Impact of Asymmetric Dimethylarginine on Mortality After Acute Myocardial Infarction

Marianne Zeller, Claudia Korandji, Jean-Claude Guilland, Pierre Sicard, Catherine Vergely, Luc Lorgis, Jean-Claude Beer, Laurence Duvillard, Anne-Cécile Lagrost, Daniel Moreau, Philippe Gambert, Yves Cottin, Luc Rochette

Objective—Asymmetrical dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide (NO) synthases. From a prospective cohort of patients with acute myocardial infarction (MI), we aimed to analyze the predictive value of circulating ADMA concentrations on prognosis.

Methods and Results—Blood samples from 249 consecutive patients hospitalized for acute MI <24 hours were taken on admission. Serum levels of ADMA and its stereoisomer, symmetrical dimethylarginine (SDMA), were determined using high-performance liquid chromatography. The independent predictors of ADMA were glomerular filtration rate, female sex, and SDMA ($R^2=0.25$). Baseline ADMA levels were higher in patients who had died than in patients who were alive at 1 year follow-up (1.23 [0.98 to 1.56] versus 0.95 [0.77 to 1.20] μmol/L, $P<0.001$). By Cox multivariate analysis, the higher tertile of ADMA (median [interquartile range]: 1.45 [1.24 to 1.70] μmol/L) was a predictor for mortality (Hazard Ratio [95% CI], 4.83 [1.59 to 14.71]), when compared to lower tertiles, even when adjusted for potential confounders, such as acute therapy, biological, and clinical factors.

Conclusion—Our study suggests that the baseline ADMA level has a strong prognostic value for mortality after MI, beyond traditional risk factors and biomarkers. (Arterioscler Thromb Vasc Biol 2008;28:954-960)

Key Words: ADMA ■ myocardial infarction ■ prognosis

Endothelium-derived nitric oxide (NO) plays a pivotal role in coronary vessel homeostasis. In diseased states, such as acute myocardial infarction (MI), endothelial dysfunction linked to reduced NO bioavailability contributes to increased vasoconstrictor responses, adhesion of platelets and monocytes, and proliferation of vascular smooth muscle cells. After an acute coronary event, a high frequency of transient endothelial vasomotor dysfunction has been reported in coronary arteries and in peripheral circulation.2 Impaired endothelial vasodilatation is strongly associated with future cardiovascular (CV) events after an acute coronary syndrome.4 Over the last decade, several studies have suggested that circulating concentrations of asymmetrical dimethylarginine (ADMA) may provide a marker of risk for atherosclerosis.5 ADMA is an endogenous competitive inhibitor of all isoforms of NO synthases and may compete with L-arginine as the substrate for the enzyme. ADMA originates from the degradation of posttranslationally methylated proteins in the course of protein turnover. A minor part of circulating ADMA is excreted via renal elimination. The major catabolic pathway of circulating ADMA occurs via dimethylarginine dimethylarginohydrolase (DDAH), which is also thought to play a major role in impaired vascular homeostasis.6 An association between high ADMA levels and a risk of acute coronary events has been demonstrated in middle-aged men.7 In patients with documented coronary artery disease, baseline serum concentrations of ADMA independently predict CV events at 2 years follow-up.8 Only few data are available on levels of ADMA during acute MI.9 Moreover, there is currently no evidence indicating any prognostic impact of this factor in the setting of acute MI, in particular in comparison with traditional risk factors and biomarkers such as C-reactive protein (CRP) and N-terminal Pro-Brain Natriuretic Peptide (NT-proBNP).

From a prospective cohort of patients with acute MI, the aim of the present study was to identify the determinants of ADMA levels and to analyze the predictive value of ADMA on mortality at long-term follow-up.

Methods

For detailed descriptions of the methods, please see supplemental material (available online at http://atvb.ahajournals.org).
Study Patients
249 consecutive patients hospitalized <24 hours after symptom onset for acute MI admitted to the coronary care unit of Dijon University Hospital between 1st March and 30th September 2006 were included. Patients under present treatment with vitamin B12 or folate or under methionine loading were excluded from the study.

