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Objective—Circulating concentrations of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthesis, are elevated in conditions associated with increased cardiovascular risk. We investigated whether elevated ADMA concentrations predict major adverse cardiovascular events (MACE) in patients with advanced peripheral artery disease (PAD).

Methods and Results—We prospectively enrolled 496 of 533 consecutive patients with PAD (median age 70 years, 279 males). ADMA and L-arginine were assessed at baseline by high performance liquid chromatography. The occurrence of MACE (myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft, stroke, carotid revascularization, death) was evaluated during a follow-up of median 19 months (interquartile range 11 to 25). One hundred eighty-two MACE were observed in 141 patients (28%). MACE occurred in 39% of the patients in the highest quartile and 26% of those in the lowest quartile of ADMA ($P=0.016$, log-rank test for all quartiles). Adjusted hazard ratios for occurrence of MACE for increasing quartiles of ADMA compared with the lowest quartile were 0.87 (95% confidence interval [CI], 0.51 to 1.48), 1.12 (95% CI, 0.62 to 1.90), and 1.70 (95% CI, 1.02 to 2.88), respectively. We observed no association between cardiovascular outcome and L-arginine.

Conclusions—High ADMA plasma concentrations independently predict MACE in patients with advanced PAD. This indicates that ADMA may be a new cardiovascular risk marker in these patients. (***Arterioscler Thromb Vasc Biol.* 2006; 26:2536-2540.)**

Key Words: asymmetric dimethylarginine ■ major adverse cardiovascular events ■ nitric oxide ■ peripheral artery disease

Peripheral artery disease (PAD) is a common condition with a prevalence of 3.6% in subjects older than 40 years in the Framingham Offspring Study.[1] Patients with advanced PAD are at increased risk for major adverse cardiovascular events (MACE).[2]

Nitric oxide (NO) is produced from L-arginine by NO synthases[3] and is essential for physiological endothelial function.[4] NO has important antiatherogenic properties and reduced NO bioavailability might substantially contribute to the development of cardiovascular disease.[5] The endogenous NO synthase inhibitor asymmetric dimethylarginine (ADMA) is increased in conditions associated with high cardiovascular risk such as hyperlipidemia,[6,7] arterial hypertension,[8] renal impairment,[9] or PAD.[10] ADMA predicts cardiovascular events in patients with end-stage renal failure and in subjects with coronary artery disease.[11,12] ADMA has not only been shown to be associated with the presence of macrovascular disease[13,14] but also might be directly involved in the formation of vascular lesions.[15] This renders ADMA as cardiovascular risk marker or even contributor to cardiovascular disease. Only inconsistent and preliminary data on the role of the NO precursor L-arginine are available in patients with coronary heart disease and PAD.[16–18]

We hypothesized that ADMA is associated with future cardiovascular events in patients with advanced PAD and a pronounced adverse cardiovascular risk profile.

Materials and Methods

Study Design

Patients with advanced PAD, who were admitted to the Angiology department of a tertiary care university hospital from March 1, 2000 to March 1, 2001, were prospectively enrolled in a cohort study. Inclusion criteria were symptomatic PAD with intermittent claudication or critical limb ischemia and asymptomatic PAD with a history of surgical or endovascular lower limb revascularization. The

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study was approved by the local Ethics Committee, and all patients gave written informed consent.

Patients

At admission, demographic data, clinical characteristics, and current medication of the patients were recorded by 2 independent observers with special attention to cardiovascular risk factors and comorbidities. Age, sex, smoking habits, hyperlipidemia, arterial hypertension, diabetes mellitus, coronary artery disease, history of myocardial infarction (MI), and stroke were assessed. Subjects with end-stage renal disease were not included. Data were evaluated for inter-observer agreement at the day of patient discharge. In case of discrepancies the respective patient was re-evaluated by both investigators in consensus. To detect undiagnosed diabetes at admission, fasting blood glucose and hemoglobin A1c (HbA1c) levels were determined. During the hospital stay repeated blood pressure measurements were applied to detect undiagnosed hypertension in patients.

