Symmetrical and Asymmetrical Dimethylarginine as Predictors for Mortality in Patients Referred for Coronary Angiography: The Ludwigshafen Risk and Cardiovascular Health Study

Andreas Meinitzer,1* Jan T. Kielstein,2 Stefan Pilz,3,4 Christiane Drechsler,5 Eberhard Ritz,6 Bernhard O. Boehm,7 Bernhard R. Winkelmann,8 and Winfried März1,9,10

BACKGROUND: Asymmetrical dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, has been linked to cardiovascular risk. The clinical role of its structural isomer symmetrical dimethylarginine (SDMA) remains largely unclear.

METHODS: We measured SDMA and ADMA in 3229 patients undergoing coronary angiography at baseline (1997–2000) and recorded total and cardiovascular mortality during a median follow-up time of 7.7 years. We investigated associations of SDMA with cardiovascular risk factors and mortality and compared its role as a cardiovascular risk factor with ADMA, which predicted mortality in previous analyses of our study.

RESULTS: In linear regression analyses including common cardiovascular risk factors as covariates, SDMA and ADMA were significantly associated with cystatin C, N-terminal pro-B–type natriuretic peptide, New York Heart Association classification, and homocysteine. The regression coefficients were higher for SDMA than for ADMA. In Cox proportional-hazards models adjusted for cardiovascular risk factors, the hazard ratios (HRs) (with 95% CI) in the second, third, and fourth SDMA quartile compared to the lowest quartile were 0.77 (0.60–0.99), 0.99 (0.78–1.25), and 1.51 (1.20–1.91) for total mortality and 0.92 (0.68–1.25), 0.93 (0.68–1.26), and 1.54 (1.14–2.01) for cardiovascular mortality. The same calculations for ADMA quartiles revealed HRs of 1.05 (0.83–1.32), 1.19 (0.86–1.63), and 1.16 (0.99–1.39) for total mortality and HRs of 1.00 (0.74–1.34), 1.26 (0.95–1.68), and 1.54 (1.18–2.02) for cardiovascular mortality.

CONCLUSIONS: Serum concentrations of SDMA are independently associated with increased cardiovascular and all-cause mortality in patients undergoing coronary angiography. The pattern of risk linked to SDMA is different from that linked to ADMA, suggesting different pathophysiological roles of these 2 methylarginine metabolites.

© 2010 American Association for Clinical Chemistry

Coronary artery disease (CAD)11 is the leading cause of death in developed countries like the United States (1). Algorithms for risk assessment in patients with CAD include several cardiovascular risk factors or a combination thereof (2). There is substantial interest in identifying new biomarkers that might help to better identify patients at increased risk for cardiovascular events who could be targeted for preventive measures. Dimethylarginines have been known to biochemists for decades (3). In the past 15 years, scientists mainly focused on asymmetrical dimethylarginine (ADMA), the most potent endogenous nitric oxide (NO) synthase inhibitor. ADMA correlates with traditional and nontraditional cardiovascular risk factors (for a review, see Cooke (4)) and predicts cardiovascular events and death in different patient populations (5–7). However,

1 Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria; 2 Department of Nephrology and Hypertension, Medical School Hannover, Hannover, Germany; 3 Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Medical University of Graz, Graz, Austria; 4 Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, University of Graz, Graz, Austria; 5 Cardiological Department, University Hospital, Ulm, Germany; 6 Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Heidelberg University, Heidelberg, Germany; 7 Division of Endocrinology, Department of Medicine, University Hospital, Ulm, Germany; 8 Cardiology Group, Frankfurt-Sachsenhausen, Germany; 9 Syntlab Center of Laboratory Diagnostics, Heidelberg, Germany; 10 Mannheim Institute of Public Health, Social and Preventive Medicine, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.

* Address correspondence to this author at: Clinical Institute of Medical and Chemical Laboratory Diagnostics, Auenbruggerplatz 29, 8036 Graz, Austria. Fax +43-316-385-13419.

Received May 19, 2010; accepted October 18, 2010.

