Asymmetric Dimethylarginine and the Risk of Cardiovascular Events and Death in Patients With Coronary Artery Disease
Results from the AtheroGene Study

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Abstract—As a competitive inhibitor of endothelial nitric oxide synthase, asymmetric dimethylarginine (ADMA) has been related to atherosclerotic disease. Little is known about the prognostic impact of baseline ADMA determination. In a prospective cohort of 1908 patients with coronary artery disease, we assessed baseline serum concentration of ADMA in 1874 consecutive patients with coronary artery disease. One hundred fourteen individuals developed the primary end point of death from cardiovascular causes or nonfatal myocardial infarction during a mean follow-up of 2.6±1.2 years. Median concentrations of ADMA levels were higher among individuals who subsequently developed the primary end point than among those who did not (0.70 versus 0.63 μmol/L; P<0.001). The risk of future cardiovascular event was associated with increasing thirds of baseline ADMA (P for trend, <0.001) such that individuals in the highest third at entry had a hazard ratio 2.48 times higher than those in the lowest third (95% confidence interval, 1.52 to 4.06; P<0.001). This relationship remained nearly unchanged after adjustment for most potential confounders. Prediction models that simultaneously incorporated ADMA, B-type natriuretic peptide, C-reactive protein, and creatinine in addition to traditional risk factors revealed B-type natriuretic peptide (hazard ratio, 1.96; 95% confidence interval, 1.3 to 3.0; P=0.002) and ADMA (hazard ratio, 1.90; 95% confidence interval, 1.3 to 2.8; P=0.001) as the strongest risk predictors. High levels of baseline ADMA independently predict future cardiovascular risk. ADMA has prognostic value beyond traditional risk factors and novel biomarkers and might guide therapeutic strategies. (Circ Res. 2005;97:e53-e59.)

Key Words: asymmetric dimethylarginine ▪ cardiovascular risk ▪ coronary artery disease

The endothelium plays a crucial role in the regulation of vascular tone.1 A factor of prime importance to maintain endothelial homeostasis is nitric oxide.2 Apart from its vasodilating effects, nitric oxide released from the endothelial nitric oxide synthase mediates many protective functions against atherosclerosis, such as reduction of adhesion molecule expression and leukocyte adhesion, as well as reduction of proinflammatory cytokines. It further controls smooth muscle cell proliferation and platelet aggregation and balances profibrinolytic activities.

Substrate for endothelial nitric oxide synthesis is the amino acid l-arginine.3 The endothelium is also capable of producing methylated amino acids such as asymmetric dimethylarginine (ADMA), which are able to out-compete l-arginine as a substrate for endothelial nitric oxide synthase, leading to endothelial dysfunction4 (for review see Cooke5). Importantly, endothelial dysfunction in coronary6 and peripheral arteries7 and the degree of oxidative stress within vascular tissue7 have been recently identified to be independent predictors of future cardiovascular events.

Evidence has emerged that circulating levels of ADMA are elevated in patients with cardiovascular risk factors and renal failure and are, in part, associated with the presence of endothelial dysfunction.4,8–11 However, it remains unclear whether measurement of levels of ADMA improves cardiovascular risk prediction, in particular, in comparison with traditional risk factors and newly discussed biomarkers such as C-reactive protein or B-type natriuretic peptide.

With the present study, we, therefore, addressed the hypothesis that circulating levels of ADMA are independently

Original received June 23, 2005; revision received July 20, 2005; accepted August 2, 2005.
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Circulation Research is available at http://circres.ahajournals.org DOI: 10.1161/01.RES.0000181286.44222.61
related to future cardiovascular events in a large prospective cohort of patients with coronary artery disease and, thus, may, as suggested, constitute a novel risk factor for cardiovascular disease.

Materials and Methods
Study Participants
Between June 1999 and February 2004, 1908 patients who underwent coronary angiography at the Department of Medicine II of the Johannes Gutenberg-University Mainz or the Bundeswehr Central Hospital Koblenz and who had at least 1 stenosis >30% diagnosed in a major coronary artery were enrolled in the AtheroGene study registry. A detailed description of the design of the AtheroGene Study has been described in detail elsewhere. Briefly, the exclusion criteria were evidence of hemodynamically significant valvular heart disease, surgery, or trauma within the previous month, known cardiomyopathy, known cancer, febrile conditions, or use of oral anticoagulant therapy within the previous 4 weeks. Patients who had received antihypertensive treatment or who had received a diagnosis of hypertension (blood pressure above 160/90 mm Hg) were considered to have hypertension. Patients were classified as currently smoking, as having smoked in the past (if they had stopped >4 weeks and <40 years earlier), or as never having smoked (if they had never smoked or had stopped ≥40 years).