Laboratory Methods
Blood samples were drawn on admission (time delay from symptom onset to blood sampling: 169 [90 to 394] min). Samples were allowed to clot at room temperature for 30 minutes and centrifuged at 2500 rpm for 10 minutes at 4°C. The serum was kept frozen at −80°C until analysis (1 week). L-arginine, ADMA, and its stereoisomer, symmetrical dimethylarginine (SDMA), were measured by high performance liquid chromatography (HPLC).

Statistical Analysis
Data are presented as median (IQR), mean±SD, as appropriate, or proportion (n[%]). All the analyses were performed using the SPSS 13.0 software package (SPSS Inc).

Results
Baseline Characteristics
The study sample consisted of 249 MI patients. The characteristics of the patients classified by ADMA tertiles are shown in Table 1. Age increased across the tertiles. Risk factors, clinical data on admission, and present medications were similar for the 3 groups. The risk score increased across the tertiles. The rate of reperfusion decreased across the tertiles; the rate of reperfusion in the higher tertile was only half that in the lower tertile (25 versus 52%). Biological data are shown in Table 2. Neither renal function, nor CRP, as an index of an inflammatory process, nor admission glucose changed according to the ADMA tertiles. In contrast, NT-proBNP concentrations showed a trend toward an increase across the tertiles (P=0.053). As expected, the SDMA and L-arginine/ADMA ratio varied with increasing ADMA.
contrast, neither homocysteine nor L-arginine changed according to the concentrations of ADMA.

Determinants of ADMA

By Spearman correlation analysis (supplemental Table I), ADMA was positively related to SDMA, age, NT-proBNP, the GRACE risk score, and homocysteine. A trend toward an inverse relationship with GFR was found. Neither systolic nor diastolic blood pressure was significantly related to ADMA concentrations. ADMA was positively related to SDMA, age, NT-proBNP, and homocysteine. A trend toward a significant relationship between ADMA concentrations and present treatments (fibrate, statin, ACE inhibitor), location of infarction, type of MI, or CV history (prior MI, diabetes, hypertension, or hypercholesterolemia). In diabetic patients, ADMA levels were similar in patients who had been taking or not taking an antiplatelet agent (acetylsalicylic acid, clopidogrel, or both agents) and were similar in patients who had diabetes, hypertension, or hypercholesterolemia. In diabetic patients, ADMA levels were similar in patients who had been taking or not taking an antiplatelet agent (acetylsalicylic acid, clopidogrel, or both agents) and were similar in patients who had diabetes, hypertension, or hypercholesterolemia.

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ADMA and Mortality

At 1-year follow-up, 34 (14%) patients had died from all cause death and 31 (12%) from CV cause. ADMA and SDMA levels were significantly higher in patients who had died than in those still alive at 1 year (respectively, 1.23 [0.98 to 1.56] versus 0.95 [0.77 to 1.20] μmol/L, P<0.001 and 0.76 [0.54 to 1.09] versus 0.46 [0.35 to 0.62] μmol/L, P<0.001; Figure 1). L-arginine concentrations were similar for the 2 groups (96.9 [72.9 to 127.5] versus 96.8 [75.1 to 130.4] μmol/L, P=0.556). The L-arginine/ADMA ratio was not significantly linked to the outcome (HR [95% CI]: 1.00 [0.99 to 1.00], P=0.471), probably because of the lack of predictive value of L-arginine (HR [95% CI]: 1.00 [0.99 to 1.00], P=0.786). ROC curve analysis revealed a significant relationship between ADMA and mortality (AUC [95% CI]: 0.69 [0.59 to 0.78], P<0.001). The optimal threshold of ADMA that maximized the combined specificity and sensitivity to predict mortality was 1.16 μmol/L. Because this value was very close to the threshold of the last tertile, we analyzed the impact of high ADMA values, as defined by the last tertile (versus other tertiles). High ADMA levels were strongly associated with excess all-cause mortality in Kaplan–Meier curve (P<0.001, log-rank test; Figure 2). High ADMA was also related with CV mortality (HR [95% CI]: 3.59 [1.80 to 7.17]). By Cox multivariate analysis, SDMA failed to independently predict mortality (HR [95% CI]: 1.67 [0.40 to 6.96]; model 2, Table 3). In contrast, high ADMA was a predictive factor for mortality (HR [95% CI]: 4.83 [1.59 to 14.71]), even when adjusted for risk score, LVEF, acute therapy, and biological variables (model 3, Table 3).