Laboratory Investigations

Routine laboratory investigations included complete blood cell count, coagulation tests and clinical chemistry. High-sensitivity C-reactive protein (hsCRP) was measured with a high-sensitivity assay (N Latex CRP Mono; DADE Behring, Vienna, Austria), with a detection level of 0.03 mg/dL, and a coefficient of variation of 4.6%. For determination of ADMA and L-arginine, venous blood was taken and plasma separated and stored at −80°C until batch analysis. Analysis was performed by high-performance liquid chromatography as described previously.[19,20] The coefficients of variation for inter- and intra-assay variations for this method are <3% for all analytes. The detection limit for methylarginines is 0.04 μmol/L.

Study End Points

The composite study end point was the occurrence of a first MACE consisting of MI, percutaneous coronary interventions (PCI), coronary artery bypass graft (CABG), stroke, carotid revascularization, and all-cause mortality. We analyzed the associations of ADMA with all-cause mortality as well as with MI, stroke, and death as secondary end points.

Follow-Up

Patients were clinically re-evaluated at 3, 6, and 12 months after hospital discharge and then annually to record clinical conditions until December 2002. Furthermore, a follow-up questionnaire was sent to each patient during December 2002 re-evaluating the occurrence of MACE during the whole follow-up period. Information from the follow-up questionnaire was validated by reviewing the original hospital discharge reports of corresponding re-admissions because of MACE. If the follow-up questionnaire was not returned, personal telephone contact to the patients, their relatives, or to the treating physicians was established. Further information was obtained by reviewing the hospital discharge reports of any other re-admission during the follow-up period. The performance of PCI, CABG, or carotid revascularization was validated by review of the original procedure protocols. Outcome was adjudicated for by 2 independent observers who were blinded with respect to the patients' baseline clinical and laboratory data.

Definitions

The diagnosis of PAD was assessed by clinical evaluation, ankle brachial index measurements (ABI <0.9), duplex sonography, and confirmed by lower limb angiography (digital subtraction technique) in all patients. Diabetes mellitus was defined as fasting blood glucose levels >126 mg/dL on 3 separate days and was considered to be present in all patients taking anti-diabetic medication. Hyperlipidemia was defined as fasting total serum cholesterol >200 mg/dL, low-density lipoprotein (LDL) cholesterol >130 mg/dL or serum triglycerides >180 mg/dL and was considered to be present in all patients receiving lipid-lowering therapy (3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase inhibitors [statins] were used routinely at our institution during the study period). Arterial hypertension was diagnosed in patients with resting blood pressure values >140/90 mm Hg, and was assumed to be present in patients with a history of hypertension taking antihypertensive drugs. Patients who were smoking >3 cigarettes per day were regarded as current smokers. Coronary artery disease was evaluated by treadmill exercise testing, dobutamine echocardiography, myocardial scintigraphy, or coronary angiography in selected cases. MI was defined according to the consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of MI as a transient increase of laboratory markers specific of myocardial necrosis (CK-MB, or troponin T) in combination with ischemic symptoms and/or typical ECG signs (development of pathologic Q-waves or ST segment elevation or depression).[21] Stroke was defined as a neurological deficit that persisted >24 hours evaluated by a neurologist according to the modified Rankin stroke scale. Mandatory cranial computed tomography or, if available, magnetic resonance imaging, were used for confirmation of the diagnosis.

Statistics

Continuous data are presented as medians (interquartile range). Categorical data are given as counts (percentages). Mann Whitney *U* test was applied for univariate comparison of continuous data and Spearman rank correlation for assessment of associations between continuous variables. Event-free survival rates until the first cardiovascular adverse event according to baseline ADMA levels (in quartiles) were calculated using the Kaplan Meier method and compared by the Log Rank test. Multivariate Cox proportional hazards analysis was used to assess the independent effect of ADMA on the composite end point and to adjust for potential confounders. Quartiles of continuous variables were included as confounders if these variables were associated with ADMA according to Spearman rank correlation coefficients. Categorical variables were entered into the model if they influenced ADMA according to univariate analysis. In addition, adjustment for traditional cardiovascular risk factors was performed. Results of the Cox proportional hazards model are presented as hazard ratio and 95% confidence interval (CI) as well as survival curves according to quartiles of ADMA. A 2-sided $P<0.05$ was considered as statistically significant. Calculations were performed with SPSS for Windows (Version 12.0; SPSS Inc, Chicago, Ill).

