dowdy K, Hendrick J, Baicu S, Muckerjee R, Manhaini M, Spinale FG. Myocardial and interstitial matrix metalloproteinase activity after acute myocardial infarction in pigs. Am J Physiol. 2001; 281:H987–H994. [PubMed: 11514263]

6. Spinale FG, Coker ML, Krombach SR, Muckerjee R, Hallak H, Houck WV, Clair MJ, Kribbs SB, Johnson LL, Peterson JT, Zile MR. Matrix metalloproteinase inhibition during the development of congestive heart failure: effects on left ventricular dimensions and function. Circ Res. 1999; 20:364–376. [PubMed: 10455065]

7. Rohde LE, Ducharme A, Arroyo LH, Aikawa M, Sukhova GH, Lopez-Anaya A, McClure KF, Mitchell PG, Libby P, Lee RT. Matrix metalloproteinase inhibition attenuates early left ventricular enlargement after experimental myocardial infarction in mice. Circulation. 1999; 99:3063–3070. [PubMed: 10368126]

8. Lindsey ML, Gannon J, Aikawa M, Schoen FJ, Rabkin E, Lopresti-Morrow L, Crawford J, Black S, Libby P, Mitchell PG, Lee RT. Selective matrix metalloproteinase inhibition reduces left ventricular remodeling but does not inhibit angiogenesis after myocardial infarction. Circulation. 2002; 105:753–758. [PubMed: 11839633]

9. Yarbrough WM, Mukherjee R, Brinsa TA, Dowdy KB, Scott AA, Escobar GP, Joffs C, Lucas DG, Crawford FA, Spinale FG. Matrix metalloproteinase inhibition modifies left ventricular remodelling

after myocardial infarction in pigs. J Thoracic Cardiovasc Surg. 2003; 125:602–610. [PubMed: 12658202]

10. Ducharme A, Frantz S, Aikawa M, Rabkin E, Lindseay M, Rohde LE, Schoen FJ, Kelly RA, Werb Z, Libby P, Lee RT. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. J Clin Invest. 2000; 106:55–62. [PubMed: 10880048]

11. Sundstrom J, Evans JC, Benjamin EJ, Levy D, Larson MG, Sawyer DB, Siwik DA, Colucci WS, Sutherland P, Wilson PWF, Vasan RS. Relations of plasma matrix metalloproteinase-9 to clinical cardiovascular risk factors and echocardiographic left ventricular measures. Circulation. 2004; 109:2850–2856. [PubMed: 15173025]

12. Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G, Meyer J, Cambien F, Tiret L, AtheroGene Investigators. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation. 2003; 109:1579–1585. [PubMed: 12668489]

13. Kai H, Ikeda H, Yusukawa H, Kai M, Seki Y, Kuwahara F, Ueno T, Sugi K, Imaizumi T. Peripheral blood levels of matrix metalloproteinases-2 and-9 are elevated in patients with acute coronary syndromes. J Am Coll Cardiol. 1998; 32:368–372. [PubMed: 9708462]

14. Thomas CV, Coker ML, Zellner JL, Handy JR, Crumbley AJ, Spinale FG. Increased matrix metalloproteinase activity and selective upregulation in LV myocardium from patients with end-stage dilated cardiomyopathy. Circulation. 1998; 97:1708–1715. [PubMed: 9591765]

15. Li YY, Feldman AM, Sun Y, McTiernan CF. Differential expression of tissue inhibitors of metalloproteinases in the human heart. Circulation. 1998; 98:1728–1734. [PubMed: 9788826]

16. Mukherjee R, Widener C, Brinsa T, Dowdy KB, Scott AA, Sample J, Hendrick J, Escobar G, Joffs C, Lucas D, Zile M, Spinale F. Myocardial infarct expansion and matrix metalloproteinase inhibition. Circulation. 2003; 107:618–625. [PubMed: 12566376]

17. Yarbrough Mukherjee R, Escobar G, Mingoia JT, Sample JT, Hendrick J, Dowdy KB, McLean JE, Lowry AS, O'Neill TP, Spinale FG. Selective targeting and timing of matrix metalloproteinase inhibition in post-myocardial infarction remodelling. Circulation. 2003; 108:1753–1759. [PubMed: 12975256]

18. Talwar S, Squire IB, Downie PF, McCullough AM, Campton MC, Davies JE, Barnett DB, Ng LL. Profile of plasma N-terminal proBNP following acute myocardial infarction: correlation with left ventricular systolic dysfunction. Eur Heart J. 2000; 21:1514–1521. [PubMed: 10973765]

