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The balance of serum matrix metalloproteinase-8 and its tissue inhibitor in acute coronary syndrome and its recurrence

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Abstract

Background Matrix metalloproteinase-8 (MMP-8) is involved in the breakdown of the extracellular matrix increasing the vulnerability of atherosclerotic lesions. We analyzed the diagnostic value of serum MMP-8 and tissue inhibitor of metalloproteinase-1 (TIMP-1) concentrations in acute coronary syndrome (ACS) and their prognostic value in ACS recurrence.

Methods The population comprised 343 patients with ACS [including 108 unstable angina pectoris and 235 acute myocardial infarctions (AMI)] and 326 healthy controls. Additionally, 157 (45.8%) patients were resampled during the recovery. The ACS patients were followed up for 6 years.

Results MMP-8, TIMP-1, and their molar ratio distinguished the cases from the controls; C-statistic of the multivariate model (95% CI, p-value) including the MMP-8/TIMP-1 ratio regarding its discriminating ability for AMI was 0.922 (0.893-0.950, $p<0.001$). After the acute phase of ACS, median MMP-8 and TIMP-1 concentrations decreased ($p<0.001$) by 34.5 and 28.7%, respectively, but ended up on a different level than those found in the controls. In the follow-up, acute phase and recovery period TIMP-1 concentrations associated with cardiovascular death with hazard ratios 4.31 (2.00-9.26, $p<0.001$) and 4.69 (1.10-20.01, $p=0.037$), respectively.

Conclusions The increase of serum MMP-8 and TIMP-1 concentrations may reflect plaque instability and tissue damage. TIMP-1 concentrations are associated with poor outcome in patients with ACS. The findings may have practical implications in both diagnostics and therapeutics.

Keywords: acute coronary syndrome, acute myocardial infarction, unstable angina pectoris, inflammation, matrix metalloproteinase-8, tissue inhibitor of metalloproteinases-1, plaque rupture

Introduction

Matrix metalloproteinases (MMP) are structurally related but genetically distinct enzymes that can degrade almost all extracellular matrix components. MMPs are secreted mainly by the inflammatory cells to remodel the matrix thereby facilitating the leukocyte traffic through tissues [1]. Their expression is rapidly induced by various cytokines and growth factors, as well as altered cell-cell or cell-matrix interactions; MMPs play an essential role in inflammation.

Several studies have implied that MMPs function in atherosclerosis by participating in the plaque rupture [2, 3] – the underlying causes for the formation of acute coronary syndrome (ACS) and sudden cardiac death [4]. The fibrous cap of the atherosclerotic plaque is rich in collagen, which the MMPs secreted by the local macrophages can degrade. MMPs localize especially in the shoulder regions of the cap; this area is the most vulnerable to rupture resulting in thrombosis [5, 6]. MMPs are endogenously inhibited by the specific tissue inhibitor of metalloproteinases (TIMP), which binds to the active MMP at a molar equivalence [7]. Therefore, the balance between MMPs and TIMPs may be crucial in atherogenesis [8].

MMP-8, also called as collagenase-2 or neutrophil collagenase, may play a role in atherosclerosis. Macrophage-associated MMP-8 protein levels are increased at the rupture site of abdominal aorta aneurysm [9, 10] compared to the normal aorta [11]. The amounts of both total and active MMP-8 are significantly higher in ruptured infarct tissue in patients with fatal myocardial infarction compared to the control infarct tissue [12]. Production of MMP-8 may reflect the disease state, since its activity is higher in asymptomatic patients with plaque progression or plaques prone to rupture compared to those with a stable disease [6, 13]. The association of serum or plasma MMP-8 concentrations with cardiovascular diseases is, however, somewhat conflicting and rarely reported. Therefore, our aim was to investigate in a case-control study the association of serum MMP-8 and TIMP-1 concentrations as well as their molar ratio with ASC and its recurrence. We assessed MMP-8 / TIMP-1 regarding specifically their i) diagnostic ability in ACS and ii) prognostic ability for recurrent ACS and cardiovascular death.

Methods

Subjects

Cases consisted of 343 patients, 270 men and 73 women, who were admitted for ACS to the heart intensive care unit in Lund University Hospital between March 1999 and April 2002 as described earlier in detail [14]. Of the invited patients, 21 died before they could be interviewed and 48 chose not to participate. After admission 12 patients were excluded from the study with the following diagnoses: unspecified precordial pain (n=7), atrial fibrillation, pericarditis, myocarditis, pulmonary embolism and aortic aneurysm. Acute myocardial infarction (AMI) was diagnosed in 235 individuals and unstable angina pectoris (UAP) in 108. An earlier AMI had been diagnosed in 87 patients. The inclusion criteria were age under 80 years, and no signs of cognitive intellectual disability. Patient history as well as information on medications such as the use of antihypertensive or lipid-lowering drugs was collected.

From the patients, 157 (45.8%) were available to give another serum sample three to six months after the acute event during the recovery period. From these, we excluded those patients with a bypass surgery (n=4), chest pain causing admission to hospital (n=3), or angioplasty with a stent (n=1) within two months before the recovery sampling. Coronary angiograms were done on 211 patients, and the percentage of coronary obstruction was determined using Cardiac Review Station, GEMnet, Version 3.1.04.2001 by General Electric Medical system information Technology. In case no flow to the periphery was seen, the arteries were interpreted to be occluded. Cardiovascular death (n=61) or hospitalization for a new ACS event (n=87) was registered during the six-year follow-up (mean 6.02, range 4.56-7.13 years).