Among the patients, 1874 (98.2%) were followed for a median of 2.6 years (maximum 5.0). Follow-up information was obtained on death from cardiovascular causes (n = 69), death from causes not related to heart disease (n = 32), nonfatal myocardial infarction (n = 45), and nonfatal stroke (n = 45). Information on the cause of death or clinical events was obtained from hospital or general practitioner charts. The study was approved by the local ethics committee. Participation was voluntary, and each subject gave written, informed consent.

Laboratory Methods
Fasting blood was drawn under standardized conditions before coronary angiography. Samples were immediately processed and stored at −80°C until analysis.

ADMA was measured from serum samples by competitive ELISA (DLD Diagnostika GmbH, Hamburg, Germany) with a standard range from 0.1 to 5.0 μmol/L. The detection limit is 0.05 μmol/L. As described before, the intraassay coefficients of variation were 7.5% at 0.81 μmol/L and 4.5% at 1.76 μmol/L; the interassay coefficient of variation ranged from 8.3% to 10.3%. Cross-reactivity with other natriuretic peptides is negligible. Plasma B-type natriuretic peptide was determined using a fluoroscent immunoassay (Biosite Inc, San Diego, Calif). The detection limit reported is <5 pg/mL. The assay has an interassay coefficient of variation of near 10%, and a recovery of 100% of added peptide was found. Cross-reactivity with other natriuretic peptides is negligible. C-reactive protein was determined by a highly sensitive, latex particle–enhanced immunoassay (detection range of 0 to 20 mg/L, Roche Diagnostics, Mannheim, Germany). Lipid serum levels were measured immediately by standardized routine methods. Glomerular filtration rate (GFR) was calculated by Cockcroft formula: (140−age)×weight/(72×serum creatinine); additionally multiplied by 0.8 for women.

Statistical Analysis
The mean levels and proportions of baseline cardiovascular risk factors were calculated for study participants in whom a cardiovascular event subsequently occurred and in those without such an event. The significance of differences between the means for the 2 groups was assessed with Student’s t test, and the significance of differences in proportions was tested with the χ² statistic. Variables with a skewed distribution were presented as medians, and the Wilcoxon rank-sum test was applied. Pearson’s (Spearman) correlation coefficients were calculated to assess the strength of the correlation of ADMA and parameters of renal function (serum creatinine/GFR) and lipid parameters (triglycerides and cholesterol levels). The cumulative event plots according to thirds of ADMA were estimated by the Kaplan–Meier method and compared with use of the log-rank test. The primary end point was death from cardiovascular causes or nonfatal myocardial infarction. Data from patients who died from other causes were censored at the time of death. To normalize the distribution of skewed variables log transformation was performed and the hazard ratio per 1-SD increment applied. In addition, hazard ratios for future coronary events according to thirds of ADMA were estimated by Cox regression models adjusted for potential confounders. Three adjusted models were constructed. We adjusted, first, for age and sex and, second, for other traditional and clinical risk factors. The final model included clinical and therapeutic variables as well as C-reactive protein and B-type natriuretic peptide. Furthermore, the strength of ADMA, B-type natriuretic peptide, C-reactive protein, and creatinine for cardiovascular risk prediction was compared. Therefore, Cox predictive models were calculated for the upper-third of each respective variable adjusted for cardiovascular confounders (model 2) and additionally for all 3 markers simultaneously (model 3). Hazard ratios and 95% confidence intervals are reported. To assess a potential increase in predictive information regarding future cardiovascular events, areas under the receiver operating curves were calculated for the combined analysis of traditional risk factors (model 1) and single-biomarker determination, as well as simultaneous assessment of all 4 markers in addition to traditional risk factors. The probability values are 2-sided; a probability value of <0.05 was considered to indicate statistical significance. All computations were performed with SPSS software, version 11.05.