The addition of ADMA significantly improved the likelihood of the model (model 3 versus model 1, -2log likelihood: 142.47 versus 134.14, respectively, P<0.05). No significant interaction between sex or smoking and ADMA was found for the outcome. As major confounders of the prognosis, such as age, the GRACE risk score, acute β-blocker therapy, and reperfusion have been shown to vary according to the ADMA levels (Table 1), we also aimed to test the impact of their interaction with ADMA on the outcome. Only age and the GRACE risk score had a significant interaction with ADMA for the outcome. High ADMA remained strongly associated
with mortality (HR [95% CI], 5.06 [1.11 to 23], \( P = 0.036 \)), even when these interactions were added to model 3 (ie, age*ADMA and GRACE risk score*ADMA), or when age, GFR, and hypertension were added to the model instead of the GRACE risk score (HR [95% CI]: 4.60 [1.71 to 12.29], \( P = 0.002 \)).

**Discussion**

These contemporary data from an observational study of MI patients were used to analyze ADMA concentrations and their impact on the prognosis. In the setting of acute MI, the measurement of ADMA concentrations has only been reported in a small series of 37 patients and was used to evaluate the short-term impact of medical therapy.\(^9\) Our results on the impact of ADMA on 1-year prognosis extend previous observations in patients with stable angina and undergoing percutaneous coronary intervention\(^1^4\) and demonstrate that ADMA is a strong and independent predictor of long-term mortality after MI.

**Determinants of ADMA**

ADMA concentrations were similar in males and females, which is in agreement with other studies in MI patients.\(^9\) In our study population, most of the subjects were male (78%) and rather young when compared to the women, most of whom were presumed to be postmenopausal (median [IQR] age: 66 [53–76] versus 71 [64–80] y). In young healthy subjects (<50 years), a lower ADMA plasma concentration is found in women than in men. Inversely, in older healthy subjects, women have higher levels of the dimethylarginine.\(^1^5\) One attractive hypothesis to explain this sex-dependent correlation between ADMA and age is derived from experimental evidence of increased DDAH activity and the subsequent fall in ADMA levels induced by estrogen,\(^1^6\) and also by the lowering effect of estrogen replacement therapy on circulating ADMA concentration in postmenopausal women.\(^1^7\) However, in our study, no data were available either on menopausal status or on estrogen replacement therapy. Moreover, because of the lack of statistical power in the population of women (n = 55), no firm conclusion can be drawn with regard to sex-related and age-related concentrations of ADMA.

ADMA levels correlated with homocysteine, which is in agreement with previous findings.\(^1^2\) Homocysteine could relate to ADMA levels by reducing DDAH activity via a redox-mediated mechanism or by directly interfering with DDAH as shown in a cell-free system.\(^1^8\) No significant association was found between ADMA and CV risk factors. This is consistent with the results reported in various pathological conditions.\(^1^9,2^0\) Lower ADMA concentrations were associated with smoking, as observed in elderly high-risk men.\(^2^1\) These paradoxical findings could in part be explained by the effect of tobacco smoke components on the upregulation of DDAH expression recently reported in human endothelium-derived cells.\(^2^2\) Treatments before hospitalization had no impact on ADMA levels in MI patients, confirming the findings of other investigators.\(^5,2^3\) In the present study, ADMA levels were independently predicted by SDMA, gender, and GFR, but these parameters accounted for only one quarter of total ADMA variance, suggesting that other unidentified factors influence ADMA levels in the setting of acute MI.