Control individuals were chosen from the same area, usually from the same blocks and were matched with age ±2 years, sex, and parish. In almost every case the control lived in the same block as the patient. Inclusion criteria were: no history of definite or suspected coronary heart disease or stroke, and no operations or chemotherapy within the previous 4 weeks. They did not have a positive history of angina i.e. chest pain in any location related to exercise and relieved by rest. None of them had any medication for diabetes, hypertension, or dyslipidemia. A research nurse made a visit to the control person within 5 days. The controls received written information about the study and signed consent to participate. A questionnaire was filled in and 70 ml of blood was collected. The blood vials were put on ice and transferred to the central laboratory for centrifugation.

The participants completed a questionnaire on general background characteristics and demographic data. The follow-up data was obtained from answers to the letters sent to the patients, hospital records or from the mortality data from the population register. The study complies with the Declaration of Helsinki, and the ethical committee of the Lund University approved it. A written informed consent was obtained from the subjects.

Diagnosis

AMI was diagnosed if two of the following criteria were fulfilled: i) typical chest pain lasting over 20 minutes, ii) ST-elevations followed by T-wave inversion or new Q-waves in the ECG, or iii) an increase in CK-MB to more than twice the upper limit of the normal value. UAP was diagnosed in patients with i) continuous ischemic chest pain, ii) transient or persistent ST-segment depression in the ECG (<1 mm), and/or iii) elevation of CK-MB ($5<CK-MB<10$ μg/l) or troponin T ($0.05<TNT<0.10$ μg/l). The location of the infarction, inferior, anterior or other location, was determined from the ECG. When admitted to the hospital, heart failure was diagnosed in patients with signs of pulmonary or peripheral oedema, shock, cyanosis, neck vein stasis, dyspnoe,

basal rhonchi, or rhonchi over the lung fields. Left ventricular dimensions were measured by echocardiography and ejection fraction computed according to the standard formula. Framingham risk scores for coronary heart disease were calculated using cholesterol concentrations as described [15].

Laboratory determinations

Sera without clot activators were collected from the patients within 24 hours after the diagnosis and three to six months after the acute event during the recovery period. From the controls the samples were collected during the home visit made by the research nurse. The laboratory determinations were done on frozen (-20°C) samples.

Serum MMP-8 concentrations were determined by a time-resolved immunofluorometric assay (IFMA) [16]. The inter-assay coefficient of variation (CV) was 7.3% (n=28) and detection limit for the assay 0.08 ng/ml. TIMP-1 ELISA (R&D Systems, Minneapolis MN, USA) was performed according to manufacturer's instructions. The inter-assay CV% was 8.2% (n=28) and the detection limit 0.08 ng/ml.

The details of the methodology of *Chlamydia pneumoniae* heat shock protein 60 (CpnHSP60) and human heat shock protein 60 (HHSP60) IgA and IgG, and high sensitive C-reactive protein (CRP) and serum amyloid A (SAA) are reported earlier [14].

Statistics

Significance of the differences in the characteristics between the cases and the controls was tested by Student's t-test or Chi-square test. Before statistical analyses, parameters with skewed distribution were log-transformed. Linear relationship between covariates was tested with Pearson correlation analyses separately for cases and controls. The diagnostic ability with the sensitivity and specificity of the MMP-8, TIMP-1, and MMP-8/TIMP-1 to distinguish between cases and controls were analysed by the receiver-operating characteristic (ROC) in crude analyses. In order to evaluate if the diagnostic value of these determinations is greater over some traditional CVD risk factors, C-statistics were used. The model included parameters significantly different between the cases and the controls, i.e. CRP, cholesterol concentration, alcohol consumption, and smoking. These results were compared to those additionally adjusted for MMP-8, TIMP-1, or their ratio with a non-parametric paired test (Wilcoxon signed ranks test) [17]. This test was also used to analyse the significance of the differences between the MMP-8 and TIMP-1 concentrations in the samples taken during the acute phase and after the recovery period. At baseline, the associations (OR) between the quartiles of the determined concentrations and the diagnosis were analysed by a multivariate logistic model adjusted for age, sex, CRP and cholesterol concentrations, alcohol consumption, and smoking.

In the follow-up of the cases for 6 years, the prognostic values of serum MMP-8 and TIMP-1 were analysed for samples taken both during the acute phase and the recovery period. The associations between the parameters and the subsequent end points (recurrent ACS and CVD death) were examined by a Cox regression model adjusted only for age and sex, and then further with CRP and cholesterol concentration, alcohol consumption and smoking. Since the risk was clearly associated with the highest quartile and no notable differences were observed between the lower three ones, the comparisons are presented as 4th quartile vs. quartiles 1st-3rd as the reference. In another model with the CVD death as a dependent, the use of lipid lowering medication, ACE inhibitors, PTCA, diabetes, and ejection fractions were additionally included as covariates. The analyses were done using SPSS version 15.0.

Results

Characteristics and determinations during the acute phase of ACS
          The characteristics of the subjects in the case-control sample are presented in Table 1. The ACS patients with either UAP or AMI had lower serum cholesterol and LDL cholesterol concentrations than the controls. The patients had higher serum concentrations of acute-phase reactants, CRP and SAA, as well as IgG-class antibody levels to both chlamydial and human HSP60. The cases smoked and used alcohol less frequently than the controls. In the coronary angiography (n=211), total occlusion in some of the branches of the coronary tree was seen in 86 (40.8%), 100 (47.4%) patients had a mean of 73% (range 36-99%) obstruction, and 25 (11.8%) patients had no obstruction.