19. Richards AM, Nicholls MG, Yandle TG, Frampton C, Espiner EA, Turner JG, Buttimore RC, Lainchbury JG, Elliott JM, Ikram H, Crozier IG, Smyth DW. Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: new neurohormonal predictors of left ventricular function and prognosis after myocardial infarction. Circulation. 1998; 1997:1921–1929. [PubMed: 9609085]

20. Kawakami R, Saito Y, Kishimito I, Harada M, Kuwakara K, Takahashi M, Nakagawa Y, Nakanishi M, Tanimoto K, Usami S, Yasuno S, Kinoshita H, Chusho H, Tamura N, Ogawa Y, Nakao K. Overexpression of brain natriuretic peptide facilitates neutrophil infiltration and cardiac matrix metalloproteinase-9 expression after acute myocardial infarction. Circulation. 2004; 110:3306–3312. [PubMed: 15545516]

21. Squire IB, Evans J, Ng LL, Loftus I, Thompson MTJ. Plasma MMP-9 and MMP-2 following acute myocardial infarction in man: correlation with echocardiographic and neurohumoral parameters of left ventricular dysfunction. Cardiac Fail. 2004; 10:328–333. [PubMed: 15309700]

22. O'Brien RJ, Squire IB, Demme B, Davies JE, Ng LL. Pre-discharge, but not admission, levels of NT-proBNP predict advers prognosis following acute LVF. Eur J Heart Fail. 2003; 5:499–506. [PubMed: 12921811]

23. Omland T, Persson A, Ng L, O'Brien R, Karlsson T, Herlitz J, Hartford M, Caidahl K. N-terminal pro-B-type natriuretic peptide and long-term mortality in acute coronary syndromes. Circulation. 2002; 106:2913–2918. [PubMed: 12460871]

24. Wagner RD, Delagardelle C, Ernens I, Rouy D, Vaillant M, Beissel J. Matrix metalloproteinase-9 is a marker of heart failure after acute myocardial infarction. J Cardiac Fail. 2006; 12:66–72. [PubMed: 16500583]

25. Matsunaga T, Abe N, Kameda K, Hagii J, Fujita N, Onodera H, Kamata T, Ishizaka H, Hanada H, Osanai T, Okumura K. Circulating level of gelatinase activity predicts ventricular remodelling in patients with acute myocardial infarction. Int J Cardiol. 2005; 105:203–208. [PubMed: 16243114]

26. Tziakis DN, Chalikias GK, Hatzinikolaou EI, Stakos DA, Tentes IK, Kortsaris A, Hatseras DI, Kaski JC. N-terminal pro B-type natriuretic peptide and matrix metalloproteinases early and late left ventricular remodeling afte acute myocardial infarction. Am J Cardiol. 2005; 96:31–34. [PubMed: 15979428]

27. Webb CS, Bonnema DD, Ahmed SH, Leonardi AH, McClure CD, Clark LL, Stroud RE, Corn WC, Finklea L, Zile MR, Spinale FG. Specific temporal profile of matrix metalloproteinase release occurs in patients after myocardial infarction: relation to left ventricular remodeling. Circulation. 2006; 114:1020–1027. [PubMed: 16923753]

28. Chen J, Tung C-H, Allport JR, Chen S, Weissleder R, Huang Pl. Near infrared fluorescent imaging of matrix metalloproteinase activity after myocardial infarction. Circulation. 2005; 111:1800–1805. [PubMed: 15809374]

29. Schmidt R, Bultmann A, Ungerer M, Joghetaei N, Bulbul O, Thieme S, Triantafyllos C, Toole BP, Gawaz M, Schomig A, May AE. Extracellular matrix metalloproteinase inducer regulates matrix metalloproteinase activity in cardiovascular cells. Circulation. 2006; 113:834–841. [PubMed: 16461815]

30. Lindsey M, Wedin K, Brown MD, Keller C, Evans AJ, Smolen J, Burns AR, Rossen RD, Lloyd M, Entman M. Matrix dependent mechanism of neutrophil-mediated release and activation of matrix metalloproteinase 9 in myocardial ischaemia/reperfusion. Circulation. 2001; 103:2181–2187. [PubMed: 11331260]

31. Tao ZY, Cavasin MA, Yang F, Liu YH, Yang XP. Temporal change in matrix metalloproteinase expression and inflammatory response associated with cardiac rupture after myocardial infarction in mice. Life Sci. 2004; 74:1561–1572. [PubMed: 14729404]

32. Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res. 2002; 53:31–47. [PubMed: 11744011]

33. Romanic AM, Burns-Kurtis CL, Gout B, Berrebi-Bertrand I, Ohlstein EH. Matrix metalloproteinase expression in cardiac myocytes following myocardial infarction in the rabbit. Life Sci. 2001; 68:799–814. [PubMed: 11205871]

34. Bril A, Percevault-Albadini J, Landais J, Toseland C, Gout B. Ventricular remodelling and tolerance to ischaemia in rabbits with myocardial infarction. J Mol Cell Cardiol. 1994; 26:322–330.