Results
Baseline Characteristics
The mean age of the study participants was 61.0±9.8 years; 79.1% were male. Patients with extreme ADMA concentrations outside 2 SD were excluded from further analysis.

ADMA concentration was slightly skewed among the study participants. It ranged from 0.12 to 3.92 with a mean of 0.68 and a median of 0.63 μmol/L with a 25th/75th interquartile range of 0.53/0.74 μmol/L. Patients presenting with acute coronary syndrome had similar ADMA concentrations compared with stable angina patients (0.64 [0.54/0.76] versus 0.63 [0.54/0.76] μmol/L; P = 0.66). The baseline concentration of ADMA was significantly higher among those who died from cardiac causes or had a nonfatal myocardial infarction than among those who did not. As expected, in patients with subsequent cardiovascular events, plasma levels of B-type natriuretic peptide (P < 0.001), C-reactive protein (P < 0.001), and creatinine (P < 0.001) were elevated. Further characteristics of the 114 study participants who developed a cardiovascular event and the 1758 who did not are outlined in Table 1.

ADMA levels were neither significantly associated with age nor gender. The strongest predictors of the level of ADMA were smoking status and diabetes. Significantly higher serum concentrations were measured in current and ever smokers versus never smokers (0.64 [0.54/0.75] versus 0.61 [0.53/0.72] μmol/L in nonsmokers; P = 0.002). Diabetic patients revealed elevated ADMA levels in comparison with nondiabetics (0.65 [0.54/0.77] versus 0.63 [0.53/0.73] μmol/L; P = 0.012). No association of serum
concentrations and a history of hypertension or an increased body mass index was detected (data not shown). No correlation was observed between ADMA levels and C-reactive protein, B-type natriuretic peptide, or any lipid level (high-density lipoprotein, \( r = 0.02 \); low-density lipoprotein, \( r = 0.01 \); triglycerides, \( r = 0.01 \)). However, a moderate interdependence was observed between C-reactive protein and B-type natriuretic peptide (\( r = 0.34 \)). Correlation coefficients for ADMA and GFR or serum creatinine were \( r = 0.03 \) and \( r = 0.025 \), respectively.

### Incidence of Subsequent Events

Table 2 provides the association of 1-SD increment in log ADMA values with future cardiovascular events. Increasing ADMA levels were associated with an elevated risk of cardiovascular death (increase of 27% for ADMA for each increment of 1-SD in log-transformed ADMA values) and cardiovascular events. By contrast, no significant association was observed between ADMA values and death of causes other than cardiovascular (hazard ratio, 1.14; 95% confidence interval, 0.86 to 1.52; \( P = 0.36 \)) and stroke (hazard ratio, 1.12; 95% confidence interval, 0.95 to 1.50; \( P = 0.12 \)). The primary end point of the study was death from cardiovascular causes and nonfatal coronary events. Figure 1 provides the Kaplan–Meier curves for event-free survival according to thirds of baseline ADMA levels. The unadjusted cardiovascular event rate increased in a stepwise fashion across increasing thirds of baseline ADMA levels (\( P < 0.001 \)). To assess the independent predictive value of ADMA concentration, we performed a series of Cox predictive models (Table 3). In an age- and
sex-adjusted model, individuals in the upper-third revealed a 2.48-fold increase (95% confidence interval, 1.52 to 4.06) in cardiovascular risk compared with those among the lowest third. Further adjustment for clinical and therapeutic variables as well as C-reactive protein, creatinine, and B-type natriuretic peptide did not attenuate the hazard ratio associated with ADMA concentration.

Comparative Analyses of Cardiovascular Biomarkers

To place the predictive value of baseline ADMA levels into the context of that obtained from measurement of B-type natriuretic peptide, C-reactive protein, and creatinine levels as a measure of renal function, we applied two Cox predictive models as outlined in Figure 2. Values above the upper-tertile of ADMA (<0.70 μmol/L), B-type-natriuretic peptide (>102.31 pg/mL), creatinine (>1.02 mg/dL), and C-reactive protein (>5.44 mg/L) were independently associated with an increase in the risk of cardiovascular events of 76%, 124%, 78%, and 91%, respectively, in a model adjusted for potential cardiovascular confounders. In multivariable analyses additionally including all markers simultaneously, ADMA (hazard ratio, 1.90; 95% confidence interval, 1.52 to 2.48) and B-type natriuretic peptide (hazard ratio, 1.96; 95% confidence interval, 1.52 to 4.06) revealed the strongest predictive value. The area under the receiver operating curve, 0.67 in the model including all traditional risk factors, moderately increased with B-type natriuretic peptide or ADMA on top (area under the curve, 0.69). When all markers were included simultaneously, the area under the receiver operating curve increased to 0.71.