          In the cases, MMP-8 (p<0.001) correlated with CRP (r=0.315) and SAA (r=0.267) concentrations. HHSP60 and CpnHSP60 IgA-class antibody levels correlated with serum TIMP-1 concentration (r=0.225, p=0.001; and r=0.197, p=0.004). These correlations were weak among the controls. The cases had significantly higher serum MMP-8 and TIMP-1 concentrations as well as MMP-8/TIMP-1 ratios than the controls (Table 2). These serum parameters had a moderate but significant correlation with the highest troponin T (TnT) and CK-MB concentrations with r~0.2. MMP-8 or TIMP-1 concentrations did not differ in patients with an anterior AMI compared to those with inferior or other location of AMI. Furthermore, the patients with signs of heart failure when admitted to the hospital (n=32) did not have significantly different MMP-8 or TIMP-1 concentrations than those without.

The diagnostic specificity and sensitivity of MMP-8, TIMP-1, and MMP-8/TIMP-1 during ACS acute phase
          The diagnostic specificity and sensitivity of the assays were tested with ROC analysis and C-statistics (Table 3). The cases and the controls could be significantly (p<0.001) distinguished from each other with the crude areas under the curve (AUC) of 0.729 for MMP-8, 0.668 for TIMP-1, and 0.757 for MMP-8/TIMP-1 ratio. UAP and AMI were distinguished from the controls by MMP-8 and MMP-8/TIMP-1 almost equally (Table 3). In C-statistics, MMP-8 and MMP-8/TIMP-1, but not TIMP-1 alone, clearly increased the sensitivity and specificity of CRP, cholesterol, and some demographic data to diagnose ACS, especially in the subgroups (Table 3, p<0.05 for all).

Association of MMP-8, TIMP-1, and MMP-8/TIMP-1 with ACS during the acute phase
          The association between ACS, UAP, and AMI and the quartiles of the determined serum values are presented in Table 4. In multivariate analysis, high serum MMP-8 concentration and MMP-8/TIMP-ratio (4th quartile vs. 1st) associated with ACS, UAP, and AMI (Table 4). In crude analyses, also high TIMP-1 concentration associated with ACS, UAP, and AMI with ORs of 1.51 (1.10-2.06, p=0.010), 1.70 (0.96-3.02, p=0.069), and 1.73 (1.19-2.53, p=0.005), respectively. In the multivariate analysis, however, the associations with TIMP-1 were abolished.

MMP-8, TIMP-1, and MMP-8/TIMP-1 levels during the recovery period
          The median MMP-8 and TIMP-1 levels decreased significantly three to six months after the ACS acute phase: the average decrease was 34.5 (p<0.001) and 28.7% (p<0.001), respectively. In all subgroups, however, the MMP-8 concentrations remained higher, and the TIMP-1 concentrations decreased to a lower level than those seen in the healthy controls (Table 1, Figure 1). As percentages, the decrease of MMP-8 and TIMP-1 was most notable in the UAP patients, who got another cardiovascular event in the six-year follow-up, 58.1 (p=0.027) and 38.7% (p<0.001), respectively.

Association of MMP-8, TIMP-1, and MMP-8/TIMP-1 levels with the recurrent ACS events

During the mean follow-up of six years, new ACS events (n=89) and deaths of CVD causes (n=61) were registered. The baseline characteristics of the patients with and without an event are summarized in Table 5. The patients with a recurrent ACS had lower (p<0.01) serum CRP, SAA, and TIMP-1 concentrations compared to those without. The patients who died due to CVD during the follow-up were older (p<0.001), more often diabetic (p<0.001), had lower ejection fractions (p<0.05), had had less often PTCA (p<0.001), and had higher serum TIMP-1 (p<0.001) than those who did not die. The Framingham risk scores were not associated with the recurrent events. MMP-8 concentration during the acute phase associated with smaller risk for a recurrent ACS with a HR (95% CI) of 0.55 (0.34-0.87, p=0.010) when adjusted for age and sex, but not in the further adjusted models (data not shown). A strong direct association was found between TIMP-1 concentration and CVD death: the HR was 4.31 (2.00-9.26, p<0.001) using the concentrations in the acute phase (Figure 2) and 4.69 (1.10-20.01, p=0.037) using the concentrations during the recovery period (Table 6). The use of lipid lowering medication or ACE-inhibitors, PTCA, diabetes, or ejection fraction did not have a notable effect on these results.

Discussion

In the present case-control study, high serum MMP-8 concentrations and MMP-8/TIMP-1 ratios were strongly associated with ACS. In the ROC analyses, MMP-8 and MMP-8/TIMP-1 were clearly able to distinguish the UAP or the AMI patients from the healthy controls despite the strongest confounding factors, smoking and CRP [16]. After the acute phase, the decrease of MMP-8 and TIMP-1 was most notable in the UAP patients, who faced another cardiovascular event in the six year follow-up. In the acute phase and during the recovery period, TIMP-1 concentration associated with CVD death in the prospective setting.

Elevation of human and chlamydial HSP60 titres at the time of an acute coronary event suggests that persistent inflammation existed before the acute event. Chlamydial HSP60 has been reported to co-localize with human HSP60 in arterial plaque macrophages and can promote TNF-α and MMP production [18]. On the other hand, TIMP-1 may also exert continuous damaging stimulus on the vascular wall, inducing stimulating smooth muscle cell proliferation [19] and promoting inflammation [20]. Inflammatory activity in the arterial wall may account for elevated TIMP-1 concentrations.

