Original Article

High Plasma Dimethylarginine Levels are Associated with Adverse Clinical Outcome After Stroke

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***Aim*: Increased levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, have been observed in patients with cardiovascular risk factors and atherosclerosis and in patients with a history of stroke. The role of ADMA and its analogue symmetric dimethylarginine (SDMA) in acute ischemic stroke is yet unclear. We hypothesized that plasma dimethylarginine levels increase in the hyper-acute phase after ischemic stroke and that their time course is related to stroke outcome.**

***Methods*: Plasma dimethylarginines ADMA and SDMA and L-arginine levels were measured in 67 patients at 6, 12, 24 hours, as well as 3 and 7 days after stroke onset using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS-MS). Data were compared to control data from 32 age-adjusted healthy volunteers. Clinical outcome was assessed using the modified Rankin Scale (mRS) at 90 days after stroke.**

***Results*: At baseline, plasma ADMA levels were higher in stroke patients than in controls, whereas plasma SDMA and L-arginine levels did not differ from control subjects. The time courses of ADMA and SDMA were related to the clinical outcome. Binary logistic regression analysis showed that ADMA levels of ≥0.566 μmol/L at day 3, ≥0.530 μmol/L at day 7 and SDMA levels of ≥0.59 μmol/L at 24 hours predicted an unfavorable clinical outcome.**

***Conclusions*: An increase of both ADMA and SDMA plasma levels within the first 72 hours after the onset of ischemic stroke predicts a poor outcome.**

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Key words; Asymmetric dimethylarginine (ADMA), Symmetric dimethylarginine (SDMA), L-arginine, Stroke, Outcome

Introduction

High levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS), are associated with the atherosclerotic burden and increase progressively with the number of stroke risk factors[1)]. Increased plasma levels have been observed in patients with hypertension[1, 2)], hyperlipidemia[1, 3)], hyperhomocysteinemia[4)], diabetes[5)], chronic kidney disease and old age[2, 6)]. Elevated ADMA plasma levels were also found in acute ischemic stroke and transient ischemic attack patients[7)],

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and in subjects with a history of stroke[8]. Currently, ADMA is considered to be not only a marker but also a mediator of oxidative stress via the inhibition and uncoupling of NOS[9]. Oxidative stress induces the expression of inflammatory genes and thereby enhances atherosclerotic processes[10, 11].

Recently, ADMA levels have been shown to be decreased in patients in the chronic stage after ischemic stroke at least 3 months after the event when treated with statins in comparison to the non-statin-treated group[12]. In another study, treatment with statins resulted in a significant reduction of markers of oxidative stress[13].

The structural isomer of ADMA, SDMA, has no effect on nitric oxide synthesis but is also related to cardiovascular events[14]. SDMA has also been shown to be an independent predictor of overall mortality after acute stroke[15]. To the best of our knowledge there has been no report on the relationship between dimethylarginine, i.e. ADMA and SDMA plasma levels during the hyper-acute phase of ischemic stroke, and functional outcome. Dimethylarginine data for the first hours after stroke onset are available only from a study where ADMA levels in the cerebrospinal fluid (CSF), not in the plasma, were directly related to stroke severity on admission and stroke outcome at 90 days after stroke[16].

In the present study we serially analyzed the dimethylarginine plasma concentration after acute ischemic stroke to elucidate its release over time and its possible relation to stroke outcome. We also investigated L-arginine, the substrate for nitric oxide synthase.

Materials and Methods

Study Population

Between January 2007 and January 2009, 67 patients with ischemic stroke were prospectively included in this study within 6 hours of symptom onset. Ischemic stroke was defined as an acute focal neurological deficit with cranial computed tomography (CCT) or magnetic resonance imaging (MRI) evidence of infarction. Exclusion criteria were transient ischemic attack (TIA), hemorrhagic stroke, history of malignant tumor, functional deficit prior to the index event and severe systemic inflammatory disease[17] with a serum C-reactive protein level >50 mg/L and/or need for intravenous antibiotic treatment.

Stroke severity on admission was assessed by the National Institute of Health Stroke Scale Score (NIHSS). NIHSS 0-1 was considered as mild stroke, 2-8 as moderate stroke and ≥ 9 as severe stroke[18]. The primary outcome was assessed by the modified Rankin scale (mRS) at 90 days after stroke. mRS 0-1 at 90 days after stroke was considered a favorable outcome, and mRS 2-6 an unfavorable outcome.