**ADMA and Mortality**

Our findings suggest the predictive value of ADMA for long-term mortality after MI, beyond that obtained with baseline determinants of prognosis. In Finnish middle-aged men elevated levels of plasma ADMA have been initially shown to be associated with risks of CV events.\(^7\) More recently, in a large cohort of patients with documented CV disease, ADMA levels were independently associated with CV death at long-term follow up, with a 27% increased risk for each increment of 1-SD in baseline ADMA values.\(^8\) ADMA was the strongest risk factor, even after adjustment for traditional risk factors and biomarkers. After MI, there is a paucity of data with regard to the value of ADMA as a
prognostic factor for outcome. Recent works in 79 patients with cardiogenic shock complicating acute MI reported that ADMA levels were the strongest independent predictor of 30-day mortality, with an odds similar to our findings (OR [95% CI]: 3.19 [1.02 to 12.81]).24 In the present study, although homocysteine was a significant predictor in univariate analysis, it was not an independent predictor of the outcome in our dataset. These findings are fully in accordance with recent data from the literature.25 NTproBNP was also strongly associated with mortality by univariate analysis (HR [95% CI]: 3.61 [2.25 to 5.79]). However, because of the small size of the study population and its interaction with some components of the GRACE risk score, ie, age, serum creatinine, heart failure, and hemodynamic parameters, NTproBNP only trend to be associated with the outcome in multivariate analysis (P=0.080). CRP, as an acute phase reactant, and NT-proBNP, as a marker integrating advanced age, renal impairment, and left ventricular dysfunction, are recognized biomarkers of prognosis after MI.26,27 Our findings, suggesting the prognostic value of ADMA beyond these biomarkers, indicate that ADMA concentrations could provide prognostic information which is complementary to the inflammatory process or ventricular dysfunction. However, further studies are needed to evaluate this marker in specific subgroups.

Whether the effects observed in the present study are mediated by NO synthase inhibition with secondary endothelial dysfunction remains to be determined. Experimental data indicated that treatment with ADMA aggravated cardiac ischemic insult, probably because of inadequate endothelial

Table 3. Cox Regression Analysis for 1-Year All-Cause Mortality

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Model 1</th>
<th>P</th>
<th>Model 2</th>
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<th>Model 3</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P</td>
<td>HR (95% CI)</td>
<td>P</td>
<td>HR (95% CI)</td>
<td>P</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>3.61 (2.25–5.79)</td>
<td>&lt;0.001</td>
<td>1.84 (0.88–3.84)</td>
<td>0.106</td>
<td>1.73 (0.81–3.70)</td>
<td>0.158</td>
<td>1.97 (0.92–4.21)</td>
</tr>
<tr>
<td>GRACE risk score</td>
<td>1.03 (1.02–1.04)</td>
<td>&lt;0.001</td>
<td>1.03 (1.01–1.05)</td>
<td>&lt;0.001</td>
<td>1.03 (1.01–1.05)</td>
<td>&lt;0.001</td>
<td>1.03 (1.01–1.05)</td>
</tr>
<tr>
<td>CRP</td>
<td>1.01 (1.00–1.01)</td>
<td>0.014</td>
<td>1.00 (0.99–1.00)</td>
<td>0.687</td>
<td>1.00 (0.99–1.01)</td>
<td>0.765</td>
<td>1.00 (0.99–1.01)</td>
</tr>
<tr>
<td>CK ×10ULN</td>
<td>1.93 (0.96–3.86)</td>
<td>0.060</td>
<td>1.33 (0.47–3.78)</td>
<td>0.588</td>
<td>1.44 (0.50–4.19)</td>
<td>0.501</td>
<td>1.48 (0.51–4.30)</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>1.05 (1.02–1.08)</td>
<td>0.002</td>
<td>1.04 (0.97–1.12)</td>
<td>0.274</td>
<td>1.02 (0.94–1.11)</td>
<td>0.632</td>
<td>0.99 (0.91–1.07)</td>
</tr>
<tr>
<td>LVEF &lt;40%</td>
<td>8.09 (3.94–16.60)</td>
<td>&lt;0.001</td>
<td>3.45 (1.34–8.89)</td>
<td>0.010</td>
<td>3.55 (1.36–9.28)</td>
<td>0.010</td>
<td>2.91 (1.11–7.66)</td>
</tr>
<tr>
<td>Statin &lt;48H</td>
<td>0.28 (0.14–0.58)</td>
<td>0.001</td>
<td>0.44 (0.14–1.35)</td>
<td>0.152</td>
<td>0.40 (0.13–1.28)</td>
<td>0.123</td>
<td>0.57 (0.16–1.97)</td>
</tr>
<tr>
<td>SDMA</td>
<td>5.14 (2.87–9.21)</td>
<td>&lt;0.001</td>
<td>...</td>
<td>...</td>
<td>1.67 (0.40–6.96)</td>
<td>0.477</td>
<td>...</td>
</tr>
<tr>
<td>High ADMA</td>
<td>3.59 (1.80–7.17)</td>
<td>&lt;0.001</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>4.83 (1.59–14.71)</td>
</tr>
</tbody>
</table>