Although the source of serum MMP-8 in the present study is not known, it may partially become liberated from ruptured or eroded atherosclerotic plaques. Type I collagen is the major load-bearing molecule of the fibrous cap, whose thinning and weakening at the shoulder regions enriched with lipid-laden macrophages establishes the mechanisms for plaque rupture [21]. This leads to acute manifestations of atherosclerosis, myocardial infarction and unstable angina pectoris [22]. On the other hand, MMP-8 concentrations might have increased due to the inflammatory process before the acute events. The elevated CRP concentrations probably reflect the degree of myocardial damage, which may also be a partial source of MMP and TIMP [23].

The association between MMP-8 concentrations and UAP has been reported in a recent article and the results are in agreement with ours [24]. In publications earlier, high circulating MMP-8 levels have been related to the presence and severity of coronary artery disease [25], carotid artery plaque progression [13], and subclinical atherosclerosis [16], whereas the concentrations have been reported to be decreased in patients with heart failure [26] or cerebral ischemia [27]. In our previous prospective study with a follow-up time of 10 years, serum MMP-8 concentrations associated with AMI as well as death from coronary heart disease, CVD, or from any cause in men free of CVD at baseline. In men with no prevalent CVD but with subclinical atherosclerosis at baseline, high serum MMP-8 concentration associated with CVD death with a relative risk of 3.03 (95% CI 1.09 to 8.44) [16]. MMP-8 did not, in agreement with the present study, associate with an increased risk of a secondary CVD end point [16].

The equilibrium between MMPs and TIMPs is believed to be crucial in the development and progression of atherosclerosis [8, 28]. Interestingly in the present study, MMP-8 and TIMP-1 decreased after the acute phase in the patients: the MMP-8 levels did not reach those found in the controls, when again the TIMP-1 declined to an even lower level than that of the controls. This suggests that after the recovery period the coronary patients still have inflammatory activity in the arterial wall. However, it remains to be explained why the decrease of MMP-8 and TIMP-1 was most significant after the acute phase in the patients facing another cardiovascular event in the follow-up. In the follow-up, high TIMP-1 concentrations correlated with CVD death but high MMP-8 displayed protective against recurrent ACS, although the significant association of MMP-8 was lost after adjusting for CRP concentrations. Due to its ability to process non-matrix bioactive substrates, i.e. chemokines, cytokines, growth factors, and immune mediators, MMP-8 may exert anti-inflammatory and defensive characteristics as recently shown by null-allele model

studies regarding lung and periodontal inflammation [29, 30]. Increased TIMP-1 concentration may be an epiphenomenon or an adaptive response to an increase in MMP activity: coronary disease maintains the MMP-8 concentration high and the body responds by elevating TIMP-1 [8]. The results imply that, if TIMP-1 is "over produced" as a response to acute phase MMP-8 expression or if its production is not suppressed enough during the recovery, the prognosis is poor. Also in two earlier follow-up studies in patients with ACS or coronary artery disease, high serum TIMP-1 concentrations have been associated with the risk of MI and cardiac death [8, 31]. As TIMP-1 functions not only as a MMP-8 inhibitor, but also exerts proinflammatory and growth factor –like characteristics, it may promote inflammation and atherogenesis [19, 20]. The situation may be different in subjects free of CVD. In them, serum TIMP-1 alone did not associate with an incident CVD event [16].

There are some limitations in the study. We did not have the blood pressure values of the controls to calculate the Framingham risk scores. In the cases, however, the scores were not associated with the recurrence of ACS, which is not that surprising since the scores were designed to be used in the primary prevention [15]. Serum MMP-8 and TIMP-1 concentrations may relate with obesity, diabetes, and use of statins, ACE-inhibitors and aldosterone antagonists [25, 32, 33]. Unfortunately, we did not have information on the body mass indexes of the subjects. This is likely to have only a restricted effect on the MMP-8 and ACS association, which was very strong and mainly confounded by inflammation markers and smoking. The assumption is also supported by the results of the Cox analyses of the follow-up data, where the use of lipid lowering medication or ACE-inhibitors did not have a notable effect on the outcome. One limitation may be the use of serum instead of plasma [34]. The clotting process is known to release MMPs from circulating leukocytes and increase their levels, especially in the presence of clot activators, which, however, were not used in the present sampling. Nevertheless, serum and plasma MMP-8 levels have a strong correlation with each other [34]. The case-control study design may have its limitations comparing the past exposures of the groups, although it is a suitable setting for finding new biomarkers. The statistical analyses were multiple, but they all were driven by the specified hypotheses resulting in highly significant p-values.

The population was collected in 1999 to 2002 after which several diagnostic, prognostic and therapeutic changes have occurred, which might influence the generability of the study. If the present diagnostic criteria based on high sensitivity troponin-T had been applied, the distribution of the patients between the groups (controls, UAP, AMI) would have been altered. Such change is unlikely to invalidate the main findings of our study, as the changes in MMP-8 and TIMP-1 were seen both in the UAP and the AMI group. The therapy given to the patients was not completely up to date, but not markedly inferior to the present standard: a majority of the patients had a coronary angiography, all patients with a coronary intervention received either ticlopidine or clopidrogrel and about two thirds were on statins. It is also possible that some of the controls may have had asymptomatic coronary artery disease considering their age. Their incidence of coronary artery disease was relatively low and unlikely to affect the strong association found between MMP-8 and ACS at baseline: in the six year long follow-up, 5.8% of the controls got ACS and 4.3% died due CVD compared to the rates of 25.4 and 17.8% observed among the patients.