Discussion

The present study provides evidence that serum ADMA independently predicts future cardiovascular risk in patients with coronary artery disease and might, thus, constitute a novel biomarker of cardiovascular risk.
ADMA represents a new and well-characterized marker that has been associated with many traditional and novel risk factors in the setting of atherosclerosis. In particular, ADMA levels are significantly elevated in patients with various cardiovascular risk factors as demonstrated for hypertension, hyperlipidemia, and hyperhomocysteinemia. In addition, they are elevated in conditions of peripheral artery disease, stroke, and end-stage renal failure in a cross-sectional manner.

In vitro experiments have provided insight into which mechanisms intracellular and/or extracellular ADMA levels may increase. Exposure of cultured endothelial cells to glucose, homocysteine, low-density lipoprotein as well as oxidized low-density lipoprotein stimulates the activity of the L-arginine–methylating enzyme and also inhibits the activity of demethylating enzymes, such as the dimethylarginine dimethylaminohydrolase, by means of reactive oxygen species. This results in increased intracellular and secondary extracellular ADMA concentrations and, subsequently, to decreased endothelial nitric oxide–mediated nitric oxide production and endothelial dysfunction. Accordingly, treatment of these cells with antioxidants has been demonstrated to restore the activity of dimethylarginine dimethylaminohydrolase, leading to a normalization of cellular ADMA levels and endothelial nitric oxide production. Infusion of ADMA in pathophysiologically relevant levels causes significant hemodynamic changes in humans. Thus, increased synthesis of ADMA and the subsequent impairment of nitric oxide synthesis may provide a common pathway by which many of the proatherogenic factors amount to clinically relevant cardiovascular risk.

Investigations aiming to elucidate the pathophysiological pathways by which ADMA interferes with endothelial function have been paralleled by in vitro as well as in vivo studies. Starting from the hypothesis to overcome the competitive inhibition by artificially increased concentrations of L-arginine, it has been demonstrated that L-arginine treatment decreases lesion development in hypercholesterolemic and low-density lipoprotein–receptor knock-out mice. Administration of L-arginine also inhibits monocyte accumulation, restores nitric oxide activity, and attenuates platelet reactivity in hypercholesterolemic rabbits.

Since the demonstration that concentration of ADMA acts as a marker (or even producer) of endothelial dysfunction, circulating levels of ADMA have been related to presence, extent, and severity of coronary artery disease and the occurrence of acute coronary syndrome in middle-aged Finnish nonsmokers. Furthermore, in patients with coronary artery disease, plasma levels of ADMA predicted subsequent adverse cardiovascular events in patients undergoing percutaneous coronary intervention. The data of our study extend this knowledge and demonstrate for the first time that ADMA is associated with the primary end points of cardiovascular death and nonfatal myocardial infarction, whereas no relationship could be observed with death from causes other than cardiovascular disease. The strength of ADMA for cardiovascular risk prediction was shown in comparison with 2 novel biomarkers, the cardiac hormone B-type natriuretic peptide, as an indicator of myocyte stretch, and C-reactive protein, as a measure of the inflammatory burden in atherosclerosis. Comparison with these 2 markers is especially interesting, as they have increasingly entered clinical routine.

In the present cohort, ADMA proved to provide independent predictive power even after adjustment for these strong markers. That any of these markers representing different pathophysiological processes related to cardiovascular disease may provide independent and additional information for cardiovascular risk prediction has been evaluated in receiver operating curve characteristics calculation. Intriguingly, simultaneous assessment of all 3 markers further increased the area under the curve from 0.67 including all traditional risk factors.