Clinical data recorded from all patients included demographic characteristics, baseline stroke severity (NIHSS on admission), stroke etiology classified according to TOAST criteria[19], stroke risk factors, such as hypertension, diabetes mellitus, hyperlipidemia and smoking status, and creatinine on admission. Nine patients had a history of stroke. In each case, the event had occurred at least 3 months previously. Two of these patients had residual symptoms without functional disability; one had unilateral sensory disturbance of the upper extremities, and the other had slight unilateral facial paresis.

Between April 2010 and July 2010, 32 healthy volunteers without malignant tumor and without an event of stroke or myocardial infarction at least during the previous two years provided blood samples for comparison with the patient data. Most were relatives of stroke patients.

The study was approved by our local ethics committee, and patients and volunteers gave informed consent.

Blood Collection

Serum and plasma samples were drawn from the patients 6h, 12h, 24h, 3d and 7d after stroke onset and stored at -80℃ until analysis.

In 34 patients, blood was collected within six hours after stroke onset ("2-6-hour samples"). Five patients were lost to follow-up over the first week, leading to 64 blood samples at 3 days and 62 samples at 7 days. In 55 patients, blood samples were also collected 90 days after stroke.

Measurement of ADMA, SDMA, L-arginine and Other Markers

Plasma ADMA, SDMA and L-arginine levels were assessed using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS-MS)[20]. The intra-day precision was 4.6% for ADMA, 6.1% for SDMA, and 4.3% for L-arginine. The inter-day precision was 4.6% for ADMA, 5.2% for SDMA, and 4.5% for L-arginine. The intra-day precision data were generated by measuring plasma quality control samples in one batch ($n=10$), whereas the inter-day precision data were derived from measurements of plasma quality control samples taken on different days ($n=6$). Creatinine and hsCRP were measured using certified laboratory tests in the Department of Clinical Chemistry at Hannover Medical School, Germany.

Table 1. Baseline Clinical Characteristics of Patients and Healthy Controls

Patients with	Favorable outcome ($n=41$)	Unfavorable outcome ($n=26$)	Controls ($n=32$)	p value
Age, years	69 (62, 74)	75.5 (58, 85)	71 (61, 76)	0.177
Gender (female), %	31.7	65.4	56.3	0.016
Hypertension, %	58.5	65.4	75	0.340
Hyperlipidemia, %	43.9	42.3	53.1	0.668
Diabetes, %	7.3	7.7	12.5	0.751
Smoking, %	19.5	11.5	18.8	0.708
BMI, kg/m^2	25.76 (24.8, 29.32)	25.1 (23.04, 27.76)	25.59 (23.92, 29.38)	0.359
Stroke severity on admission, %				
Mild	26.8	11.5		
Moderate	65.9	34.6		0.000
Severe	7.3	53.8		
Stroke etiology				
Atherothrombotic, %	22.0	11.5		
Cardioembolic, %	31.7	38.5		0.566
Lacunar, %	36.6	46.2		
Others, %	9.8	3.8		
eGFR, mL/min per 1.73 cm^2	80 (64.25, 92.95)	74.1 (46.55, 89.425)	71.3 (61.55, 89.9)	0.219

BMI: body mass index; eGFR: estimated glomerular filtration rate.
The data were tested for statistically significant difference between groups by one-way ANOVA for continuous data and Fisher's exact test or Pearson's Chi-square test for categorical data.

Statistical Analyses

Statistical analysis was performed with the SPSS software package version 11.5. Data are presented as a portion for categorical variables and as the median with interquartile range for continuous variables. The data were tested for statistically significant differences between groups by one-way ANOVA for continuous data and Pearson's Chi-square test or Fisher's exact test for categorical data. Differences between patient levels of ADMA, SDMA and L-arginine and levels in the control group were calculated by Student's t test. Within-group comparisons of the biomarkers at different time points were analyzed by repeated measures ANOVA.

Cut-off points of dimethylarginines and L-arginine were determined using the receiver operating characteristics (ROC) curve and Youden index.