MMP-8 and its ratio to TIMP-1 participate in the mechanisms involved in the acute coronary syndrome. Elevated TIMP-1 concentrations during the acute phase and the recovery period are associated with CVD death in patients with ACS. The results imply that MMP-8 may have a strong role in diagnostics of ACS. At the same time its counterpart, TIMP-1, does not alone have that important diagnostic role but a significant prognostic role in ACS recurrence. The present findings may have practical implications in both diagnostics and therapeutics.

Disclosure of interests: none declared

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Figure 1
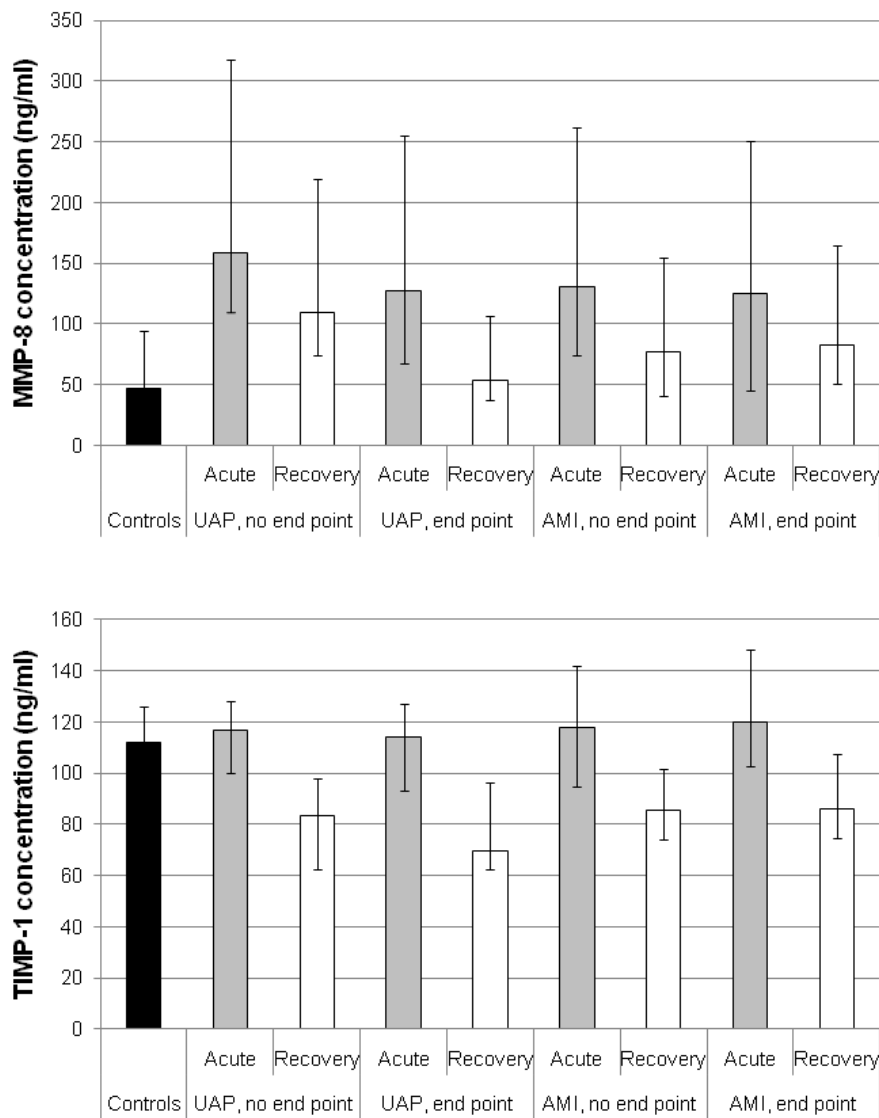
Figure 1. Serum MMP-8 and TIMP-1 concentrations in 157 patients during the acute coronary syndrome and after a six month recovery period. The median and interquartile ranges are shown for unstable angina pectoris (UAP) patients with (n=23) and without (n=32) and acute myocardial infarction (AMI) patients with (n=39) and without (n=63) a new coronary event during the six following years. The median serum levels of the healthy controls (n=326) are also shown for reference.

Figure 2
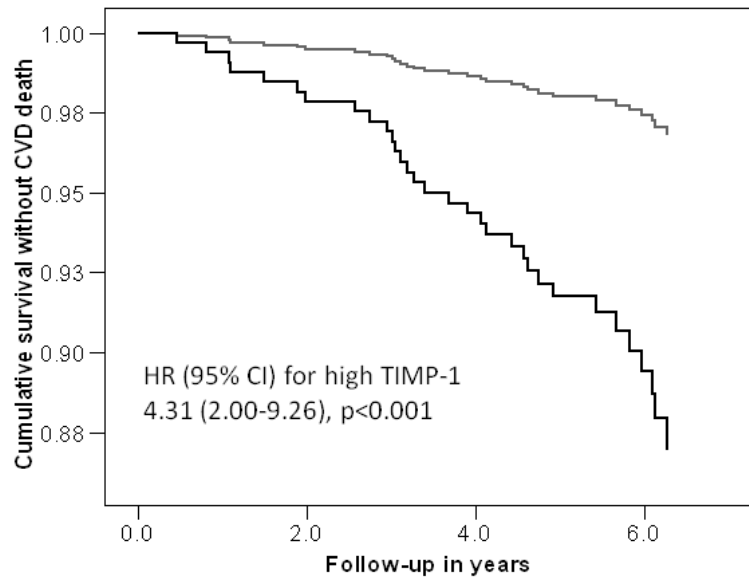
Figure 2. Association of serum TIMP-1 concentrations with CVD death in the follow-up. The patients with ACS (n=343) sampled during the acute phase were followed up for 6 years and CVD deaths (n=61) were registered. The associations of TIMP-1 quartiles (4th vs. 1st-3rd) with the end point were analysed by Cox regression model adjusted for age, sex, CRP and cholesterol concentration, alcohol consumption, and smoking.

