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Relation of Baseline Plasma MMP-1 Levels to Long-term All-Cause Mortality in Patients with Known or Suspected Coronary Artery Disease Referred for Coronary Angiography

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Abstract

Objectives—To investigate the long-term prognostic significance of baseline plasma MMP-1 levels in a group of well-characterized male patients with known or suspected coronary artery disease, including those presenting with the acute coronary syndrome.

Background—MMP-1 is an interstitial collagenase that is considered the primary enzyme responsible for collagen degradation. In addition, MMP-1 can lead to platelet activation through the PAR1 pathway that is independent of thrombin.

Methods—Baseline plasma MMP-1 levels were measured in 364 male patients who were referred for coronary angiography and followed prospectively for the development of all-cause mortality for 5 years.

Results—After adjustment for a variety of baseline clinical, angiographic and laboratory parameters, baseline plasma MMP-1 levels (analyzed as a continuous variable) was an independent predictor of all-cause mortality at 5 years (HR, 1.49; 95% CI, 1.23-1.80; P<0.0001). Furthermore, in 3 additional multivariate models that included a wide variety of contemporary biomarkers with established prognostic efficacy (i.e., ST2, GDF-15, cystatin C, hs-CRP, myeloperoxidase, TIMP-1, adiponectin, RDW, hemoglobin, erythropoietin), MMP-1 remained an

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independent predictor of all-cause mortality at 5 years. Similar results were obtained when the analyses were restricted to the subpopulation of patients presenting with acute coronary syndrome.

Conclusions—Elevated levels of MMP-1 are associated with an increased risk of long-term all-cause mortality in patients with known or suspected coronary disease that is independent of a variety of clinical, angiographic, laboratory variables, including a whole host of contemporary biomarkers with established prognostic efficacy representing multiple different pathophysiologic processes.

Keywords

matrix metalloproteinase 1; biomarkers; acute coronary syndrome; prognosis; mortality

Introduction

Matrix metalloproteinases (MMPs) are a family of extracellular matrix degrading enzymes implicated in plaque destabilization (1,2). MMPs are expressed by macrophages, VSMC, and endothelial cells in response to inflammatory stimuli (2,3). MMP-1 is an interstitial collagenase that is considered the primary enzyme responsible for collagen degradation (3). MMP-1 is primarily detected in the vulnerable shoulder region and areas of foam cell formation in the atherosclerotic plaques where it colocalizes with degraded collagen fragments (1,4-6). Accordingly, MMP-1 has been associated with total plaque burden (2). Furthermore, platelets are known to harbor MMP-1, which is released after exposure of platelets to thrombin (3,7). MMP-1 is then subsequently capable of directly cleaving PAR1 on the platelet surface (3,8). PAR1 is a G-protein-coupled receptor that is classically activated through cleavage of the N-terminal exodomain by the serine protease thrombin (9). Thus, MMP-1 can lead to platelet activation through the PAR1 pathway that is independent of thrombin. Collectively, these basic observations suggest that MMP-1 may play a pathogenic role in atherosclerosis and its complications through two different, yet complementary, mechanisms – namely, matrix-degradation in the vessel wall and thrombin-independent effects on PAR1 and platelet activation (3).

Despite these basic science observations potentially linking MMP-1 to the development and propagation of atherosclerosis, no studies to date have specifically examined the prognostic significance of baseline plasma levels of MMP-1 in patients with ischemic heart disease. Accordingly, the objective of the current study was to investigate the long-term prognostic significance of baseline MMP-1 levels in a group of well-characterized male patients with known or suspected coronary artery disease who were referred for coronary angiography, including those presenting with the acute coronary syndrome. An additional and specific objective of the study was to compare the utility of MMP-1 in this regard with other well-established and contemporary biomarkers representing multiple different pathophysiologic processes, such as hemodynamic stress, inflammation, and myocardial necrosis, in an attempt to better delineate the clinical significance of the basic science observations pertaining to MMP-1. The aim was to test whether this novel protein could improve risk prediction above and beyond established and existing biomarkers.