The influence of ADMA, SDMA and L-arginine levels on functional outcome was assessed using binary logistic regression after adjusting for main baseline variables related to outcome in univariate analyses (enter approach and probability of entry $p<0.05$). Results are presented as adjusted odds ratios (OR) with the corresponding 95% confidence intervals (95% CI). A p value <0.05 was considered significant.

Results

Baseline clinical characteristics, vascular risk factors, stroke type, stroke severity and renal function at baseline are shown in **Table 1**.

Dimethylarginine Time Courses After Acute Ischemic Stroke

The dimethylarginine levels in the control group were 0.447 (range: 0.349-0.606) μmol/L for ADMA, 0.546 (range: 0.473-0.630) μmol/L for SDMA, and 74.7 (range: 40.7-104.4) μmol/L for L-arginine. ADMA levels were significantly higher in the stroke patient group at any time point compared to controls while SDMA and L-arginine were not (**Table 2**). ADMA ($p=0.008$, $F=3.858$) and SDMA ($p=0.008$, $F=3.868$) levels increased significantly during the first 3 days after stroke onset, while L-arginine levels ($p=0.247$, $F=1.400$) tended to decrease (**Fig. 1**).

Forty-one of the 67 patients had a favorable outcome 90 days after stroke, but 26 patients had an unfavorable outcome. In patients with a favorable outcome, ADMA increased during the first 12 hours after stroke onset and then remained stable until day 3 before it decreased again. In patients with an unfavorable outcome, the ADMA level increased until day 3, remained at that level until day 7 and had decreased

Table 2. Levels of Dimethylarginine in patients

	6h	12h	24h	3d	7d
ADMA					
Median	0.470	0.499	0.501	0.532	0.498
(umol/L)	(0.429, 0.548)	(0.444, 0.586)	(0.460, 0.569)	(0.459, 0.588)	(0.431, 0.591)
p	0.032	0.000	0.000	0.000	0.000
SDMA					
Median	0.500	0.495	0.535	0.517	0.486
(umol/L)	(0.421, 0.619)	(0.425, 0.641)	(0.457, 0.690)	(0.451, 0.699)	(0.429, 0.616)
p	0.200	0.633	0.620	0.594	0.642
L-Arg					
Median	70.50	70.00	65.20	65.65	68.80
(umol/L)	(56.30, 87.90)	(51.17, 86.55)	(52.50, 76.80)	(50.10, 82.00)	(56.05, 82.65)
p	0.884	0.254	0.079	0.059	0.222

ADMA: asymmetric dimethylarginine; SDMA: symmetric dimethylarginine; L-Arg: L-arginine
Data are presented as the median and quartile. *P*-values indicate differences between patient levels of ADMA, SDMA and L-Arginine and levels in the control group calculated by Student's *t* test.