Table 1. Characteristics of the subjects in the case-control sample. Cases with ACS include patients with UAP or AMI.

|	Controls (n=326)	Cases (n=343)		
		All ACS	UAP (n=108)	AMI (n=235)
		Mean (SD)		
Age (years)	63.0 (9.2)	63.3 (9.2)	63.3 (9.2)	63.4 (9.2)
Cholesterol (mmol/l)	5.8 (1.1)	5.3 (1.3)‡	5.3 (1.5) †	5.3 (1.2) ‡
LDL cholesterol (mmol/l)	3.68 (1.05)	3.27 (1.09)‡	3.09 (0.96)*	3.34 (1.14) †
CRP (mg/l)	2.30 (2.41)	26.1 (41.7) ‡	10.5 (20.3) ‡	33.2 (46.7) ‡
SAA (mg/l)	4.07 (4.17)	90.2 (223.8) ‡	23.8 (66.0) †	120.4 (260.9) ‡
Cigarettes (/day)	16.9 (12.4)	15.8 (9.0)	16.0 (10.4)	15.7 (8.2)
CpnHSP60-IgA (EU)	0.37 (0.36)	0.41 (0.39)	0.41 (0.40)	0.41 (0.39)
CpnHSP60-IgG (EU)	0.32 (0.47)	0.38 (0.55)*	0.41 (0.63)	0.38 (0.51)*
Human HSP60-IgA (EU)	0.36 (0.25)	0.33 (0.18)	0.32 (0.16)	0.34 (0.18)
Human HSP60-IgG (EU)	0.67 (0.48)	0.70 (0.48)*	0.61 (0.41)	0.74 (0.51)*
Creatine kinase-MB (IU/l)	ND	110.1 (153.4)	7.29 (19.8)	157.2 (164.6)
Troponin T (µg/l)	ND	3.0 (5.1)	0.17 (0.37)	4.2 (5.7)
		n (%)		
Sex (% men)	254 (77.9)	275 (78.7)	90 (83.3)	180 (76.6)
Current smoker	68 (20.9)	55 (16.0)	10 (9.3)*	45 (19.2)
Alcohol consumption (yes)	277 (85.0)	232 (67.6) †	71 (65.7) †	161 (68.5)*
Enclosed in inclusion criteria[§]:				
Diabetic	0 (0)	46 (13.4)	12 (11.1)	34 (14.5)
Earlier PTCA/CABG	0 (0)	64 (18.7)	34 (31.5)	30 (12.8)
Earlier AMI	0 (0)	87 (25.4)	41 (38.0)	46 (19.6)

Medicines, treatments, characteristics[§]:
When entering hospital   -

Lipid lowering medication	-	77 (22.5)	40 (37.0)	37 (15.7)
ACE/ATII blockers	-	58 (16.9)	18 (16.7)	40 (17.0)
Beta blockers	-	128 (37.3)	51 (47.2)	77 (32.8)
Calcium blockers	-	54 (15.7)	19 (17.6)	35 (14.9)
Digitalis	-	15 (4.4)	6 (5.6)	9 (3.8)
Diuretics	-	55 (16.0)	15 (13.9)	40 (17.0)
Medication for diabetes	-	38 (11.0)	8 (7.4)	30 (12.8)
Long-acting nitroglycerin	-	61 (17.8)	34 (31.5)	27 (11.5)
In the hospital	-			
Systolic blood pressure (mmHg)	-	140 (20)	141 (17)	139 (21)
Diastolic blood pressure (mmHg)	-	80 (11)	79 (10)	80 (12)
Framingham risk score	-	10.6 (3.4)	10.3 (3.3)	10.7 (3.4)
Ejection fraction (%)		66.6 (17.7)	73.1 (14.6)	64.1 (18.2)
Inotropic support	-	9 (2.6)	1 (0.9)	8 (3.4)
Isoprenaline	-	2 (0.6)	0 (0)	2 (0.8)
Acute PTCA	-	35 (10.2)	0 (0)	35 (14.9)
Heart failure	-	32 (9.3)	12 (11.1)	20 (8.5)
Re-AMI	-	2 (0.6)	0 (0)	2 (0.9)
PTCA	-	144 (42.0)	36 (33.3)	108 (46.0)
CABG	-	63 (18.4)	23 (21.3)	40 (17.0)
Defibrillation	-	1 (0.3)	0 (0)	1 (0.4)
Ventricular tachycardia ≥10 in a row	-	17 (4.9)	1 (0.9)	16 (6.8)
Asystole	-	8 (2.3)	1 (0.9)	7 (2.9)
Died	-	4 (1.2)	1 (0.9)	3 (1.3)
PTCA class (n=211)	-			
no significant stenosis	-	25 (11.8)	12 (19.7)	13 (8.7)
obstruction	-	100 (47.4)	29 (47.5)	71 (47.3)
occlusion	-	86 (40.8)	20 (32.8)	66 (44.0)
Follow-up for 6 years[§]:				
Lipid lowering medication	0 (0)	228 (66.5)	72 (66.7)	156 (66.4)
ACE/ATII blockers	0 (0)	139 (40.5)	28 (25.9)	111 (47.2)
Beta blockers	0 (0)	253 (73.8)	72 (66.7)	181 (77.0)
Calcium blockers	0 (0)	47 (13.7)	22 (20.3)	25 (10.6)