Methods

The study population and design have been described in detail elsewhere (10). The study database was generated at an urban Veterans Administration (VA) Medical Center and was approved by the local IRB. Written informed consent was obtained from all patients. All patients who were referred to the Cardiac Catheterization Laboratory for coronary angiography at the Bronx Veterans Affairs Medical Center between January 13, 1999 and October 17, 2002 were eligible for inclusion in the database. Patients with active gastrointestinal bleeding or a hemoglobin concentration less than 8 gm/dL were excluded. During the period of study enrollment, a total of 523 unique and consecutive male patients underwent diagnostic coronary angiography. Of these 523 patients, 50 could not be enrolled because of an unexpected loss of key study personnel between January 29, 2001 and July 2, 2001. Of the remaining 473 patients, 84 were either unwilling or unable to provide informed consent. Thus, a total of 389 patients provided informed consent and constituted the final study population. Of these 389 patients, MMP-1 values were not available for 25 patients. The present study represents an analysis of both the entire cohort of 364 patients as well as the subpopulation of 180 patients who underwent diagnostic coronary angiography for the evaluation of ACS (12% with ST-segment elevation MI, 43% with non-ST-segment elevation MI, and 45% with unstable angina pectoris).

Fasting whole blood was obtained from all patients at the time of angiography prior to injection of contrast. The whole blood was centrifuged (4g) for 10 minutes at 4°C, the plasma aliquoted into 1.5 mL vials and then stored at -70°C for subsequent analysis. The samples were thawed only once when ready for analysis. All patients were followed prospectively for the development of all-cause mortality.

Patients were divided into tertiles according to baseline MMP-1 values (≤ 0.503 ng/mL, >0.503 to <1.082 ng/mL, and ≥ 1.082 ng/mL). Summary statistics for continuous variables were presented both as means (with standard deviations) as well as medians (with interquartile ranges), and comparisons between the three groups were performed with the non-parametric Kruskal-Wallis test. All biomarkers were log transformed and normalized to reduce skewness and kurtosis of data. Categorical data were summarized as frequencies and percentages, and comparisons between the 3 groups were performed with Pearson chi-square test or Fisher's exact test.

Predictors of all-cause mortality at 60 months were identified by univariate Cox proportional hazards regression with results were presented as hazards ratios (HR) and corresponding 95% confidence intervals. For the entire cohort as well as the ACS subpopulation, each of the clinical, angiographic and laboratory variables (including biomarkers) listed in Table 1 were studied for their association with all-cause mortality; predictors with $p < 0.05$ on unadjusted analyses were subsequently entered into multivariate Cox proportional hazards regression models. Independent predictors were then identified using the backward elimination procedure. All biomarkers were analyzed as continuous variables and the hazards ratios represented an increase in one standard deviation in the respective log transformed biomarker. Four different multivariate models were created for the entire cohort and for the ACS subpopulation. The first model adjusted only for baseline clinical,

angiographic and laboratory variables. The other three “biomarker only” models, adjusted for a variety of biomarkers with established prognostic efficacy in the literature (hs-CRP, MPO, Cystatin-C, ST2, NT-proBNP, Adiponectin, TIMP-1, hemoglobin, RDW, and Erythropoietin). All biomarkers were checked for collinearity and three different “biomarker only” models were created so as not to include any 2 markers whose Spearman correlation was > 0.5 . In this way, we sought to determine the relative prognostic significance of MMP-1 with respect to contemporary biomarkers with established prognostic significance and to better understand the pathophysiologic basis for the predictive power of MMP-1. Because this study was exploratory in nature and the objective was to determine the relationship between various covariates (including MMP-1) and the outcome of all-cause mortality in this specific patient population, there were no *a priori* intentions to create predictive models that could subsequently be used to predict outcomes in validation cohorts. Accordingly, prediction metrics such as the c-index were not reported for the current analyses.

Time-to-event at 60 months was presented with Kaplan-Meier curves for the individual endpoint of all-cause mortality. Comparisons between the 3 groups identified by tertiles of MMP-1 as described above were performed using the log-rank test.

All analyses used two-sided tests with an overall significance level of $\alpha = 0.05$. All statistical analyses were performed using SAS version 8 (SAS Institute Inc, Cary, NC).