Previously published online at DOI: 10.1373/clinchem.2010.150854

11 Nonstandard abbreviations: CAD, coronary artery disease; ADMA, asymmetrical dimethylarginine; NO, nitric oxide; SDMA, symmetrical dimethylarginine; GFR, glomerular filtration rate; LURIC, Ludwigshafen Risk and Cardiovascular Health; NT-proBNP, N-terminal pro-B–type natriuretic peptide; CRP, C-reactive protein; eGFR, estimated GFR; HR, hazard ratio; PTH, parathyroid hormone; AUC, area under the curve; PRMT, protein methyltransferase.
little attention has been paid to the structural isomer of ADMA, symmetrical dimethylarginine (SDMA). Kikimoto and Akazawa (3) showed that both ADMA and SDMA are excreted in the urine, but experimental evidence indicated that SDMA, in contrast to ADMA, is almost completely eliminated by renal excretion (8). ADMA showed that SDMA correlates tightly with the glomerular filtration rate (GFR) in humans (9). The same holds true for several animal species (10). In addition to being an excellent marker of renal function, SDMA may indirectly interfere with NO synthesis, suggesting a role for cardiovascular diseases (11). In this context, recently published results showed that SDMA is of clinical significance as an independent cardiovascular risk factor (12–16). In high-risk patients with coronary events as the leading cause of death, however, long-term follow-up data from a large, well-characterized cohort are still missing.

We aimed to evaluate the correlation of SDMA concentrations with renal function, cardiovascular risk factors, and mortality in a large cohort of 3229 patients referred for coronary angiography. In the same study cohort, we previously demonstrated that ADMA is a significant risk factor for mortality after a median follow-up time of 5.45 years (7). In our present analyses, with a median follow-up time of 7.7 years, we particularly aimed to elucidate whether SDMA and ADMA differ in their association with cardiovascular risk in patients with coronary heart disease and normal or slightly impaired renal function.

Methods

STUDY DESIGN AND PARTICIPANTS

We studied participants of the Ludwigsafen Risk and Cardiovascular Health (LURIC) study (17). Inclusion criteria were German ancestry, clinical stability except for acute coronary syndromes, and availability of a coronary angiogram. The indications for coronary angiography in individuals in clinically stable condition were chest pain and/or noninvasive test results consistent with myocardial ischemia. We excluded individuals suffering from acute illness other than acute coronary syndromes, chronic noncardiac diseases, or malignancy within the 5 past years and those unable to understand the purpose of the study. Hence patients with clinical evidence for chronic kidney disease were excluded. The study was approved by the ethics committee of the Medical Association of Rheinland-Pfalz (Ärztekammer Rheinland-Pfalz). We obtained informed written consent from all participants.

We assessed CAD by angiography and estimated maximum luminal narrowing by visual analysis. Clinically relevant CAD was defined as the occurrence of ≥1 stenosis of ≥20% in ≥1 of 15 coronary segments. Individuals with stenoses <20% were considered not to have CAD. The definition for CAD with a stenosis ≥20% is rather conservative compared with more common definitions such as ≥50% or ≥75%. Use of the ≥20% definition is based on the consideration to identify completely CAD-free patients in whom the calculated quartiles for ADMA and SDMA concentrations may resemble those ranges obtained in a coronary healthy control group. Results for quartile ranges of such a subpopulation might be more comparable to other cohorts without an indication for coronary angiography (as in the LURIC study). The diagnostic criteria of heart failure have been described in the baseline LURIC article (17). In detail, the diagnosis was based on the combined presence of symptoms of dyspnea on exertion and impaired left ventricular function assessed by echocardiography and/or ventricular angiography. Semiquantitatively graded left ventricular function assessed by contrast ventriculography was available in 3194 patients of the LURIC study and correlated significantly with N-terminal pro-B-type natriuretic peptide (NT-proBNP) (r = 0.3; P < 0.001) and with the calculated ejection fraction (r = 0.8; P < 0.001), which was available in 1360 study participants.

Diabetes mellitus was diagnosed if plasma glucose was >1.25 g/L in the fasting state or >2.00 g/L 2 h after an oral glucose load (18) or if individuals were receiving antidiabetic treatment. Hypertension was diagnosed if the systolic and/or diastolic blood pressure exceeded 140 and/or 90 mmHg or if there was a history of hypertension and use of antihypertensive drugs. Women were categorized into pre-, peri-, or postmenopausal according to menstrual bleeding history and the concentrations of follicle-stimulating hormone (17).