35. Heymans S, Luttun A, Nuyens D, Theilmeier G, Creemers E, Moons L, Dyspersin GD, Cleutjens JP, Shipley M, Angellilo A, Levi M, Nube O, Baker A, Keshet E, Lupu F, Herbert JM, Smits JF, Shapiro SD, Baes M, Borgers M, Collen D, Daemen MJ, Carmeliet P. Inhibition of plasminogen activators or matrix metalloproteinases prevent cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure. Nat Med. 1999; 10:1135–1142. [PubMed: 10502816]

36. Schaffer CJ, Nanney B. Cell biology of wound healing. Int Rev Cytol. 1996; 169:151–181. [PubMed: 8843654]

37. Hudson MP, Armstrong RW, Ruzyllo W, Brum J, Cusmano L, Krzeski P, Lyon R, Quinones M, Theroux P, Sydlowski D, Kim HE, Garcia MJ, Jaber WA, Weaver WD. 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. 2006; 48:15–20. [PubMed: 16814643]

38. Shapiro SD. Proteinases in chronic obstructive pulmonary disease. Biochem Soc Trans. 2002; 30:98–102. [PubMed: 12023833]

39. Hautamaki RD, Kobayashi DK, Senior RM, Shapiro SD. Requirement for macrophage elastase for cigarette smoke induced emphysema in mice. Science. 1997; 277:2002–2004. [PubMed: 9302297]

40. Kirchvink N, Vincke G, Fievez L, Onclinx C, Wirth D, Belleflamme M, Louis R, Cataldo D, Peck MJ, Gustin P. Repeated cadmium nebulisations induce pulmonary MMP-2 and MMP-9 production and emphysema in rats. Toxicology. 2005; 211:36–48. [PubMed: 15863246]