Fasting blood was obtained from all patients at the time of angiography for subsequent analysis. Commercially available kits were used to measure the plasma levels of high-sensitivity C-Reactive Protein (hs-CRP; Life Diagnostics, West Chester, PA, USA), N-Terminal-Pro-B-Type Natriuretic Peptide (NT-proBNP; Diagnostic Automation, Calabasas, CA, USA), Myeloperoxidase (MPO; Assay Designs, Ann Arbor, Michigan, USA), Adiponectin (R & D Systems, Minneapolis, MN, USA), total TIMP-1 (EMD Biosciences, San Diego, CA, USA), GDF-15 (R & D Systems, Minneapolis, MN, USA), ST2 (R & D Systems, Minneapolis, MN, USA), Cystatin C (BioVendor, Asheville, NC, USA), erythropoietin (R & D Systems, Minneapolis, MN, USA), and MMP-1 (R & D Systems, Minneapolis, MN, USA).

Patients were followed for the occurrence of all-cause mortality. The information regarding the date of death was obtained using the following modalities: death certificate, social security death index, conversation with next of kin and/or primary physician, and review of medical records.

Results

Baseline characteristics

A total of 364 male patients were enrolled in the study. Five-year clinical data in the form of all-cause mortality were available for all of the patients. The baseline clinical, laboratory, and angiographic characteristics of the study population stratified by the lower, middle, and upper tertiles of MMP-1 values are shown in Table 1.

Association of MMP-1 with baseline clinical variables and other biomarkers

Elevated MMP-1 levels were associated with older age, lower BMI, lower GFR, and lower hemoglobin values. In addition, the levels of MMP-1 were also positively correlated with those of NT-proBNP, erythropoietin, TIMP-1, myeloperoxidase, adiponectin, ST2, Cystatin C, GDF-15 and RDW.

Clinical outcomes for the entire population

There were a total of 109 deaths (28.02%) at 5 years. The following baseline variables were significant for their association all-cause mortality at 5 years with $p < 0.05$ on univariate analysis: age/10 years, a family history of premature coronary artery disease, diabetes mellitus, myocardial infarction on presentation, congestive heart failure on presentation, atrial fibrillation, ACE-inhibitor use, left ventricular systolic function, serum creatinine, GFR-MDRD, the number of diseased coronary arteries, a history of CABG surgery, as well as the following biomarkers all analyzed as continuous variables: TIMP-1, hs-CRP, Fibrinogen, Adiponectin, NT-proBNP, hemoglobin, Erythropoietin and RDW. Together with MMP-1 (analyzed as a continuous variable), these significant univariate predictors of outcome were entered into multivariate models to identify the independent predictors of all-cause mortality at 5 years (Table 2). Congestive heart failure on presentation was not included as a covariate in multivariate models because of collinearity with NT-proBNP and left ventricular systolic function. Model 1 adjusted for clinical and angiographic variables only, Models 2-4 adjusted only for other established biomarkers. TIMP-1 and Cystatin C, GDF-15 and Cystatin C, and TIMP-1 and GDF-15 were not included in the same multivariate models due to a high degree of collinearity (>0.5) between these biomarkers. In each and every one of these models, MMP-1 emerged as a strong and independent predictor of all-cause mortality at 5 years.

Kaplan-Meier survival analysis showed a significantly reduced survival in patients in the highest MMP-1 tertile (Figure 1). At 5 years, the all-cause mortality rate was 18.2% in the lowest tertile, 22.1% in the middle tertile, and 43.8% in the highest tertile ($P < 0.0001$ by log-rank test).

Clinical outcomes for the ACS subpopulation

To determine whether the predictive power of MMP-1 was either related to or affected by baseline risk, similar analyses were performed in the subgroup of patients who had presented with an acute coronary syndrome at baseline. The ACS group, which consisted of 180 patients, was comprised of those with unstable angina, NSTEMI and STEMI. For this group, in whom follow-up data for all-cause mortality were similarly available for 100% of the patients, there were a total of 59 deaths.