ACS	19 (5.8)	87 (25.4)	35 (32.4)	54 (23.0)
CVD death	14 (4.3)	61 (17.8)	17 (15.7)	44 (18.7)

* p<0.05, † p<0.01, ‡ p<0.001 compared to the control group; § parameters enclosed to inclusion criteria, no statistical testing

ACS, acute coronary syndrome; UAP, unstable angina pectoris; AMI, acute myocardial infarction; LDL, low density lipoprotein; CRP, C-reactive protein; SAA, serum amyloid A; Cpn, *Chlamydia pneumoniae*; HSP, heat shock protein; ND, not determined; PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass graft

Table 2. Serum concentrations for MMP-8, and TIMP-1 and MMP-8/TIMP-1 ratios in the case-control sample. Cases with ACS include patients with UAP or AMI.

Group	MMP-8 (ng/ml)	TIMP-1 (ng/ml)	MMP-8/TIMP-1
		Median (IQR), p-value	
Controls (n=326)	47.1 (22.8-98.4)	112 (97-126)	0.16 (0.07-0.31)
ACS (n=343)	118.2 (56.2-221.1), <0.001*	119 (99-146), <0.001*	0.34(0.17-0.60), <0.001*
UAP (n=108, 31.5%)	81.8 (45.5-184.2), <0.001*	116 (99-142), 0.042*	0.28 (0.13-0.57), <0.001*
AMI (n=235, 68.5%)	124.3 (68.9-232.5), <0.001*	122 (99-151), <0.001*	0.36 (0.20-0.65), <0.001*
ST elevation - (n=113, 48%)	140.7 (70.5-250.2), 0.168†	125 (102-163), 0.117†	0.40 (0.21-0.77), 0.381†
ST elevation + (n=122, 52%)	115.4 (66.1-219.6)	120 (97-144)	0.34 (0.18-0.58)
Inferior (n=74, 60.7%)	143.0 (64.3-292.5), 0.624 ‡	119 (92-154), 0.152 ‡	0.43 (0.16-0.91), 0.992 ‡
Anterior (n=33, 27.0%)	142.2 (101.8-238.3)	130 (106-171)	0.38 (0.25-0.61)
Other (n=15, 12.3%)	154.1 (68.4-266.6), 0.795 ‡	111 (105-147), 0.230 ‡	0.40 (0.15-0.92), 0.992 ‡

* p-value compared to the control group, † p-value compared to the group with ST elevation AMI; ‡ p-value compared to the group with anterior AMI; ACS, acute coronary syndrome; UAP, unstable angina pectoris; AMI, acute myocardial infarction; ST, ST-segment

Table 3. Analyses of the diagnostic value of serum MMP-8 and TIMP-1 concentrations and MMP-8/TIMP-1 ratio for ACS, UAP, and AMI in the case-control sample.

	ACS (n=343)	UAP (n=108)	AMI (n=235)
	Area under the curve (95% CI)		
ROC			
MMP-8	0.729 (0.692-0.767)	0.576 (0.533-0.620)	0.707 (0.668-0.746)
TIMP-1	0.668 (0.610-0.726)	0.540 (0.473-0.608) ‡	0.660 (0.602-0.719)
MMP-8/TIMP-1	0.757 (0.718-0.797)	0.593 (0.543-0.643)	0.729 (0.686-0.770)
C-statistics			
Basic *	0.859 (0.824-0.894)	0.773 (0.697-0.849)	0.901 (0.868-0.934)
Basic +MMP-8†	0.891 (0.861-0.920)	0.824 (0.757-0.891)	0.920 (0.892-0.948)
Basic +TIMP-1†	0.867 (0.833-0.901)	0.772 (0.696-0.849)	0.907 (0.875-0.940)
Basic +MMP-8/TIMP-1†	0.893 (0.863-0.922)	0.823 (0.755-0.891)	0.922 (0.893-0.950)

*Including C-reactive protein, cholesterol concentration, alcohol consumption, and smoking
† Additionally included MMP-8 or TIMP-1 concentration or MMP-8/TIMP-1 ratio
‡ p=0.211; all other p-values <0.001
ACS, acute coronary syndrome; UAP, unstable angina pectoris; AMI, acute myocardial infarction; ROC, receiver operating characteristic

Table 4. Odds ratios for the cardiovascular event in the quartiles of serum MMP-8 and TIMP-1 concentrations and MMP-8/TIMP-1 ratio.

	Odds ratio (95% CI) compared to the 1st quartile			
	2nd	3rd	4th	p for trend*
Acute coronary syndrome				
MMP-8	2.17 (1.32-3.59)	3.03 (1.86-4.93)	3.30 (2.03-5.38)	**<0.001**
TIMP-1	1.10 (0.74-1.63)	1.00 (0.66-1.51)	1.15 (0.76-1.76)	0.507
MMP-8/TIMP-1	2.20 (1.34-3.63)	2.77 (1.70-4.53)	3.42 (2.13-5.51)	**<0.001**
Unstable angina pectoris				
MMP-8	2.95 (1.08-8.10)	4.21 (1.57-11.3)	7.28 (2.78-19.1)	**0.001**
TIMP-1	1.13 (0.51-2.46)	1.02 (0.46-2.27)	1.10 (0.42-2.86)	0.990
MMP-8/TIMP-1	4.29 (1.56-11.8)	3.67 (1.27-10.7)	8.29 (3.11-22.1)	**<0.001**
Acute myocardial infarction				
MMP-8	2.13 (1.18-3.84)	3.19 (1.80-5.67)	3.39 (1.90-6.05)	**<0.001**
TIMP-1	1.03 (0.64-1.65)	0.94 (0.57-1.55)	1.18 (0.72-1.94)	0.829
MMP-8/TIMP-1	2.09 (1.16-3.79)	2.94 (1.67-5.18)	3.59 (2.03-6.32)	**<0.001**