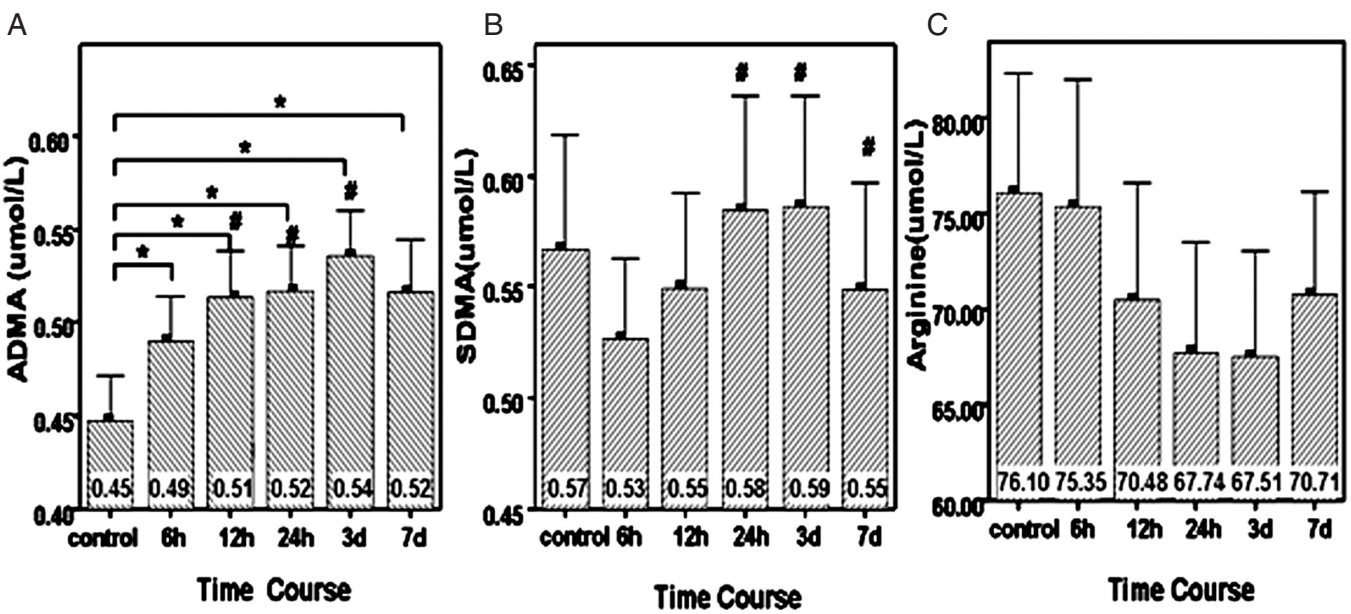

Fig. 1. Time courses of dimethylarginine levels after acute ischemic stroke onset (mean ± SD). A: ADMA; B: SDMA; C: L-arginine. *compared to control, $p<0.05$, #compared to 6 hours, $p<0.05$

again 90 days after stroke. At day 3 ($p=0.025$) and day 7 ($p=0.001$), ADMA levels were significantly higher in patients with an unfavorable outcome than in those with a favorable outcome (**Fig. 2A**).

SDMA levels remained unchanged in patients with a favorable outcome while they continuously increased over the first 24 hours after stroke onset in those with unfavorable outcome and then decreased again. The SDMA level in patients with an unfavorable outcome was significantly higher at 12 hours ($p=0.003$), 24 hours ($p=0.001$) and 3 days ($p=0.023$) compared to those with a favorable outcome (**Fig. 2B**).

L-arginine levels did not significantly differ between the two groups ($p>0.05$) (**Fig. 2C**).

Association of ADMA and SDMA with Neurological Outcome in Stroke Patients

ROC analysis was performed for ADMA 3d and 7d and SDMA 12h, 24h and 3d after stroke onset (**Fig. 3**). A possible cut-off point for the discrimina-