In the ACS subpopulation, the following baseline variables were significant for their association all-cause mortality at 5 years with $p < 0.05$ on univariate analysis: age/10 years, diabetes mellitus, CHF on presentation, myocardial infarction on presentation, left ventricular systolic function, serum creatinine, GFR-MDRD, the number of diseased coronary arteries, as well as the following biomarkers all analyzed as continuous variables: TIMP-1, hs-CRP, adiponectin, NT-proBNP, hemoglobin, and RDW. As was done for the

entire cohort of patients, MMP-1 (analyzed as a continuous variable) and these significant univariate predictors of outcome were entered into multivariate models to identify the independent predictors of all-cause mortality at 5 years (Table 3). Congestive heart failure on presentation was not included as a covariate in multivariate models because of a very high and significant correlation between this variable and both NT-proBNP and left ventricular systolic function). Model 1 adjusted for clinical and angiographic variables only, Models 2-4 adjusted only for other established biomarkers. TIMP-1 and Cystatin C, GDF-15 and Cystatin C, and TIMP-1 and GDF-15 were not included in the same multivariate models due to a high degree of collinearity (>0.5) between these biomarkers. In each and every one of these models, MMP-1 emerged as a strong and independent predictor of all-cause mortality at 5 years. As was the case for the entire cohort of patients, MMP-1 emerged as a strong and independent predictor of all-cause mortality at 5 years in each and every one of these models.

Kaplan-Meier survival analysis showed a significantly reduced survival in patients in the highest MMP-1 tertile (Figure 2). At 5 years, the all-cause mortality rate was 16.7% in the lowest tertile, 30.0% in the middle tertile, and 51.7% in the highest tertile ($P = 0.0002$ by log-rank test).

Discussion

The most significant finding of our study was that elevated baseline plasma levels of MMP-1 were strong and independent predictors of long-term all-cause mortality in a cohort of male patients with known or suspected coronary artery disease referred for coronary angiography. This association remained significant even after adjustment for a wide variety of clinically relevant covariates, including a whole host of contemporary biomarkers with established prognostic significance in the literature. Furthermore, the independent relation between MMP-1 levels and all-cause mortality was also evident in the subgroup of patients presenting with an acute coronary syndrome at baseline. It is particularly noteworthy that MMP-1 emerged as an independent predictor of all-cause mortality even after adjustment for numerous well-established biomarkers representing different pathophysiologic processes and shown to have prognostic significance in a variety of cardiac populations. To this end, we methodically performed several different multivariate models which included in the selection traditional biomarkers of inflammation (such as hs-CRP and MPO) (11,12), biomarkers of prognostic value in heart failure (such as NT-proBNP, ST2, cystatin C) (13-15), biomarkers of hematologic significance with known prognostic utility (such as RDW, Hb and erythropoietin) (16-18), and recently-described novel biomarkers with incompletely understood and/or multiple different mechanisms (such as adiponectin, TIMP-1 and GDF-15) (10,19-20). In all of these multivariate models, MMP-1 emerged as the most significant and only independent biomarker which predicted all-cause mortality at 5 years. This was true despite the fact that the biomarkers included in these analyses were significant univariate predictors of all-cause mortality. Not only do these data suggest that MMP-1 is an especially powerful biomarker but we believe that the consistency of the association between MMP-1 and mortality in all of these multivariate models is a testimony to the validity of the findings.

Matrix metalloproteinases are a family of zinc-dependent endopeptidases that have recently emerged as important mediators of platelet and endothelial function, and atherothrombotic disease (3). MMP-1 is an interstitial collagenase that is considered the primary enzyme responsible for collagen degradation (3,5,7). It is expressed in most human tissues, including cells of the blood vessel wall, inflammatory cells, and platelets (3). The matrix-degrading functions of MMP-1 in the vessel wall have been considered to play a critical role in the pathogenesis of atherosclerosis and its complications. Indeed, consistent with a role for MMP-1 in atherothrombosis, MMP-1 expression has been shown to be increased in atherosclerotic plaques, and in particular in the cells in the vulnerable region of the plaque (4-6). Furthermore, platelets express several metalloproteinases on their surface, including MMP-1 (3,7). MMP-1 has been demonstrated to have agonist activity for PAR1 (8), which is a G-protein-coupled receptor that is classically activated through cleavage of the N-terminal exodomain by the serine protease thrombin (3,9). Exposure of platelets to collagen results in activation of MMP-1, which in turn directly cleaves PAR1 on the surface of platelets (8). This in turn leads to collagen-dependent thrombogenesis, arterial thrombosis, and clot retraction. Thus, there may be a dual role for MMP-1 in the pathogenesis of atherosclerosis: destabilization of the collagenous structure of plaques coupled with the induction of a procoagulant state through its effects on platelets (3). This unique dual role could potentially explain our data on how and why MMP-1 emerged as a particularly powerful and independent biomarker in a cardiac population. Furthermore, with respect to therapeutic implications of these findings, blockade of the MMP-1/PAR-1 pathway with an MMP-1 inhibitor greatly curtailed arterial thrombosis in an animal model of arterial denudation, suggesting that such inhibition may represent a point of early intervention in preventing arterial thrombosis (3,8). Thus, inhibition of the MMP-1/PAR-1 axis may represent a novel approach to the treatment of vascular thrombosis.