ADMA indicates asymmetric dimethylarginine; CRP, C-reactive protein; CK, creatine Kinase; GFR, glomerular filtration rate; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; SDMA, symmetric dimethylarginine; ULN, upper limit of normal.
NO production. NO is crucial for the preservation of organ blood flow by regulating vascular tone and influencing the interactions of white blood cells and platelets with the endothelium. Severe impairment of platelet responsiveness to NO also occurs at admission for an acute coronary syndrome and is an independent predictor of worse outcome. ADMA is now considered the most important regulator of the L-arginine/NO pathway in vivo. We may think that the link between ADMA and a worse outcome is at least partly associated with NO bioavailability, because SDMA, which is not a competitive inhibitor of NO synthase, failed to show any independent prognostic value, in accordance with findings in critically ill patients. Moreover, a perinfarct accumulation of ADMA mediated by reduced eNOS activity and phosphorylation and associated with inflammatory response has recently been reported in an experimental model. However, we cannot exclude the possibility that, as the vascular effects of ADMA may also relate to activation of the renin-angiotensin system and oxidative stress, ADMA might also affect coronary vessel integrity through mechanisms independent of eNOS synthase.

Study Limitations
No data are available on the kinetics of ADMA levels at the acute phase of MI. Bae et al found a significant decrease (of 50%) in ADMA levels after 2 weeks of medical therapy for acute coronary syndrome. In critically ill patients, only a modest increase in ADMA levels was reported from admission to day 2 (P = 0.043). In the present study, no correlation was found between ADMA levels and the time delay to blood sampling (P = 0.99), suggesting that sampling time had little or no influence on the results. Furthermore, no control group was included in the present study. Nevertheless, ADMA levels were about 45% higher than those in healthy subjects. HPLC assay is considered a robust method to quantify ADMA with a high degree of accuracy in a large number of subjects. However, ELISA, which allows relatively large numbers of samples to be analyzed more quickly than HPLC, would be more suitable for routine use. Although some HPLC assays appear to overestimate ADMA concentrations, the values reported here are within ranges of values found in acute pathological states. Neither the index of NO levels nor coronary endothelial function was measured directly. In experimental conditions, ADMA concentrations ranging from 1 to 10 μmol/L inhibit NO production by cultured macrophages and increase endothelial adhesiveness to monocytes in a dose-dependent manner. It is plausible to think that the levels of ADMA reported here may have pathophysiological significance, as its concentration falls into the range shown to interfere with NO bioavailability. Moreover, even higher intracellular levels of ADMA, associated with the inhibition of endothelial NO production and a marked loss of vasodilatory function, have been reported in pathological states. Unlike traditional cardiac biomarkers used to predict adverse outcomes, ADMA, as a hallmark of reduced NO bioavailability may be linked to many critical pathways involved in atherogenesis, glucose-related disorders and renal function, all of which affect the outcome after MI. Our study suggests that measuring ADMA levels at baseline might improve cardiovascular risk prediction beyond traditional risk factors and biomarkers. These insights also suggest the potential interest of new research pathways, such as modulators of DDAH activity or expression, for therapeutic intervention in acute myocardial infarction.