Results

Five hundred thirty-three subjects with PAD were eligible for the study and 496 subjects (93%) with complete baseline and follow-up data were included in the present analysis. In 19 patients laboratory values for ADMA and L-arginine were missing and in 18 patients clinical follow-up data of MACE were not available. Demographic data and clinical characteristics at baseline did not differ between patients included and those not included in the study (data not shown). The median age of the patients included was 70 years (interquartile range [IQR], 60 to 76), 279 (56%) were male and follow-up duration was median 19 months (IQR, 11 to 25).

Median ADMA and L-arginine plasma concentration of all patients were 0.55 μmol/L (IQR, 0.48 to 0.64) and 78 μmol/L (IQR, 61 to 96), respectively. ADMA was elevated in females, patients with history of MI, and patients without hyperlipidemia, and correlated positively with age and creatinine but not with other continuous variables (Tables 1 and 2).

During follow-up 182 MACE occurred in 141 subjects (28%) including 18 (3.6%) MIs, 41 (8.3%) PCIs, 7 (1.4%) CABGs, 27 (5.4%) carotid revascularizations, 25 (5.0%) strokes, and 64 (12.9%) deaths. Cumulative event free survival rates at 6, 12, 18, and 24 months were 90%, 84%, 79%,

TABLE 1. Median (Interquartile Range) Plasma Concentrations of Asymmetric Dimethylarginine (ADMA) Grouped According to Categorical Baseline Variables

	N	ADMA (μmol/L)	*P*
Sex			
Male	279	0.54 (0.47–0.62)	
Female	217	0.56 (0.48–0.69)	0.008
Previous myocardial infarction			
Yes	111	0.56 (0.49–0.70)	
No	385	0.54 (0.47–0.63)	0.042
Previous stroke			
Yes	63	0.55 (0.48–0.64)	
No	433	0.55 (0.48–0.64)	0.551
Current Smoker			
Yes	201	0.54 (0.48–0.62)	
No	295	0.55 (0.48–0.66)	0.164
Hyperlipidemia			
Yes	388	0.54 (0.47–0.63)	
No	108	0.57 (0.49–0.68)	0.048
Arterial hypertension			
Yes	364	0.54 (0.48–0.64)	
No	132	0.55 (0.48–0.65)	0.789
Diabetes mellitus			
Yes	188	0.55 (0.48–0.65)	
No	308	0.55 (0.48–0.63)	0.585
Statin therapy			
Yes	256	0.54 (0.47–0.63)	
No	240	0.55 (0.48–0.66)	0.139

and 70%, respectively. Thirty-nine percent of the patients with ADMA concentrations in quartile 4, 29% of those with ADMA in quartile 3, 24% of those with ADMA in quartile 2, and 26% of those with ADMA in quartile 1 had a MACE during the observation period ($P=0.016$, log-rank test). Patients with ADMA concentrations in quartile 4 had a significantly higher hazard ratio for MACE during follow-up compared with patients in quartile 1 before and after adjustment for age, sex, history of MI, current smoking, hypertension, diabetes mellitus, statin therapy, body mass index, hsCRP, LDL cholesterol, and creatinine (Figure 1, Table 3). L-arginine (in quartiles) or the L-arginine/ADMA ratio (in quartiles) showed no significant association with MACE during follow-up ($P=0.87$ and $P=0.33$, respectively; log-rank test).