![Figure 2. Hazard ratio and 95% confidence interval of cardiovascular events associated with the upper-third in the concentration of each biomarker in comparison with the lower thirds. The traditional risk factors (model 1) comprised age, sex, body mass index, a history of hypertension, diabetes, smoking status, and high-density lipoprotein (as continuous variable). Model 2 provides the hazard ratio of each biomarker adjusted for the traditional risk factors as outlined in model 1 as well as angiotensin-converting enzyme inhibitors, statin, and β-blocker therapy, and extent of vessel disease. Model 3 is additionally adjusted for the 4 respective markers simultaneously. Further provided is the area under the receiver operating curve for prediction of future cardiovascular events by traditional risk factors (model 1), single-marker determination in addition to traditional risk factors (model 2), and simultaneous biomarker evaluation (model 3).](http://circres.ahajournals.org/DownloadedFrom)
factors to 0.71, which suggests a better model. However, whether or not this increase of the area under the receiver operating curve necessarily indicates a clinically relevant improvement in cardiovascular risk prediction has to be further evaluated in larger prospective epidemiological studies of primary and secondary prevention. As a further limitation, only data from baseline medication are available, and changes of ADMA-levels under cardiac medication cannot be assessed. It would be interesting to demonstrate whether ADMA concentration is influenced by standard therapies.

Plasma ADMA levels also correlated with other markers for atherosclerosis such as the carotid intima media thickness in asymptomatic subjects and patients with end-stage renal disease. For this reason, elevated ADMA levels and their implications on outcome and cardiovascular risk have been intensively studied in this patient population with up to 10-fold increased plasma concentrations. Uniformly, ADMA was reported to contribute to presence and severity of cardiovascular disease in cross-sectional studies and to mortality in patients with renal failure.

For the present study population, a strong relation of serum creatinine and outcome could be documented, as has recently been described. To exclude that measurement of ADMA might only mirror renal function, and, to include an acceptably accurate index of mild impairment of renal function, GFR was introduced in all regression models. The results support the notion that ADMA provides risk information that is, at least in part, independent of renal function, although a correlation with reduction in renal function has been described. The latter article also reports an association between ADMA concentration and smoking, which can be confirmed by present data.

In recent studies, a strong correlation of ADMA with serum cholesterol has been described, which the present investigation cannot confirm. This might, at least in part, be explained by administration of statin medication in half of the population, which might blur the results of “ADMA-lipid” correlation in this cohort.

Circulating levels of ADMA have been analyzed so far by means of a high-performance liquid chromatography–based technique. With the present studies, we used a recently introduced ELISA technique that has been extensively evaluated against liquid chromatography mass spectrometry. It represents a reliable procedure that is suitable for the determination of ADMA in large sample series.

From a clinical perspective, assessment of ADMA might aid cardiovascular risk assessment. This biomarker represents nitric oxide bioavailability and also oxidative stress and, thus, identifies individuals at high cardiovascular risk even in an early stage, apart from traditional risk factors and inflammatory biomarkers. In addition, results from small trials indicate that ADMA levels may decrease in response to treatment with angiotensin-converting enzyme inhibitors, statins, or antioxidants, suggesting that this biomarker may be used in the future, similar to B-type natriuretic peptide in conditions of chronic congestive heart failure, as an indicator of reduced oxidative stress within vascular tissue and, therefore, successful treatment.

Our study has important implications for risk stratification. It emphasizes the importance of markers of left-ventricular dysfunction, inflammation, and oxidative stress as being paramount and suggests that modifying these risk factors are likely to lead to the greatest clinical and population benefit in patients with vascular disease. Whether or not these specific markers represent an independent causal pathway for atherogenesis and its clinical complications will require interventional trials with specific agents that primarily affect inflammation or oxidative stress.

In conclusion, the results of this prospective study suggest that circulating ADMA adds independent prognostic information with regard to cardiovascular risk beyond that obtained from classical risk factors and novel biomarkers.

Acknowledgments
This work was supported by the “Stiftung Rheinland-Pfalz für Innovation,” Ministry for Science and Education (AZ 15202–386261/545), Mainz, Germany; and by the MAIFOR grant 2001 of the Johannes Gutenberg-University Mainz. The test assay for B-type natriuretic peptide was kindly performed by Biosite Inc, San Diego, Calif. We are indebted to Margot Neuser for graphical work.

References


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Circ Res. 2005;97:e53-e59; originally published online August 11, 2005;
doi: 10.1161/01.RES.0000181286.44222.61
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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