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Disclosures
None.

References
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Study patients

CV risk factors (history of diagnosed diabetes, history of hypertension or treated high blood pressure, treated or total cholesterol > 6.45 mmol/L, current or recently-stopped (< 3 months) smoking) were collected. The presence of clinical heart failure as defined by Killip class > 1 and current treatments prior to hospitalization were collected on admission. Patients with ST-segment elevation or suspected new left bundle branch block on the admission ECG were defined as having ST-segment elevation myocardial infarction (STEMI). Left ventricular ejection fraction (LVEF) was measured at day 3 ± 2 by echocardiography using the Simpson method and was cut at 40% for more clinical relevance. Acute reperfusion procedures (thrombolysis or primary percutaneous coronary intervention) and acute medications (<48h) were also collected. The Global Registry of Acute Coronary Event (GRACE) risk score was calculated with admission variables including age, heart rate, serum creatinine, systolic blood pressure, Killip class, cardiac arrest, ST-segment deviation and cardiac markers (http://www.outcomes-umassmed.org/grace/acs_risk.cfm) (11). The GRACE risk model has shown excellent characteristics as a predictor of mortality, providing most (>90%) of the prognostic information (11). Information at one-year follow-up (all-cause or CV mortality) was obtained at 341 ± 51 days by telephone interview or mail to the patients, the patient’s relatives or the treating physician. The study was approved by the Ethics Committee of the Hospital University and conducted in accordance with the Declaration of Helsinki. All subjects gave their written consent to participate in the study.

Laboratory methods

Briefly, serum was added with N-monomethyl L-arginine as the internal standard, and applied to a cation-mixed mode polymeric sorbent. The analytes were eluted with concentrated ammonia/water/methanol by vacuum suction, dried under nitrogen, derivatized with orthophthaldehyde reagent and injected into the HPLC system, which was equipped with a
fluorescent detector ($\lambda_{\text{exc}}$ 340 nm, $\lambda_{\text{em}}$ 455 nm) and a LiChrospher®100 RP-18 column protected by a guard-column. The intra sample variation was 6.3 and 7.3 % at 1.2 µmol/L and 9.5 and 9% at 0.6 µmol/L for ADMA and SDMA respectively. The recovery rate was > 94%. The detection limit of the assay was 0.1 µmol/L.

Homocysteine concentrations were determined by chemiluminescence on an Immulite 2000 analyzer (Diagnostic Products Corporation, Los Angeles, USA). Plasma NT-proBNP was determined by ELISA using Elecsys NT-proBNP sandwich immunoassay on Elecsys 2010 (Roche Diagnostics). CRP was measured on Dimension Xpand (Dade Behring) using immunonephelometry assay (analytical range 0.1–16 mg/dL). Plasma glucose concentrations (enzymatic method (glucose oxidase)) and creatinine levels were measured on a Vitros 950 analyzer (Ortho Clinical Diagnostics, Rochester, NY). The glomerular filtration rate (GFR) was estimated on the formula / [0.814 x creatinine (µmol/L)] x 0.85 for women (13). Total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations were measured on a Dimension analyzer (Dade Behring, Newark, NE). The level of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula. Peak plasma Creatine Kinase (CK) was assessed by sampling every 8H during the first 2 days after admission and was expressed as % of values 10 times higher than the upper limit of normal (ULN) (men: 170 UI/L and women: 135 IU/L).