LABORATORY PROCEDURES

Blood was drawn in the fasting state before coronary angiography. Standard laboratory methods have been outlined in detail (17). We measured ADMA and SDMA in frozen serum (−80 °C) with the reversed-phase HPLC method (19), with slight modifications (20). Within-day CVs for SDMA were 4.6% (0.60 μmol/L) and 1.9% (1.0 μmol/L), and between-day CVs were 9.8% (0.60 μmol/L) and 6.1% (1.0 μmol/L). Within-day CVs for ADMA were 3.1% (0.62 μmol/L) and 1.0% (2.0 μmol/L), and between-day CVs were 9% (0.62 μmol/L) and 1.5% (2.0 μmol/L). Fibrinogen was measured by use of the Clauss method (Dade Behring). We used immunonephelometry for measurement of C-reactive protein (CRP) (N High Sensitivity CRP; Dade Behring) and cystatin C, the latter being a sensitive parameter to determine GFR (21, 22). We measured NT-proBNP, a marker of ventricular dysfunction and cardiovascular risk (23), by electrochemiluminescence on an Elecsys 2010 (Roche Diag-
nositics). We calculated estimated GFR (eGFR) with the Chronic Kidney Disease Epidemiology Collaboration equation (24).

FOLLOW-UP
During follow-up, information on vital status was obtained from local registries. Death certificates as well as medical records of local hospitals and autopsy data were reviewed to classify causes of death. Deceased patients were classified according to whether they died from cardiovascular or noncardiovascular causes. Cardiovascular deaths included sudden cardiac death, fatal myocardial infarction, death due to congestive heart failure, death immediately after intervention to treat CAD, fatal stroke, and other deaths due to cardiac causes. Three experienced clinicians who were blinded to the baseline characteristics of the study participants independently classified the causes of death. In cases of disagreement or uncertainty concerning the coding of a specific cause of death, classification was based on the majority opinion.

STATISTICAL ANALYSIS
Baseline characteristics are presented according to ADMA and SDMA quartiles, which were formed according to the concentrations observed in patients without CAD. Comparisons across these quartiles were done by $\chi^2$ test with $P$ for linear test for proportions and by ANOVA with $P$ for linear trend for continuous variables. For these analyses, concentrations of triglycerides, CRP, NT-proBNP, and homocysteine were logarithmically transformed to reduce skewness. We used linear regression analyses to explore associations of ADMA and SDMA with markers of kidney function (eGFR, cystatin C), parameters of myocardial dysfunction [NT-proBNP, New York Heart Association (NYHA) class], and others (albumin, hemoglobin, homocysteine, fibrinogen), adjusted for common cardiovascular risk factors (age, sex, body mass index, triglycerides, LDL and HDL cholesterol, CRP, smoking status, diabetes mellitus, hypertension). We used Cox proportional hazards models to compare the risk of all-cause and cardiovascular mortality between the ADMA and SDMA quartiles using the first ADMA and SDMA quartile as the reference. We calculated hazard ratios (HRs) and 95% CIs, which were adjusted for potential confounders as indicated. All statistical tests were 2-sided, and a $P$ value $<0.05$ was considered significant. The analyses were performed using SPSS 15.0 statistical package (SPSS Inc.).

Results
ADMA and SDMA concentrations were available in 3229 study participants (97.3% of the entire study cohort). Baseline clinical and laboratory characteristics according to ADMA and SDMA quartiles are shown in Table 1.

In linear regression analyses including common cardiovascular risk factors as covariates, SDMA concentrations were significantly associated with cystatin C ($\beta$ coefficient 0.715), eGFR ($-0.687$), NT-proBNP (0.321), NYHA class ($-0.095$), albumin ($-0.087$), hemoglobin ($-0.112$), homocysteine (0.331), ADMA (0.487), and fibrinogen (0.083) (all $P$ values $\leq 0.001$).

The same analyses with ADMA concentrations showed lower $\beta$ coefficients compared with SDMA, but associations remained statistically significant with the exception of fibrinogen. In detail we obtained the following results with ADMA concentrations: cystatin C ($\beta$ coefficient 0.414), eGFR ($-0.197$), NT-proBNP (0.163), NYHA class ($-0.083$), albumin ($-0.094$), hemoglobin (0.019), and homocysteine (0.169) (all $P$ values $<0.001$). ADMA in contrast to SDMA showed a significant relation to arginine, with a $\beta$ coefficient of 0.278 ($P < 0.001$).