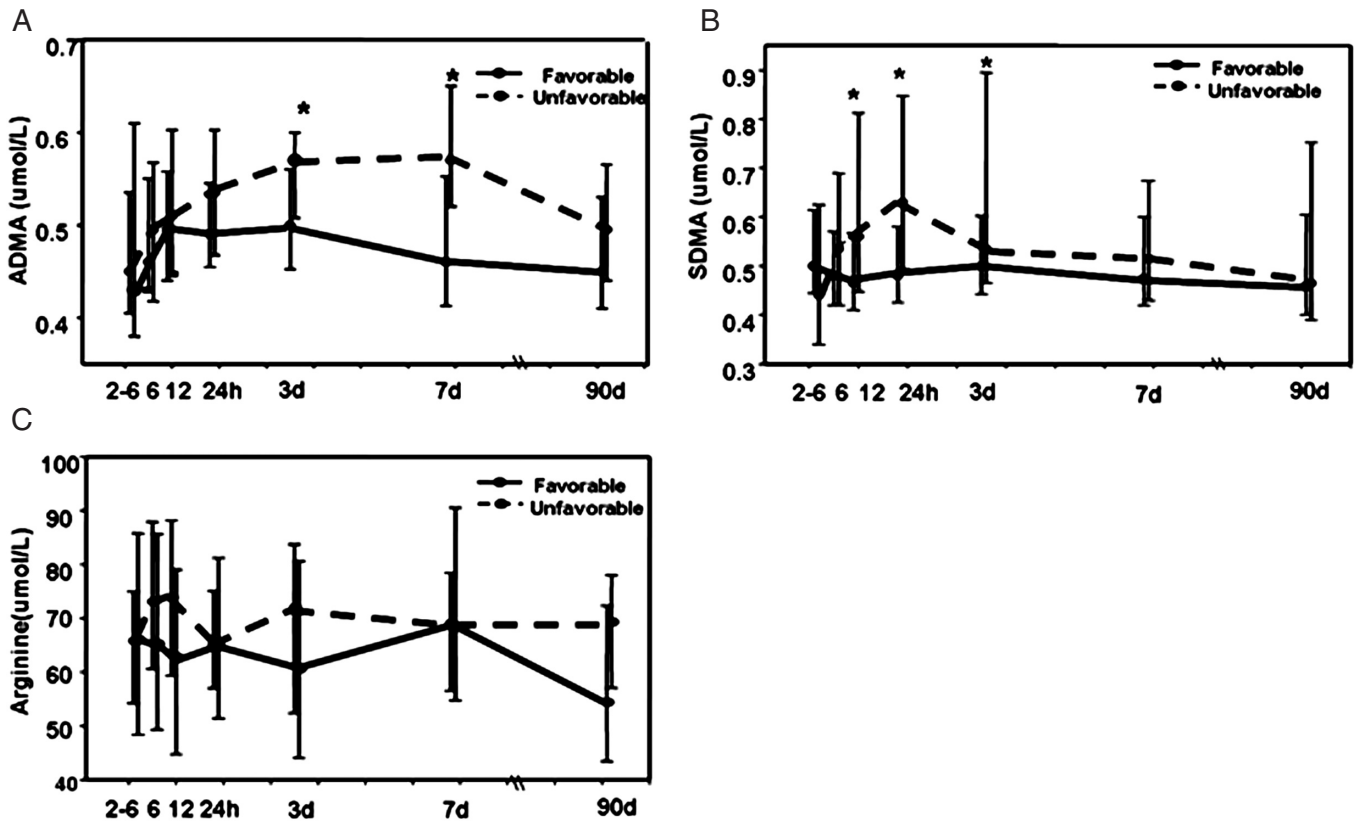

Fig. 2. Dimethylarginine time course in patients with favorable and unfavorable Outcomes. A: ADMA; B: SDMA; C: L-Arginine

*ADMA or SDMA levels are significantly different between the two groups.
Data are given as median and 25-75% range.

tion of dichotomized neurological outcome (favorable outcome: mRS 0 or 1 vs. nonfavorable outcome: mRS 2-6) for ADMA at 3d after stroke was calculated as 0.566 μmol/L, and at 7d as 0.530 μmol/L. For SDMA, the cut-off points were 0.519 μmol/L at 12h and 0.591 μmol/L at 24h. We categorized ADMA 3d and 7d, SDMA 12h and 24h into two levels according to the cut-off points determined above. Univariate analysis found that ADMA 3d ≥0.566 μmol/L, ADMA 7d ≥0.530 μmol/L, SDMA 12h ≥0.519 μmol/L and SDMA 24h ≥0.591 μmol/L predicted an unfavorable outcome on 90d after stroke onset. Higher ADMA levels at 3d and 7d and SDMA levels at 24h were independent markers of an unfavorable outcome in multivariate analysis considering all parameters, which were significant in univariate analysis as well as gender and stroke severity on admission (Model 1). The results did not change when age, hypertension and eGFR (estimated glomerular filtration rate) were also considered (Model 2) (**Table 3**).

Discussion

To the best of our knowledge, this is the first study to investigate the time course of plasma dimethyl-arginines and L-arginine after acute ischemic stroke as well as their relation to the clinical outcome at 90 days.

The pertinent findings of our study are: 1) Plasma ADMA levels increased in the hyperacute phase of ischemic stroke irrespective of stroke outcome, SDMA levels increased in patients with an unfavorable outcome, exclusively, while L-arginine levels did not change; 2) the time profile of ADMA and SDMA alterations was related to stroke outcome; 3) high ADMA and SDMA predicted an unfavorable outcome.