**Statistical analysis**

For the tests across tertiles, we performed either the Kruskal-Wallis one-way analysis of variance by rank, for non-normally distributed values or one way ANOVA for normally distributed values. Categorical variables were compared by the chi-square test for trends. The Mann-Whitney test was used to test for continuous data in the 2 groups (deaths or survivors). Spearman correlation was applied to test for associations between ADMA and continuous variables. The independent predictors of ADMA were examined by stepwise multiple linear
regression analyses. Variables entered into the models were chosen because of their significant relationship in univariate analysis (SDMA, age, NT-proBNP), or because they can affect risk prediction after MI (female gender, GRF, prior MI, anterior wall location, LVEF, HR, and CRP), and classical risk factors (hypercholesterolemia, diabetes, hypertension, smoking). Receiver-operating characteristics (ROC) analysis was used to assess the ability of ADMA levels to predict mortality. The ROC curve indicates the probability of a true-positive result as a function of the probability of a false-positive result for all possible threshold values. Multivariate Cox regression analyses were used for the prediction of mortality at 1 year, in models including variables that were predictive by univariate analysis. The variables tested in univariate analysis were 1) the variables that are known to potentially affect the outcome after MI (GRACE risk score, NT-proBNP, CRP, LVEF, peak CK levels, GFR, reperfusion and acute statin therapy), 2) variables showing a variation according to ADMA tertiles (p<0.10) (age, current statin therapy, acute betablocker, STEMI) and 3) risk factors that have been previously shown to be associated with significant variations in ADMA levels (gender, homocysteine, diabetes, smoking, hypertension, hypercholesterolemia). Among these, 10 variables were predictors of prognosis (age, GFR, hypertension, NT-proBNP, GRACE risk score, CRP, homocysteine, LVEF, peak CK, acute statin therapy), and were therefore included as covariates in multivariate analysis (model 1) plus either SDMA (model 2) or ADMA (model 3) as dichotomic data (higher ADMA tertile (T3) vs lower tertiles (T1+T2)). As age, GFR and hypertension are major determinants of the prognosis, they were also tested as individual variables by Cox regression analysis. They were tested in separate analyses, instead of the risk score, in order to avoid multicolinearity since they are components included in the calculation of the risk score. The assumption of a linear relationship between continuous variables and mortality was assessed by univariate Cox proportional hazard models including the variable in its simple and square-rooted form in the
regression equation. The goodness of the fit was tested by the -2log likelihood $\chi^2$ criteria. The additional prognostic information of ADMA was tested by comparing the -2log likelihoods.
Table I. Spearman correlation analysis for the association for ADMA and study variables.

<table>
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<tbody>
<tr>
<td>SDMA</td>
<td>0.563</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.216</td>
<td>0.001</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>0.168</td>
<td>0.008</td>
</tr>
<tr>
<td>GRACE risk score</td>
<td>0.154</td>
<td>0.021</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>0.143</td>
<td>0.032</td>
</tr>
<tr>
<td>GFR</td>
<td>-0.126</td>
<td>0.050</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.119</td>
<td>0.070</td>
</tr>
<tr>
<td>L-arginine</td>
<td>0.108</td>
<td>0.089</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>-0.075</td>
<td>0.266</td>
</tr>
<tr>
<td>Heart rate</td>
<td>0.057</td>
<td>0.383</td>
</tr>
<tr>
<td>BMI</td>
<td>0.039</td>
<td>0.544</td>
</tr>
<tr>
<td>SBP</td>
<td>0.038</td>
<td>0.562</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.036</td>
<td>0.577</td>
</tr>
<tr>
<td>CRP</td>
<td>0.010</td>
<td>0.876</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>0.005</td>
<td>0.940</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.033</td>
<td>0.617</td>
</tr>
<tr>
<td>DBP</td>
<td>-0.049</td>
<td>0.459</td>
</tr>
<tr>
<td>LVEF</td>
<td>-0.057</td>
<td>0.419</td>
</tr>
</tbody>
</table>

ADMA: Asymmetric dimethylarginine; BMI: Body Mass Index; CRP: C-reactive protein; DBP: Diastolic blood pressure; GFR: Glomerular filtration rate; HDL-C: high density lipoprotein; LDL-C: low density lipoprotein; LVEF: Left ventricular ejection fraction; NT-proBNP: N-terminal Pro-Brain Natriuretic Peptide; SBP: Systolic blood pressure; SDMA: Symmetric dimethylarginine.