In addition, we performed multivariate linear regression analyses using forward selection methods. We thereby included all variables that showed a significant association with ADMA and SDMA in the previous analyses except eGFR, which was excluded owing to collinearity with cystatin C. In the final model of SDMA, predictors were cystatin C $>$ ADMA $>$ NT-proBNP $>$ homocysteine $>$ CRP $>$ hemoglobin, with an overall $R^2$ of 0.58. The order of ADMA predictors was SDMA $>$ arginine $>$ cystatin C $>$ albumin $>$ hemoglobin $>$ CRP $>$ NYHA. The overall $R^2$ for ADMA was 0.35, suggesting that 35% of the variation of ADMA concentrations was explained by the selected covariates. In contrast, for SDMA, $>50\%$ was explained by the described variables.

Our prospective analyses included a median follow-up time of 7.7 years, during which 749 patients died (22.5% of the study population). We recorded 469 deaths (14.1%) due to cardiovascular causes. Eighteen people were lost during follow-up, and for 24 deceased individuals we did not obtain sufficient data to classify cause of death. These latter individuals were included in the analyses for all-cause mortality but were excluded from analyses for cardiovascular mortality.

For SDMA quartiles, we observed a J-shaped characteristic for the association with total as well as cardiovascular mortality. After adjustments for cardiovascular risk factors, the HRs (95% CIs) in the second, third, and fourth quartiles compared to patients in the lowest SDMA quartile were 0.77 (0.60–0.99), 0.99 (0.78–1.25), and 1.51 (1.20–1.91) for all-cause mortality and 0.92 (0.68–1.25) 0.93 (0.68–1.26), and 1.54 (1.14–2.01) for cardiovascular mortality (Tables 2 and 3; Fig. 1, A and C). Additional inclusion of some indicators of
Table 1. Baseline characteristics according to SDMA and ADMA quartiles.a

<table>
<thead>
<tr>
<th></th>
<th>SDMA quartile</th>
<th>ADMA quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First (≤0.45 μmol/L)</td>
<td>Second (0.46–0.53 μmol/L)</td>
</tr>
<tr>
<td>n</td>
<td>791</td>
<td>797</td>
</tr>
<tr>
<td>ADMA, μmol/L</td>
<td>0.73 (0.10)</td>
<td>0.79 (0.11)</td>
</tr>
<tr>
<td>SDMA, μmol/L</td>
<td>0.48 (0.11)</td>
<td>0.53 (0.14)</td>
</tr>
<tr>
<td>Arginine, μmol/L</td>
<td>81.9 (20.2)</td>
<td>82.0 (20.6)</td>
</tr>
<tr>
<td>Age, years</td>
<td>67.8</td>
<td>68.7</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>76.4</td>
<td>78.3</td>
</tr>
<tr>
<td>eGFR, mL·min⁻¹·(1.73 m²)⁻¹</td>
<td>90.0 (12.4)</td>
<td>84.9 (13.7)</td>
</tr>
<tr>
<td>Cystatin C, mg/L</td>
<td>0.82 (0.13)</td>
<td>0.89 (0.14)</td>
</tr>
<tr>
<td>Coronary artery disease, %</td>
<td>76.4</td>
<td>78.3</td>
</tr>
<tr>
<td>CAD50, %b</td>
<td>66.2</td>
<td>66.4</td>
</tr>
<tr>
<td>Deaths, n (%)</td>
<td>129 (16.3)</td>
<td>120 (15.1)</td>
</tr>
<tr>
<td>CVD deaths, n (%)</td>
<td>76 (9.7)</td>
<td>85 (10.7)</td>
</tr>
<tr>
<td>Arterial hypertension, %</td>
<td>70.3</td>
<td>71.0</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.0 (4.2)</td>
<td>27.3 (3.9)</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>31.7</td>
<td>29.7</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>141 (23)</td>
<td>141 (23)</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>81 (11)</td>
<td>81 (11)</td>
</tr>
<tr>
<td>Current cigarette smoking, %</td>
<td>26.3</td>
<td>21.7</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>117 (35)</td>
<td>117 (34)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>39.0 (10.6)</td>
<td>39.0 (10.6)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>184 (143)</td>
<td>170 (97)</td>
</tr>
<tr>
<td>NT-proBNP, mg/L</td>
<td>0.43 (1.06)</td>
<td>0.62 (1.02)</td>
</tr>
<tr>
<td>NYHA class 3 or 4, %</td>
<td>14.9</td>
<td>15.2</td>
</tr>
<tr>
<td>LV function, %</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Continued on page 116
malnutrition (albumin and hemoglobin) in our Cox regression analyses did not attenuate the predictive value of SDMA concentrations for total and cardiovascular mortality (data not shown).