High ADMA in Hyperacute Ischemic Stroke

ADMA and SDMA originate from the degradation of methylated proteins, which are catalyzed by protein arginine methyltransferases (PRMTs). After proteolysis of the methylated protein, ADMA and

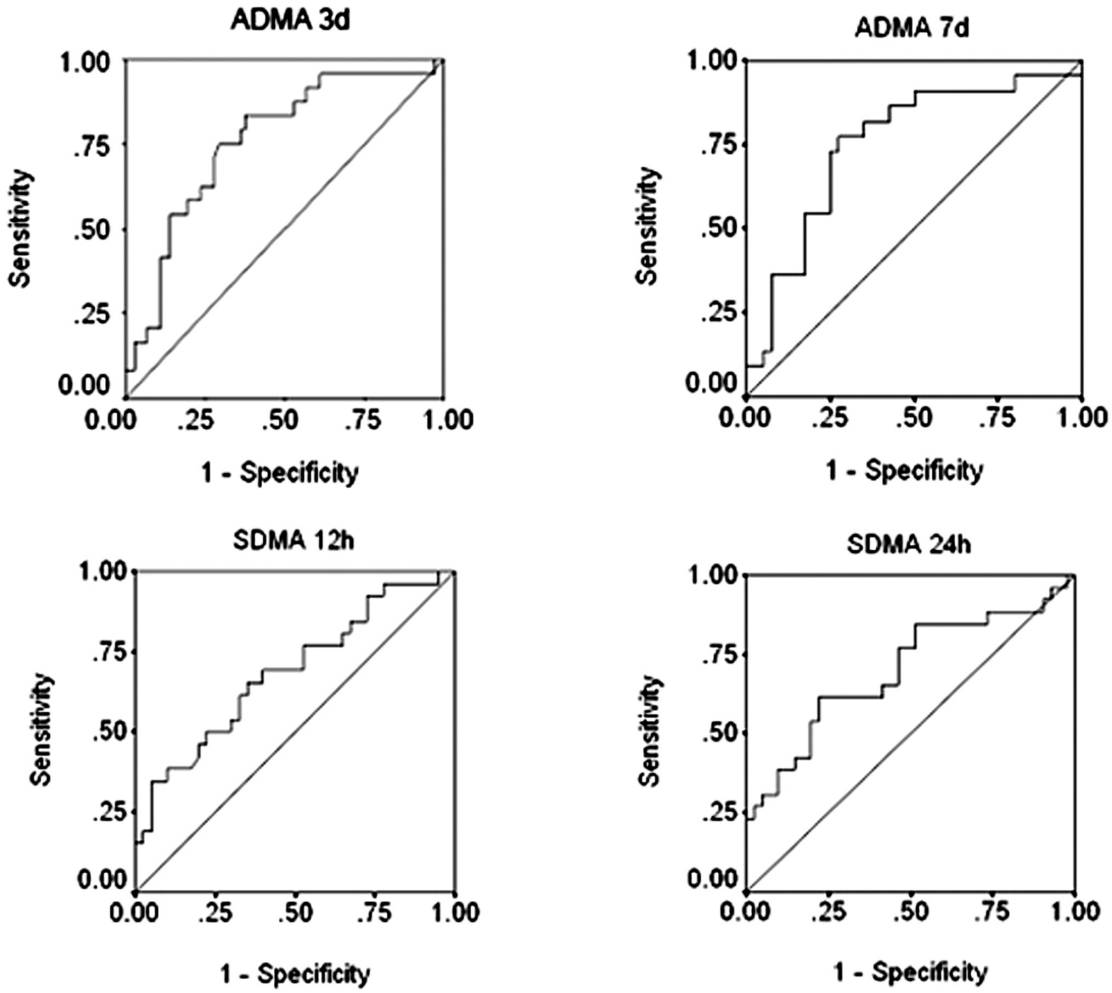

Fig. 3. ROC curve of ADMA at 3 and 7 days, and SDMA at 12 and 24 hours.

SDMA are released. More than 90% of ADMA is metabolized through DDAHs (dimethylaminohydrolases) while, in contrast, SDMA is completely excreted by the kidney[21]. Acute ischemic stroke induces oxidative stress in the region of the lesion, which causes an increase in the expression of PRMTs and decreased enzymatic activity of DDAHs[9, 21]. Both mechanisms - one by increased production, the other by decreased metabolization-may be responsible for the elevation of ADMA and SDMA.

Aside from ADMA, SDMA production/release should also be increased in stroke patients, but SDMA increases may be concealed and SDMA concentrations could have remained quite stable after stroke, at least in patients without severe renal dysfunction due to renal excretion.