* Adjusted for age, sex, CRP, cholesterol concentration, alcohol consumption, and smoking by a logistic regression model. The samples of the patients were taken during the acute phase of ACS.
MMP-8 quartiles: 0.48-1.53, 1.53-1.88, 1.88-2.19, and 2.20-3.34 ng/ml. TIMP-1 quartiles: 61.0-97.0, 98.0-114.0, 115.0-135.0, and 136.0-298.0 ng/ml. MMP-8/TIMP-1 quartiles: 0.01-0.11, 0.11-0.23, 0.23-0.47, and 0.47-5.93.

Table 5. Baseline characteristics of the ACS patients with and without registered CVD event in the follow-up.

	No recurrent ACS	Recurrent ACS	No CVD death	CVD death
	Mean (SD)			
Age (years)	63.4 (9.5)	63.1 (8.2)	62.2 (9.0)	69.1 (7.4) ‡
Cholesterol (mmol/l)	5.3 (1.1)	5.3 (1.6)	5.3 (1.1)	5.0 (1.1)
LDL cholesterol (mmol/l)	3.28 (1.00)	3.24 (1.00)	3.33 (1.09)	2.95 (1.09)
CRP (mg/l)	30.1 (45.9)	14.7 (22.8) ‡	23.9 (40.4)	37.1 (46.6) *
SAA (mg/l)	110.0 (253.3)	34.4 (79.3) †	86.7 (230.3)	111.8 (199.9)
Creatine kinase-MB (IU/l)	115.9 (154.2)	100.0 (155.2)	115.0 (160.4)	101.5 (128.2)
Troponin T (µg/l)	3.4 (5.5)	2.0 (4.0)*	2.9 (5.0)	3.8 (6.0)
Hemoglobin (g/l)	128.1 (15.2)	130.7 (15.2)	129.3 (15.1)	122.1 (14.6)
Systolic blood pressure (mmHg)	140 (21)	139 (19)	139 (19)	146 (28)
Diastolic blood pressure (mmHg)	79.4 (12)	81 (10)	80 (11)	77 (14)
Framingham risk score	10.6 (3.4)	10.6 (3.2)	10.6 (3.3)	10.3 (3.6)
Ejection fraction (%)	65.6 (19.0)	68.6 (15.2)	67.2 (17.6)	59.8 (20.8) *
	Median (IQR)			
MMP-8 (ng/ml)	117.1 (55.7-233.6)	118.2 (64.4-185.9)	115.4 (55.0-212.5)	130.1 (68.2-264.2)
TIMP-1 (ng/ml)	123.0 (101.0-152.0)	109.0 (94.0-127.0) †	113.5 (95.0-137.0)	158.0 (125.0-188.5) ‡
MMP-8/TIMP-1	0.34 (0.16-0.61)	0.37 (0.19-0.59)	0.34 (0.17-0.61)	0.33 (0.18-0.54)
	n (%)			
Sex (% men)	202 (79.5)	68 (76.4)	222 (79.9)	45 (73.8)
Current smoker	45 (17.7)	10 (11.2)	49 (17.6)	5 (8.2)*
Alcohol consumption (yes)	168 (81.6)	64 (76.2)	206 (80.8)	23 (71.9)
Diabetic	35 (14.2)	11 (12.8)	27 (10.0)	18 (30.5) ‡
PTCA class (n=211)				
no significant stenosis	18 (11.7)	7 (12.7)	22 (12.2)	1 (4.0)

obstruction	73 (47.4)	25 (45.5)	87 (48.3)	11 (44.0)
occlusion	63 (40.9)	23 (41.8)	71 (39.4)	13 (52.0)
PTCA	98 (39.7)	41 (47.7)	125 (46.3)	12 (20.3) ‡
CABG	43 (17.4)	10 (11.6)	43 (15.9)	10 (16.9)*

* p<0.05, † p<0.01, ‡ p<0.001; no recurrent ACS compared to recurrent ACS; no CVD death compared to CVD death

Table 6. Hazard ratios for CVD death according to serum MMP-8 and TIMP-1 concentrations and MMP-8/TIMP-1 ratio in the 6 year follow-up of the cases.

End point	n/tot n	Hazard ratio (95% CI) in the 4th quartile compared to the quartiles 1st-3rd	
		HR	p*
CVD death			
Sampled during the acute phase	61/343		
MMP-8		0.94 (0.40-2.20)	0.882
TIMP-1		4.31 (2.00-9.26)	**<0.001**
MMP-8/TIMP-1		0.52 (0.20-1.35)	0.176
Sampled during the recovery period	14/157		
MMP-8		0.82 (0.38-1.77)	0.614
TIMP-1		4.69 (1.10-20.06)	**0.037**
MMP-8/TIMP-1		0.29 (0.03-3.09)	0.304

* Analyzed by the Cox regression model adjusted by age, sex, CRP, cholesterol concentration, alcohol consumption, and smoking. The cases were sampled during the acute phase and the recovery phase.