For ADMA, we observed a gradual increase of all-cause and cardiovascular mortality across quartiles. After adjustments for cardiovascular risk factors, the HRs (95% CIs) in the second, third, and fourth quartiles compared to patients in the lowest ADMA quartile were 1.05 (0.83–1.32), 1.19 (0.95–1.50), and 1.61 (1.30 –1.99) for all-cause mortality and 1.00 (0.74 –1.34), 1.26 (1.03–1.68), and 1.54 (1.18 –2.02) for cardiovascular mortality (Tables 2 and 3; Fig. 1, B and D). Inclusion of albumin and hemoglobin as additional covariates changed these HRs to 1.18 (0.91–1.53), 1.30 (1.02–1.67), and 1.73 (1.37–2.19) for all-cause mortality and 1.17 (0.84 –1.62), 1.41 (1.03–1.92), and 1.73 (1.29 –2.33) for cardiovascular mortality. Inclusion of the 5 different medication classes as covariates into the Cox regression analyses did not materially change our results (data not shown).

Parathyroid hormone (PTH), a predictor of cardiovascular mortality (25) that has previously been associated with ADMA concentrations (26), was significantly associated with SDMA (r = 0.34, P < 0.001) and ADMA (r = 0.18, P < 0.001) and ADMA concentrations to our Cox proportional hazard analyses. We found no significant interaction between PTH and SDMA or ADMA as assessed by the C statistic (equivalent to the area under the ROC curve) as described (25, 27). We calculated this C statistic for all-cause mortality according to the multivariate adjusted model (model 3) with and without ADMA as well as SDMA. Parameters from model 3 (age, sex, CAD on angiography, body mass index, hypertension, smoking status, LDL cholesterol, HDL cholesterol, triglycerides, and eGFR) showed discrimination for all-cause mortality of 0.728 (95% CI 0.707–0.748) area under the curve (AUC). Addition of ADMA modified AUC to 0.739 (0.719 – 0.754). Removal of ADMA and addition of SDMA changed the AUC to 0.725 (0.704–0.746).

To quantitatively assess the discriminative predictive power of ADMA and SDMA for mortality, we calculated the area under the curve (AUC) of the Cox regression analyses. We found no significant difference between ADMA and SDMA as assessed by the C statistic (equivalent to the area under the ROC curve) as described (25, 27). We calculated this C statistic for all-cause mortality according to the multivariate adjusted model (model 3) with and without ADMA as well as SDMA. Parameters from model 3 (age, sex, CAD on angiography, body mass index, hypertension, smoking status, LDL cholesterol, HDL cholesterol, triglycerides, and eGFR) showed discrimination for all-cause mortality of 0.728 (95% CI 0.707–0.748) area under the curve (AUC). Addition of ADMA modified AUC to 0.739 (0.719–0.754). Removal of ADMA and addition of SDMA changed the AUC to 0.725 (0.704–0.746).

Table 1. Baseline characteristics according to SDMA and ADMA quartiles. (Continued from page 115)
AUC to 0.753 (0.733–0.772). Hence, there is no significant difference between ADMA and SDMA in improving mortality risk prediction, although results for SDMA were tentatively better. Similar results for the C statistic were obtained for cardiovascular mortality (data not shown).

Discussion

The key findings of the largest clinical study so far measuring SDMA and ADMA in patients who underwent coronary angiography confirm that SDMA correlates with parameters of renal function and document that it is independently associated with all-cause and cardiovascular mortality. The prediction is quantitatively different from that provided by ADMA.

Our univariate analyses of SDMA and different markers of renal function confirm earlier studies. Marescau et al. (28) described the close relationship between SDMA and eGFR by the Cockroft–Gault equation 13 years ago. This was confirmed by other studies summarized by a recent metaanalysis (9, 11, 29, 30). The correlations of SDMA with cystatin C in small patient cohorts were reported in 2006 (31, 32).

Table 2. HRs (95% CIs) for death from all causes according to SDMA and ADMA.