The mechanism(s) by which high MMP-1 levels are associated with adverse outcomes in patients with known or suspected coronary artery disease is unknown. While it is certainly tempting to attribute the prognostic utility of MMP-1 to its known effects on platelet activation and/or its destabilizing actions on the collagenous structures of the atherosclerotic plaque, demonstration of such a cause-and-effect association is far beyond the scope of the present study. Furthermore, it is also conceivable that the prognostic power of MMP-1 may in fact be completely unrelated to any of its cardiovascular effects. For example, MMP-1 levels have been shown to have prognostic significance in patients with malignancy (21-23). Finally, as is the case for the vast majority of biomarkers in the published literature, it is unknown whether MMP-1 represents an actual mediator of increased risk or simply a marker of that risk. Regardless, however, what is clearly evident from the current study is that MMP-1 likely represents a novel and powerful prognostic biomarker for the prediction of long-term all-cause mortality that is independent not only of traditional cardiovascular risk factors but also independent of many of the well-established biomarkers in the literature.

There are several limitations to this study. First, the study was exploratory and observational in nature and as such is subject to the limitations of this type of analysis. However, we had formulated the hypothesis that elevated MMP-1 levels might be associated with adverse outcomes (based on review of the available literature in this regard) before performing

analyses on our database. We therefore feel that this approach reduced the risk of spurious conclusions. Second, the study did not have a 'control' group consisting of patients who were not referred for cardiac catheterization. Third, the size of our population was small and the study was not designed with a priori calculations with respect to sample size or statistical power. As such, the findings need to be confirmed in larger and prospectively designed studies. Fourth, the population was a high-risk cohort as evidenced by both clinical and laboratory parameters as well as by the high event rate for death. Therefore, it is unknown whether MMP-1 levels would be similarly predictive of events in a low-risk population. Finally, this study was conducted in an exclusively male population and the results cannot be extrapolated to women.

Conclusions

In conclusion, we found that elevated MMP-1 levels were a strong and independent predictor of all-cause mortality in a broad population of male patients referred for coronary angiography, including those with the acute coronary syndrome. Furthermore, in multivariate models adjusting for numerous other contemporary biomarkers with established prognostic utility in a variety of cardiac populations, MMP-1 consistently emerged as the most important and only independent predictor of all-cause mortality at 5 years. While these data are certainly consistent with the known biologic actions of MMP-1 on a basic science level, it remains unknown whether MMP-1 represents a marker or a mediator of risk. The answer to this question could have potential therapeutic implications, as there are a variety of MMP inhibitors currently under development.

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Abbreviations

MMP-1	matrix metalloproteinase 1
eGFR	estimated glomerular filtration rate

PAR1	protease-activated receptor-1
MI	myocardial infarction
NSTEMI	non-ST-segment elevation MI
STEMI	ST-segment elevation MI
ACS	acute coronary syndrome
CAD	coronary artery disease
CABG	coronary artery bypass graft surgery
CHF	congestive heart failure
LV	left ventricular
EF	ejection fraction
BMI	body mass index
hs-CRP	high-sensitivity C-Reactive Protein
TIMP-1	tissue inhibitor of matrix metalloproteinase 1
NT-proBNP	N-Terminal-Pro-B-Type Natriuretic Peptide
MPO	myeloperoxidase
GDF-15	Growth Differentiation Factor 15
RDW	red blood cell distribution width
ACE-I	angiotensin converting enzyme inhibitor
ARB	angiotensin receptor blocker

- MMP-1 is an interstitial collagenase that is considered the primary enzyme responsible for collagen degradation
- MMP-1 can lead to platelet activation through the PAR1 pathway that is independent of thrombin
- Elevated levels of MMP-1 are associated with an increased risk of long-term all-cause mortality in patients with known or suspected coronary disease that is independent of a variety of clinical, angiographic, laboratory variables

Figure 1.