Dimethylarginine Levels and Stroke Outcome

In this study, stroke outcome was worse in patients with increased ADMA levels than in those with stable ADMA levels. The mechanism behind this is not clear, but it can be hypothesized that increased ADMA levels impair the activity of endothelial NO synthase (eNOS) and thereby inhibit the production of endothelial NO and cerebral perfusion. This hypothesis is supported by several *in vitro* and animal studies showing that ADMA increases vascular tone in cerebral blood vessels[22, 23]. Moreover, ADMA infusion in healthy volunteers reduced cerebral perfusion significantly[24]. Furthermore, there is an association of ADMA levels in the CSF and cerebral vasospasm in a primate model of subarachnoid hemorrhage[25]. Accordingly, ADMA may impair perfusion in the penumbra and might therefore be pathophysiologically relevant.

Table 3. Separate Logistic Regression Models for ADMA (3 and 7 Days) and SDMA (12 and 24 Hours) and Stroke Outcome at 90 Days after Stroke Onset

	Univariate	Model 1	Model 2
ADMA 3 days	4.727 (1.549, 14.427) $p = 0.007$	7.201 (1.772, 29.263) $p = 0.006$	6.527 (1.537, 27.723) $p = 0.011$
ADMA 7 days	8.964 (2.660, 20.207) $p = 0.000$	6.529 (1.802, 23.664) $p = 0.004$	7.191 (1.734, 29.82) $p = 0.007$
SDMA 12 hours	3.044 (1.073, 8.633) $p = 0.045$	1.884 (0.571, 6.217) $p = 0.298$	1.677 (0.475, 5.918) $p = 0.281$
SDMA 24 hours	5.689 (1.928, 16.788) $p = 0.002$	5.177 (1.508, 17.769) $p = 0.009$	7.159 (1.670, 30.693) $p = 0.008$

Covariant factors included in model 1: gender and stroke severity on admission.
Covariant factors included in model 2: age, gender, stroke severity on admission, hypertension and CKD-EPI-GFR.

The role of SDMA is less clear than that of ADMA. SDMA has been shown to be a good marker of renal function with high temporal resolution to identify changes in renal function[26], so the increase in SDMA might be a reflection of (minor) changes in renal function that were not detected by creatinine measurements. This seems possible as even small changes in renal function have been shown to be related to adverse outcomes in neurological ermergencies[27] as well as long-term outcome after acute stroke[28]. Aside from being a marker of renal dysfunction, SDMA interferes with the uptake of L-arginine and may thereby (indirectly) inhibit NO production[29, 30]. Brouns *et al*. reported that higher SDMA levels in the CSF were correlated with a poor outcome at 3 months after stroke onset[16], but after multivariate analysis this correlation remained only weak[16]. Recently, Schulze *et al*. reported that SDMA predicted all-cause mortality in stroke patients during 7.4 years of follow-up even after adjustment for renal function[15]. In this study SDMA has also been shown for the first time to affect cerebrovascular mortality. Experimental data suggest that SDMA triggers vascular pathology by modulating store-operated calcium channels in monocytes[31]. It might also be important in modulating the function of monocytes recruited to the site of the ischemic lesion after stroke onset[32]. SDMA may enhance reactive oxygen species (ROS) production in those monocytes[31], thereby causing blood brain barrier damage and neuronal cell death[32]. Furthermore, inhibited L-arginine uptake by SDMA may induce L-arginine deficiency in neuronal cells. In consequence, neuronal NOS produces huge amounts of superoxide instead of NO[33], which leads to lipid, protein and DNA damage[34] in the cells and contributes to a poor outcome after stroke.

Despite being the first prospective clinical study in the field, our study has some limitations. Firstly, the number of patients included is rather small considering the multifactorial pathology of ischemic stroke, but we were able to collect serial blood samples from all of our patients, which had never been achieved. Secondly, patients with mild stroke predominated in the present study (NIHSS range 0 to 25, median 4), although the neurological examination was augmented by brain imaging in all of our patients. Thirdly, at the time of blood withdrawal, ischemic stroke patients were not in a fasting state since this conflicted with the strict time course of blood sampling, particularly during the first day. Lastly, the very nature of our work does not allow the elucidation of pathophysiological concepts, i.e. clarify whether elevated ADMA and SDMA just indicate or actually cause an adverse clinical outcome.