<table>
<thead>
<tr>
<th>All individuals (n = 3229)</th>
<th>SDMA, µmol/L</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>First quartile (&lt;0.44)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Second quartile (0.45–0.53)</td>
<td>0.92 (0.72–1.18)</td>
<td>0.509</td>
<td>0.80 (0.63–1.03)</td>
<td>0.082</td>
</tr>
<tr>
<td>Third quartile (0.54–0.62)</td>
<td>1.32 (1.05–1.67)</td>
<td>0.018</td>
<td>1.24 (0.81–1.29)</td>
<td>0.804</td>
</tr>
<tr>
<td>Fourth quartile (≥0.62)</td>
<td>2.62 (2.14–3.21)</td>
<td>&lt;0.001</td>
<td>1.68 (1.31–2.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADMA, µmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First quartile (&lt;0.72)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Second quartile (0.72–0.80)</td>
<td>1.20 (0.95–1.52)</td>
<td>0.129</td>
<td>1.06 (0.84–1.35)</td>
<td>0.614</td>
</tr>
<tr>
<td>Third quartile (0.81–0.89)</td>
<td>1.48 (1.18–1.87)</td>
<td>0.001</td>
<td>1.29 (1.02–1.62)</td>
<td>0.033</td>
</tr>
<tr>
<td>Fourth quartile (≥0.89)</td>
<td>2.37 (1.93–2.93)</td>
<td>&lt;0.001</td>
<td>1.82 (1.47–2.25)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3. HRs (95% CIs) for death from cardiovascular causes according to SDMA and ADMA.

<table>
<thead>
<tr>
<th>All individuals (n = 3205)</th>
<th>SDMA, µmol/L</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>First quartile (&lt;0.44)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Second quartile (0.45–0.53)</td>
<td>1.11 (0.81–1.51)</td>
<td>0.519</td>
<td>0.98 (0.72–1.33)</td>
<td>0.871</td>
</tr>
<tr>
<td>Third quartile (0.54–0.62)</td>
<td>1.27 (0.94–1.72)</td>
<td>0.123</td>
<td>1.00 (0.74–1.36)</td>
<td>0.997</td>
</tr>
<tr>
<td>Fourth quartile (≥0.62)</td>
<td>2.82 (2.17–3.67)</td>
<td>&lt;0.001</td>
<td>1.86 (1.42–2.44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADMA, µmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First quartile (&lt;0.72)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Second quartile (0.72–0.80)</td>
<td>1.15 (0.85–1.56)</td>
<td>0.366</td>
<td>1.03 (0.76–1.39)</td>
<td>0.856</td>
</tr>
<tr>
<td>Third quartile (0.81–0.89)</td>
<td>1.61 (1.21–2.15)</td>
<td>0.001</td>
<td>1.41 (1.06–1.88)</td>
<td>0.019</td>
</tr>
<tr>
<td>Fourth quartile (≥0.89)</td>
<td>2.32 (1.77–3.02)</td>
<td>&lt;0.001</td>
<td>1.81 (1.38–2.36)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

AUC to 0.753 (0.733–0.772). Hence, there is no significant difference between ADMA and SDMA in improving mortality risk prediction, although results for SDMA were tentatively better. Similar results for the C statistic were obtained for cardiovascular mortality (data not shown).

Discussion

The key findings of the largest clinical study so far measuring SDMA and ADMA in patients who underwent coronary angiography confirm that SDMA correlates with parameters of renal function and document that it is independently associated with all-cause and cardiovascular mortality. The prediction is quantitatively different from that provided by ADMA.

Our univariate analyses of SDMA and different markers of renal function confirm earlier studies. Marescau et al. (28) described the close relationship between SDMA and eGFR by the Cockroft–Gault equation 13 years ago. This was confirmed by other studies summarized by a recent metaanalysis (9, 11, 29, 30). The correlations of SDMA with cystatin C in small patient cohorts were reported in 2006 (31, 32).
The consistent increase in mortality with increasing ADMA concentrations in our present analysis is in line with results from other studies (5, 6) and confirms our previous LURIC study results with a shorter follow-up (7). In contrast to ADMA, the association of SDMA and mortality was J-shaped, with the highest mortality risk for patients in the fourth SDMA quartile and a decreased risk of death in the second compared to the first SDMA quartile. Interestingly, the J-shaped association of SDMA and mortality increased upon multivariable adjustment with cardiovascular risk factors (see model 3, Table 2). Furthermore, if we related ADMA to SDMA by multiplication, the rank order of the quartiles in total mortality was also significantly reduced in the second compared to the first quartile (data not shown). Given the observed role of SDMA as a cardiovascular risk factor, we believe that further studies are needed to confirm and extend our findings regarding SDMA and elucidate the underlying mechanisms for the differences of the relationship of ADMA and SDMA with mortality and fatal cardiovascular events. In this context, it may be of interest that increased plasma SDMA concentrations correlated better with the total sequential organ failure assessment than ADMA (33). If our finding is confirmed by independent studies, the measurement of SDMA may emerge as a diagnostic tool for assessment of adverse cardiovascular outcome.