Kaplan-Meier curves for all-cause mortality according to baseline plasma MMP-1 levels stratified by tertiles in the entire cohort of patients. At 5 years, the number of events (deaths) in the lowest tertile was 22 (18.2%), 27 (22.1%) in the middle tertile, and 53 (43.8%) in the upper tertile ($p < 0.0166$ by log-rank test). The upper numbers on the x-axis represent the number of patients at risk.

Figure 2.

Kaplan-Meier curves for all-cause mortality according to baseline plasma MMP-1 levels stratified by tertiles in the ACS subpopulation of patients. At 5 years, the number of events (deaths) in the lowest tertile was 10 (16.7%), 18 (30.0%) in the middle tertile, and 31 (51.7%) in the upper tertile ($p = 0.0002$ by log-rank test). The upper numbers on the x-axis represent the number of patients at risk.

Table 1
Baseline Characteristics of the Entire Population Stratified by Tertiles of Plasma MMP-1 Values

Characteristic	Statistics	1 st tertile (<=0.503 ng/mL) N=121	2 nd tertile (>0.503 to <1.082 ng/mL) N=122	3 rd tertile (>=1.082 ng/mL) N=121	p Value
Age	Mean (SD)	62.6(10.2)	65.9(10.2)	67.1(9.2)	
	Median (25 th , 75 th)	62.5(53.9, 69.9)	67.3(57.2, 74.3)	67.3(61.8, 74.2)	0.0018
Race	Black, N (%)	37(30.6)	33(32.0)	41(33.9)	0.4412
	Hispanic, N (%)	41(33.9)	29(23.8)	32(26.5)	
	White, N (%)	43(35.5)	54(44.3)	48(39.7)	
Family History of Premature CAD	N (%)	37(30.6)	31(25.4)	22(18.2)	0.0804
Diabetes mellitus	N (%)	51(42.2)	55(45.1)	53(43.8)	0.8987
Hypertension	N (%)	95(78.5)	103(84.4)	105(86.8)	0.2073
Any history of tobacco use	N (%)	98(81.0)	97(79.5)	105(86.8)	0.2910
Active tobacco use	N (%)	39(32.2)	36(29.5)	42(34.7)	0.6857
Hyperlipidemia *	N (%)	69(57.0)	61(50.0)	64(52.9)	0.5444
Atrial fibrillation	N (%)	8(6.6)	8(6.6)	13(10.7)	0.3855
CHF on presentation	N (%)	29(24.0)	27(22.1)	41(33.9)	0.0838
MI on presentation	N (%)	41(33.9)	28(23.0)	35(28.9)	0.1678
Aspirin	N (%)	106(87.6)	105(86.1)	97(80.2)	0.2384
Beta blocker	N (%)	83(8.6)	82(67.2)	83(68.6)	0.9650
ARB	N (%)	6(5.0)	9(7.4)	7(5.8)	0.7236
ACE inhibitor	N (%)	70(57.9)	73(59.8)	76(62.8)	0.7302
Statin	N (%)	63(52.1)	59(48.4)	64(52.9)	0.7531
Fibrate	N (%)	7(5.8)	5(4.1)	4(3.3)	0.6372
LV function	EF >= 55%, N(%)	43(38.7)	45(38.5)	35(31.0)	0.8777
	EF 45-54%, N(%)	25(22.5)	29(24.8)	28(24.8)	
	EF 31-44%, N(%)	26(23.4)	26(22.2)	32(28.3)	
	EF <= 30%, N(%)	17(15.3)	17(14.5)	18(15.9)	
No. of diseased coronary arteries **	0, N(%)	31(25.6)	18(14.8)	21(17.4)	0.1068
	1, N(%)	24(19.8)	22(18.0)	13(10.7)	