TABLE 2. Spearman Correlation Coefficients for Asymmetric Dimethylarginine and Continuous Baseline Variables of Patients With Peripheral Artery Disease (n=496)

	Median (IQR)	*R*	*P*
Age, y	70 (60–76)	0.213	<0.001
Body mass index, kg/m^2	25.7 (23.4–28.4)	0.006	0.887
Systolic blood pressure, mm Hg	145 (118–167)	0.008	0.853
hsCRP, mg/dL	0.41 (0.18–0.91)	0.084	0.060
Triglycerides, mg/dL	149 (103–212)	0.009	0.838
LDL cholesterol, mg/dL	124 (101–152)	<0.001	0.997
Creatinine, mg/dL	1.06 (0.93–1.28)	0.268	<0.001
HbA1c, %	6.2 (5.7–6.9)	0.040	0.373
Ankle brachial index	0.57 (0.42–0.71)	0.038	0.321

IQR denotes interquartile range (range from the 25th to the 75th percentile).

TABLE 3. Cox Regression Analysis Assessing the Univariate and Multivariate Hazard Ratios for Quartiles of Asymmetric Dimethylarginine (ADMA) Compared to the Lowest Quartile

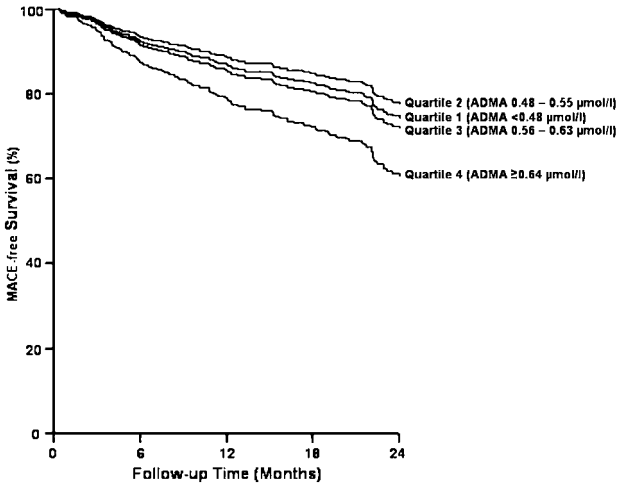
	ADMA (μmol/L)	Hazard Ratio	95% CI	*P*
Univariate model				
Quartile 1	<0.48	1.00		
Quartile 2	0.48–0.55	0.83	0.49–1.42	0.500
Quartile 3	0.56–0.63	1.21	0.74–1.97	0.453
Quartile 4	≥0.64	1.62	1.03–2.56	0.038
Multivariate model				
Quartile 1	<0.48	1.00		
Quartile 2	0.48–0.55	0.87	0.51–1.48	0.582
Quartile 3	0.56–0.63	1.12	0.62–1.90	0.681
Quartile 4	≥0.64	1.70	1.02–2.88	0.043

Multivariate model. Adjustment for age, sex, history of myocardial infarction, hypertension, diabetes mellitus, current smoking, statin therapy, BMI, hsCRP, creatinine, and LDL cholesterol.

Analyzing the associations of ADMA with all-cause mortality as well as with MI, stroke and death consistent findings were observed. Adjusted hazard ratios for increasing quartiles of ADMA as compared with the lowest quartile were 1.25 (95% CI, 0.54 to 2.90), 2.15 (0.99 to 4.62), and 2.43 (1.16 to 5.10) for all-cause mortality, and 1.11 (0.62 to 1.97), 1.45 (0.84 to 2.51), and 1.77 (1.06 to 2.98) for MI, stroke, and death, respectively.

Discussion

Elevated plasma ADMA concentrations were related to MACE in this prospective study in subjects with advanced PAD. Patients in the highest quartile of ADMA had >65%



Cox regression survival curves for major adverse cardiovascular events (MACE) according to quartiles of asymmetric dimethylarginine (ADMA) at baseline adjusted for confounders and traditional cardiovascular risk factors ($P=0.043$).

greater relative risk for future MACE compared with those in the lowest quartile. Traditional cardiovascular risk factors like hypertension, hyperlipidemia, diabetes mellitus, or increased body mass index did not influence these results. Our results therefore confirm the clinical importance of ADMA in patients with advanced PAD[10] and indicate a predictive value of elevated ADMA concentrations for future cardiovascular events.