What are the possible pathophysiological mechanisms linking SDMA and mortality? In part, this association can be explained by the fact that SDMA is excreted by glomerular filtration. This strong association of SDMA with eGFR might also partially explain the J-shaped SDMA mortality curve when considering that eGFR shows a U-shaped association with mortality. Toward this, the linear association between renal function and mortality is well documented for eGFR levels <60 mL·min⁻¹·(1.73 m²)⁻¹ (34). In >33 000 patients, however, it was shown that compared to eGFRs of 60–90 mL·min⁻¹·(1.73 m²)⁻¹ there was a 29% and 163% increase in mortality for patients with eGFRs of 90–119 and 120–150 mL·min⁻¹·(1.73 m²)⁻¹, respectively (35). Hence, the SDMA mortality association in our cohort may reflect the association of renal function and mortality. The much weaker association of ADMA with eGFR might be responsible for the differences in the mortality risk prediction by ADMA and SDMA. SDMA, however, retained its predictive power in multivariate analyses, including eGFR or cystatin C, suggesting that mortality prediction by SDMA is not driven only by its association with parameters of renal function. There is experimental evidence that SDMA may indirectly interfere with NO synthesis. SDMA inhibits the y⁺ transporter that mediates the intracellular uptake of L-arginine (36) and decreases renal tubular arginine absorption (37). These 2 mechanisms
SDMA and ADMA as Predictors for Mortality

SDMA is increased in states of high protein turnover and ADMA have been associated with poor outcomes and whose relationship with ADMA and SDMA deserves further in-depth studies (25, 26). Finally, we want to note that our present results confirm recently published data indicating that SDMA is a marker of increased risk for cardiovascular disease and mortality (12–16).

We wish to point out 2 crucial limitations of our study. It was not designed to elucidate the pathophysiological pathways linking SDMA to survival, but addressed the potential clinical value of SDMA as a predictor of survival in patients with cardiovascular disease. The design of our study precludes conclusions on cause and effect. Moreover, we did not use methods measuring true glomerular filtration, which would not have been feasible in such a large number of patients. Above all, as pointed out by Manolio (46) there are problems inherent to new biomarkers. Although initial reports about novel markers provide exciting clues into the pathophysiology of diseases and enable us to improve diagnostic capabilities, translating these into clinical application requires replication in multiple settings, ideally with an intervention trial proving that modification of the new marker improves outcome. The time-consuming measurement of SDMA by HPLC might be the least problematic component in employing this new marker for routine clinical use.

In summary, this is the first clinical study showing that SDMA is a predictor of survival in patients referred for coronary angiography but with a different impact than its structural isomer ADMA. Future studies should measure and report both methylarginines, i.e., ADMA and SDMA, as both may be independently predictive.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: B.O. Boehm, LURIC; B. Winkelmann, LURIC; W. März, LURIC.

Consultant or Advisory Role: None declared.

Stock Ownership: None declared.

Honoraria: None declared.

Research Funding: LURIC has received funding through the Sixth Framework Programme (integrated project Bloodomics, grant LSHM-CT-2004-503485) and Seventh Framework Programme (integrated project Atheroremo, Grant Agreement number 201668) of the European Union. C. Drechsler, research grants from the European Renal Association–European Dialysis and Transplant Association and the Deutsche Forschungsgemeinschaft (German Research Foundation).

Expert Testimony: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Acknowledgments: The authors extend sincere appreciation to the participants of the Ludwigshafen Risk and Cardiovascular Health Study; without their collaboration this article would not have been written. We thank Gabriele Gartner for excellent technical assistance; the LURIC study team either temporarily or permanently involved in patient recruitment and sample and data handling; the laboratory staff at the Ludwigshafen General Hospital and the Universities of Freiburg and Ulm, Germany; and the German registration offices and local public health departments for their assistance.

References