41. Perlstein TS, Lee RT. Smoking, metalloproteinases and vascular disease. Arterioscler Thromb Vasc Biol. 2006; 26:250–256. [PubMed: 16339501]
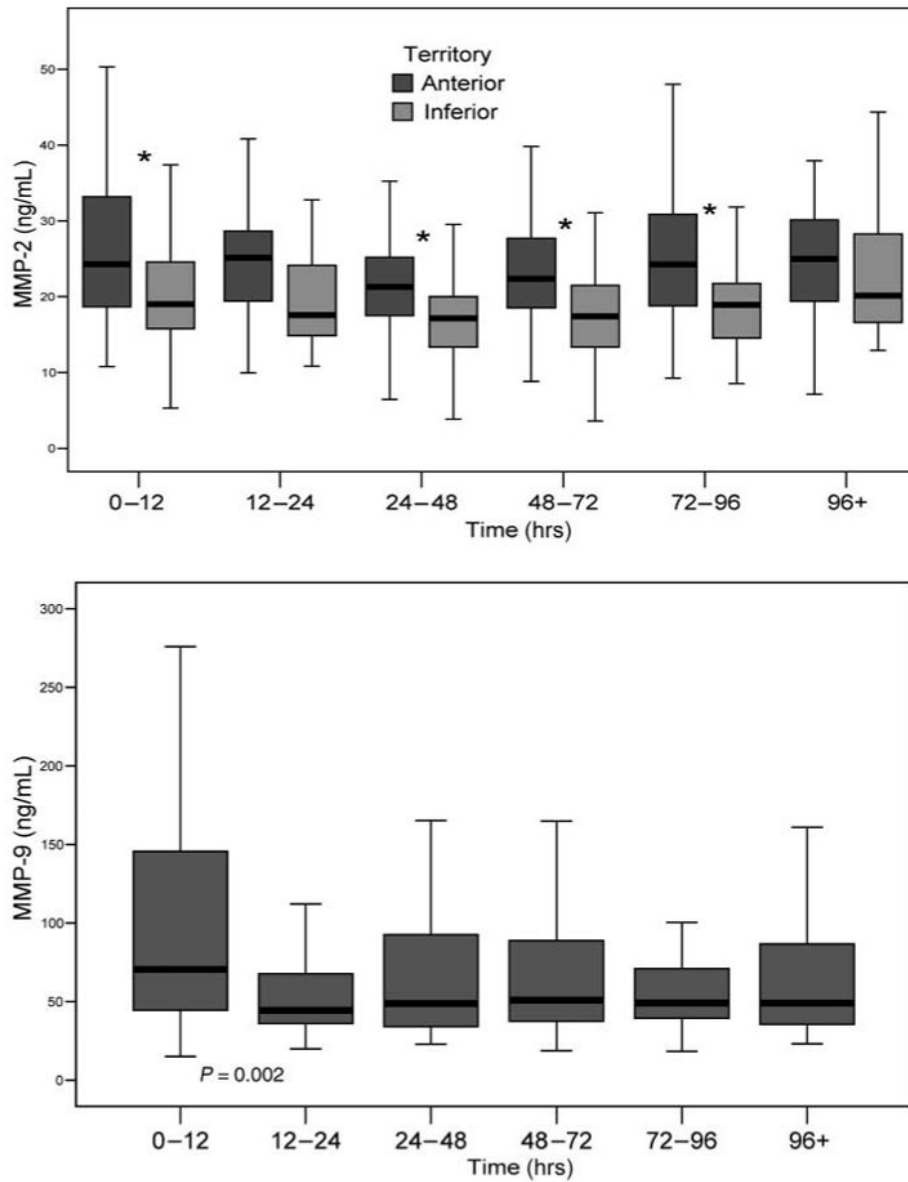
Figure 1.
Temporal profiles of MMP-2 (upper) and MMP-9 (lower) post-AMI. Data are median with upper and lower quartiles and range. Asterisk indicates $P < 0.05$ anterior vs. inferior territory of AMI.
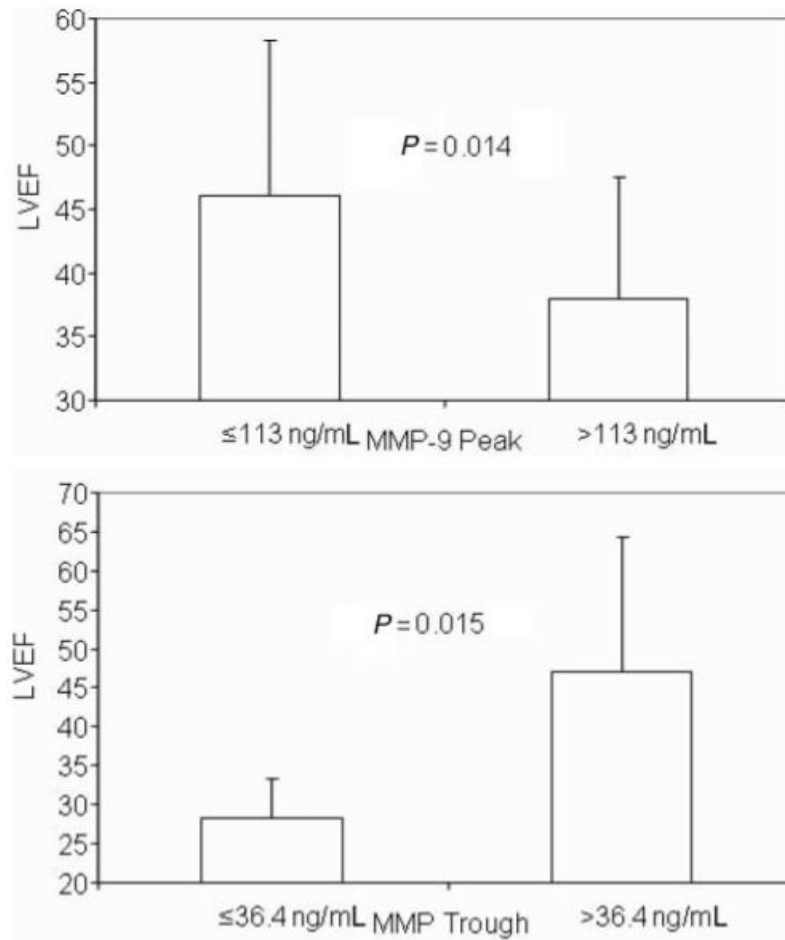
Figure 2.
Upper panel: LVEF according to peak MMP-9 below or above the median value (113 ng/mL). Lower panel: LVEF according to plateau MMP-9 below or above the median value (36.4 ng/mL).
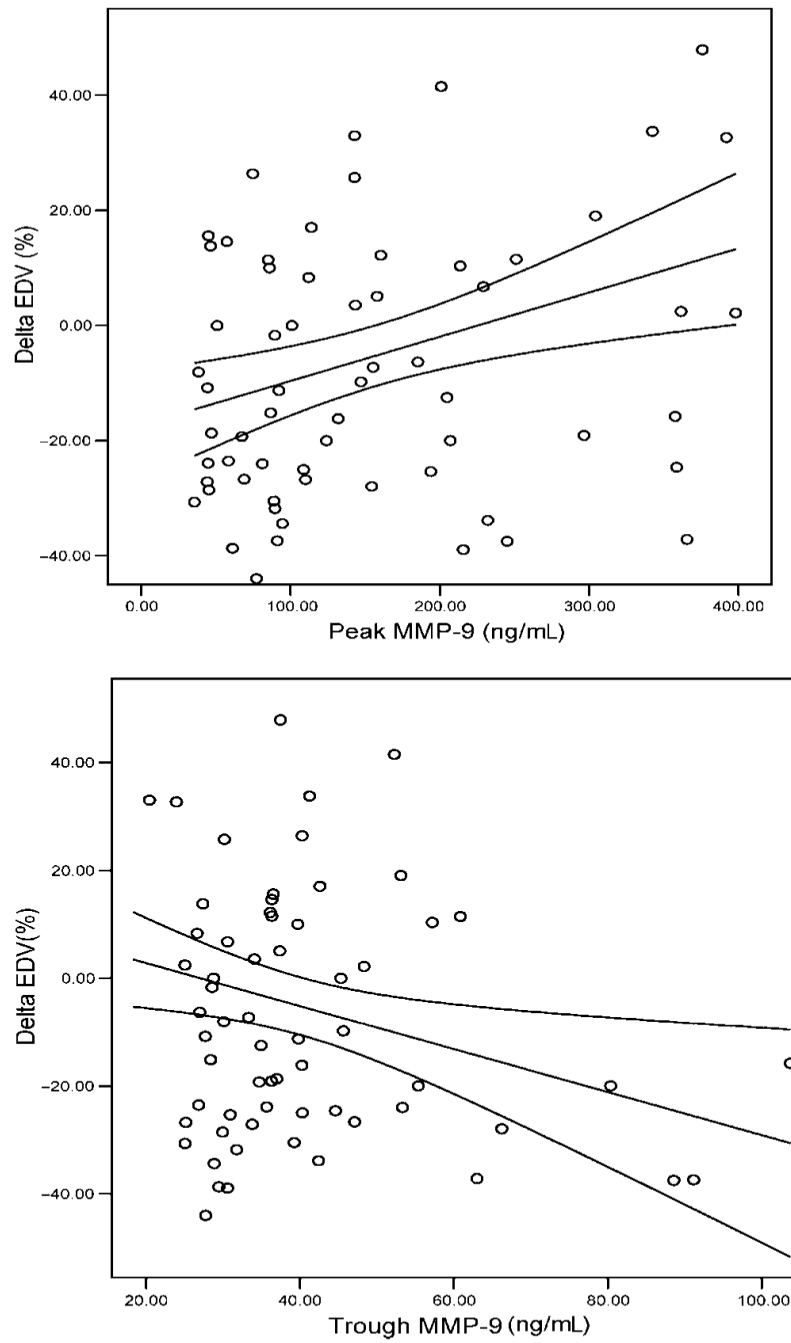
Figure 3.
Relationship between peak MMP-9 (upper) and plateau MMP-9 (lower) and the change in
LVEDV (ΔEDV).