The mechanisms regulating ADMA concentrations are well described.[22] Impaired renal function is of major relevance because ADMA is excreted via the kidneys.[9] Consistent with these data we found a significant correlation between ADMA and creatinine. However, the relationship between increased ADMA and MACE was not affected by creatinine which indicates that kidney function only partly influences ADMA and hence the association with cardiovascular outcome. Degradation of ADMA by dimethylarginine dimethylaminohydrolase is considered the principal determinant for ADMA concentrations in subjects with normal renal function.[23]

High concentrations of cholesterol, glucose, or inflammatory stimuli inhibited dimethylarginine dimethylaminohydrolase activity and consequently increased ADMA in in vitro experiments.[24,25] ADMA was not related to LDL cholesterol, hsCRP, or diabetes mellitus in our study. Although that does not exclude a clinical impact of these conditions, a confounding influence in our cohort of PAD patients is unlikely. Surprisingly, patients with hyperlipidemia had lower ADMA concentrations than other subjects, which is in contrast with previous clinical data.[6,7] Although concomitant lipid-lowering therapy could have biased our findings, the effect of statins on ADMA is controversial as most statins do not affect circulating ADMA concentrations in clinical studies,[26–30] with the exception of rosuvastatin.[31] These results and the fact that statin use was not associated with ADMA in our study renders lipid-lowering therapy an unlikely reason for the lower ADMA concentrations found in subjects with hyperlipidemia. It is however unclear if stringent pharmacological cardiovascular risk factor management could have contributed to lower ADMA in the hyperlipidemic subjects. Treatment with rosiglitazone, metformin, angiotensin-converting enzyme inhibitors, and angiotensin 2 receptor blockers has been shown to decrease ADMA concentrations in humans.[32–36] Therefore, an effect of diabetes mellitus or hypertension on ADMA in our study might be obscured by the use of anti-diabetic or antihypertensive drugs.

Women had higher ADMA than men in the present cohort which is consistent with results from a study in healthy subjects that demonstrated that women older than 50 years of age have elevated ADMA compared with age-matched men.[37] These sex differences could be related to hormonal changes in the postmenopause as younger women have lower ADMA than men[37] and elevated ADMA is normalized by hormone replacement therapy in postmenopausal women.[38]

Recently, prospective studies in patients with end-stage renal disease and coronary artery disease have identified ADMA as an important cardiovascular risk marker.[11,12] Our present findings are compatible with these data and add to the evidence that ADMA may be regarded as an independent predictor of future cardiovascular events also in a different group of patients. Considering the fact that ADMA is a competitive NO synthase inhibitor and that NO is a major antiatherogenic molecule it may be speculated that increased ADMA levels could even represent a therapeutic target and therefore be regarded as risk factor rather than plasma marker alone. This is supported by the fact that infusion of ADMA causes hypertension and increases systemic vascular resistance in healthy volunteers.[23] However, this hypothesis cannot be tested as selective ADMA-lowering drugs are not available yet. Further prospective studies in patients with mild PAD and other cohorts are mandatory to validate the clinical role of ADMA. In addition, it is necessary to develop new reliable methods for easy and efficient determination of ADMA plasma concentrations to establish ADMA as cardiovascular risk marker.

Several studies demonstrated that administration of the NO precursor L-arginine improves endothelial function in subjects at increased cardiovascular risk[39–41] and can reverse the actions of NO synthase inhibitors.[42] This effect has been attributed to structural antagonism with preserved NO release. In contrast, a recent study found adverse effects of L-arginine supplementation in patients with acute MI.[16] Our results do not support a role of circulating endogenous L-arginine for cardiovascular outcome.

In conclusion, this study has identified ADMA as an independent predictor for MACE in patients with advanced PAD. ADMA may represent a useful new risk marker for future adverse cardiovascular outcome in this cohort of subjects.

Disclosures

None.

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