Characteristic	Statistics	1 st tertile (<=0.503 ng/mL) N=121	2 nd tertile (>0.503 to <1.082 ng/mL) N=122	3 rd tertile (>=1.082 ng/mL) N=121	p Value
	2, N(%)	24(19.8)	35(28.7)	35(28.9)	
	3, N(%)	34(28.1)	42(34.4)	47(38.8)	
	4, N(%)	8(6.6)	5(4.1)	5(4.1)	
Prior CABG	N(%)	8(6.6)	14(11.5)	11(9.1)	0.4182
ACS	N(%)	61(50.4)	57(46.7)	62(51.2)	0.7545
STEMI	Yes	11(9.1)	6(4.9)	6(5.0)	0.3083
NSTEMI	Yes	30(24.8)	22(18.0)	29(24.0)	0.3843
Unstable angina	Yes	20(16.5)	29(23.8)	27(22.5)	0.3347
BMI	Mean(sd)	29.3(6.2)	29.3(5.9)	27.3(4.8)	
	Med(25 th , 75 th)	28.2(25.6, 31.8)	28.6(24.6, 32.6)	27.1(23.8, 30.2)	0.0461
hs-CRP	Mean (SD)	20.7(33.3)	26.3(47.2)	25.9(41.9)	
	Median (25 th , 75 th)	8.9(4.7, 18)	8.4(3.2, 17.3)	11.5(4.8, 21.9)	0.1962
Troponin	Mean (SD)	13.8(68.1)	5.9(20.3)	12.2(43.3)	
	Median (25 th , 75 th)	0.3(0.2, 3.1)	0.3(0.2, 1.7)	0.3(0.2, 1.5)	0.8277
Serum creatinine	Mean (SD)	1.08(0.29)	1.13(0.42)	1.55(2.10)	
	Med(25 th , 75 th)	1.0(0.9, 1.2)	1.0(0.9, 1.2)	1.1(0.9, 1.3)	0.0515
NT-pro-BNP	Mean(SD)	1252(982)	1196(940)	1591(1230)	
	Med(25 th , 75 th)	974(488, 1723)	848(529, 1580)	1215(646, 2198)	0.0247
Erythropoietin	Mean(SD)	16.1(13.3)	13.5(10.5)	19.4(20.4)	
	Med(25 th , 75 th)	11.8(7.8, 18.1)	10.5(7.7, 15.5)	12.6(8.7, 19.4)	0.0405
GFR-MDRD	Mean(SD)	83.7(22.6)	82.4(25.3)	76.0(28.2)	
	Med(25 th , 75 th)	81(70, 94)	79(69, 94)	72(62, 90)	0.0249
TIMP-1	Mean(SD)	81.5(38.1)	87.8(41.4)	110.7(52.3)	
	Med(25 th , 75 th)	70.2(63.1, 90.7)	78.4(64.6, 93.8)	96.1(78.3, 125.7)	<0.0001
Myeloperoxidase	Mean(SD)	25.3(23.0)	22.1(13.1)	28.9(19.8)	
	Med(25 th , 75 th)	18.4(13.3, 28.1)	17.9(12.8, 27.4)	22.5(16.0, 35.5)	0.0041
Adiponectin	Mean(SD)	7.5(5.7)	7.0(4.7)	9.7(6.9)	
	Med(25 th , 75 th)	5.8(3.5, 9.4)	5.7(3.7, 8.7)	7.7(4.9, 14.0)	0.0028

Characteristic	Statistics	1 st tertile (<=0.503 ng/mL) N=121	2 nd tertile (>0.503 to <1.082 ng/mL) N=122	3 rd tertile (>=1.082 ng/mL) N=121	p Value
ST2	Mean(SD)	22.9(13.6)	23.5(14.3)	28(15.5)	
	Med(25 th , 75 th)	19.7(15.8, 26.7)	19.2(15.6, 26.2)	21.8(17.7, 34.7)	0.0041
Cystatin-C	Mean(SD)	957(240)	1055(348)	1289(671)	
	Med(25 th , 75 th)	941(770,1055)	997(814,1160)	1104(921, 1356)	<0.0001
GDF-15	Mean(SD)	1212(787)	1342(886)	1859(1391)	
	Med(25 th , 75 th)	992(757, 1367)	1100(856, 1532)	1492(1034,2056)	<0.0001
RDW	Mean(SD)	14.1(1.2)	13.9(1.2)	14.9(2.6)	
	Med(25 th , 75 th)	13.9(31.1, 14.7)	13.7(13.2, 14.6)	14.2(13.3, 15.3)	0.0094
Hemoglobin	Mean(SD)	13.7(1.6)	13.6(1.6)	13.1(1.9)	
	Med(25 th , 75 th)	13.8(12.8, 14.7)	13.7(12.6,14.7)	13.2(12.0, 14.3)	0.0364

* Hyperlipidemia was diagnosed in patients who had been given lipid-lowering medication or had a history of total cholesterol levels ≥ 240 mg/dl [19].