Table 1

Population demographics

	Median	Range
Age (years)	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)	
Previous revascularization	2 (2.2)	
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-I	82 (90)	
ARB	8 (8.8)	
Statin	87 (96)	
Loop diuretic	14 (15.4)	
Spironolactone	2 (2.2)	
Ca antagonist	11 (12.1)	

NR, normal range; ACE-I, angiotensin converting enzyme-inhibitor; ARB, angiotensin receptor blocker.

Table 2

Correlations (R) with significance (P) between peak MMP-9 (above) and trough MMP-9 (lower) and ECG measures predischarge[1] and at follow-up[2] and changes in LV volume (Δ)

	EDV[1]	ESV[1]	LVEF[1]	WMIS[1]	EDV[2]	ESV[2]	LVEF[2]	WMIS[2]	ΔEDV	ΔESV
$R =$	−0.051	0.087	−0.316	0.217	0.149	0.137	−0.131	0.186	0.30	0.123
$P =$	0.648	0.438	0.004	0.048	0.221	0.260	0.281	0.117	0.016	0.331
$R =$	0.106	0.116	0.002	−0.122	0.158	0.318	0.290	−0.230	−0.280	−0.40
$P =$	0.341	0.298	0.984	0.158	0.195	0.008	0.015	0.032	0.024	0.001