** Takes into account the left main, left anterior descending, left circumflex, and right coronary arteries (minimum = 0, maximum = 4)

Table 2
Multivariate Cox Regression Models for All-Cause Mortality at 5 Years for the Entire Cohort

Variable	Model 1		Model 2		Model 3		Model 4	
	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)
MMP-1	<0.0001	1.49 (1.23,1.80)	<0.0001	1.47(1.22, 1.77)	0.0136	1.25(1.05, 1.50)	0.0160	1.26(1.04, 1.51)
Age/10 y	0.0490	1.28 (1.00,1.62)						
Family history	NS	-						
DM	NS	-						
MI	NS	-						
ACE-I	NS	-						
LV function	0.0001	1.48 (1.22,1.81)						
CAD extent	0.0170	1.28 (1.05,1.56)						
Prior CABG	NS	-						
GFR-MDRD	0.0307	0.78 (0.63,0.98)						
hs-CRP			NS	-	0.0013	1.37(1.13, 1.66)	NS	-
MPO			NS	-	NS	-	NS	-
NT-proBNP			NS	-	NS	-	NS	-
Cystatin C			NS	-				
ST2			NS	-	NS	-	NS	-
Hemoglobin			NS	-	NS	-	NS	-
RDW			NS	-	NS	-	NS	-
Erythropoietin			NS	-	NS	-	NS	-
GDF-15							<0.0001	1.92(1.57, 2.34)
TIMP-1					<0.0001	1.63(1.35, 1.96)		
Adiponectin			<0.0001	1.67(1.36, 2.06)	0.0016	1.43(1.15, 1.78)	0.0087	1.34(1.08, 1.67)

All variables included in the selection were significant predictors of all-cause mortality at 5 years on univariate analysis, $p < 0.05$

Model 1 included only clinical and angiographic variables in the selection, along with MMP-1

Model 2 included MMP-1 with other biomarkers, except GDF-15 and TIMP-1 due to a high degree of collinearity between them

Model 3 included MMP-1 with other biomarkers, except Cystatin C and GDF-15, due to a high degree of collinearity between them

Model 4 included MMP-1 with other biomarkers, except Cystatin C and TIMP-1, due to a high degree of collinearity between them

Table 3
Multivariate Cox Regression Models for All-Cause Mortality at 5 Years for the ACS Subpopulation

Variable	Model 1		Model 2		Model 3		Model 4	
	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)
MMP-1	0.0035	1.41 (1.12, 1.77)	0.0233	1.35(1.04, 1.75)	0.0052	1.40(1.11, 1.77)	0.0022	1.44(1.14, 1.83)
Age/10 y	0.0490	1.28 (1.00, 1.62)						
DM	NS	-						
MI	0.0062	2.18 (1.25, 3.82)						
LV function	NS	-						
CAD extent	NS	-						
GFR-MDRD	0.0001	0.57 (0.43, 0.76)						
hs-CRP			NS	-	0.0133	1.37(1.07, 1.76)	NS	-
NT-proBNP			NS	-	NS	-	NS	-
Cystatin C			<0.0001	1.65(1.30, 2.08)				
ST2			NS	-	NS	-	NS	-
Hemoglobin			NS	-	NS	-	NS	-
RDW			NS	-	NS	-	NS	-
GDF-15							<0.0001	1.83(1.42, 2.35)
TIMP-1					<0.0001	1.76(1.39, 2.24)		
Adiponectin			NS	-	NS	-	NS	-

All variables included in the selection were significant predictors of all-cause mortality at 5 years on univariate analysis, $p < 0.05$

Model 1 included only clinical and angiographic variables in the selection, along with MMP-1

Model 2 included MMP-1 with other biomarkers, except GDF-15 and TIMP-1, due to a high degree of collinearity between them

Model 3 included MMP-1 with other biomarkers, except GDF-15 and Cystatin C, due to a high degree of collinearity between them

Model 4 included MMP-1 with other biomarkers, except Cystatin C and TIMP-1, due to a high degree of collinearity between them