In conclusion, our study is hypothesis generating in nature and suggests that both an increase of ADMA known as a marker of cardiovascular risk and atherosclerosis and an increase of SDMA plasma levels after acute ischemic stroke predict poor functional outcome at 90 days after stroke onset. Based on our findings, we believe that the prognostic value and pathophysiological role of ADMA and SDMA in this setting deserve further investigation in larger patient populations.

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Disclosures

Dr. Kielstein runs and hosts the website www.adma.com.

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References

1) Nishiyama Y, Ueda M, Katsura KI, Otsuka T, Abe A, Nagayama H, Katayama Y: Asymmetric dimethylarginine (adma) as a possible risk marker for ischemic stroke. J Neurol Sci, 2010; 290: 12-15
2) Kielstein JT, Bode-Boger SM, Frolich JC, Ritz E, Haller H, Fliser D: Asymmetric dimethylarginine, blood pressure, and renal perfusion in elderly subjects. Circulation, 2003; 107: 1891-1895
3) Vladimirova-Kitova L, Terzieva D, Marinov B: Intima-media thickness and flow-mediated vasodilation in asymptomatic subjects with newly detected severe hypercholesterolemia. Echocardiography, 2009; 26: 1060-1068
4) Boger RH, Lentz SR, Bode-Boger SM, Knapp HR, Haynes WG: Elevation of asymmetrical dimethylarginine may mediate endothelial dysfunction during experimental hyperhomocyst(e)inaemia in humans. Clin Sci (Lond), 2001; 100: 161-167
5) Abbasi F, Asagmi T, Cooke JP, Lamendola C, McLaughlin T, Reaven GM, Stuehlinger M, Tsao PS: Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. Am J Cardiol, 2001; 88: 1201-1203
6) Kielstein JT, Zoccali C: Asymmetric dimethylarginine: a cardiovascular risk factor and a uremic toxin coming of age? Am J Kidney Dis, 2005; 46: 186-202
7) Wanby P, Teerlink T, Brudin L, Brattstrom L, Nilsson I, Palmqvist P, Carlsson M: Asymmetric dimethylarginine (adma) as a risk marker for stroke and tia in a swedish population. Atherosclerosis, 2006; 185: 271-277
8) Yoo JH, Lee SC: Elevated levels of plasma homocyst(e)ine and asymmetric dimethylarginine in elderly patients with stroke. Atherosclerosis, 2001; 158: 425-430
9) Sydow K, Munzel T: Adma and oxidative stress. Atheroscler Suppl, 2003; 4: 41-51
10) Kondo T, Hirose M, Kageyama K: Roles of oxidative stress and redox regulation in atherosclerosis. J Atheroscler Thromb, 2009; 16: 532-538. Epub 2009 Sep 14. Review
11) Hirose H, Kawabe H, Komiya N, Saito I: Relations between serum reactive oxygen metabolites (ROMs) and various inflammatory and metabolic parameters in a Japanese population. J Atheroscler Thromb, 2009; 16: 77-82
12) Nishiyama Y, Ueda M, Otsuka T, Katsura KI, Abe A, Nagayama H, Katayama Y: Statin Treatment Decreased Serum Asymmetric Dimethylarginine (ADMA) Levels in Ischemic Stroke Patients. J Atheroscler Thromb, 2010; Nov 11. [Epub ahead of print]
13) Kirmizis D, Papagianni A, Dogrammatzi F, Skoura L, Belechri AM, Alexopoulos E, Efstratiadis G, Memmos D: Effects of simvastatin on markers of inflammation, oxidative stress and endothelial cell apoptosis in patients on chronic hemodialysis. J Atheroscler Thromb, 2010; 17: 1256-1265. Epub 2010 Sep 25
14) Meinitzer A, Kielstein JT, Pilz S, Drechsler C, Ritz E, Boehm BO, Winkelmann BR, März W: Symmetrical and Asymmetrical Dimethylarginine as Predictors for Mortality in Patients Referred for Coronary Angiography: The Ludwigshafen Risk and Cardiovascular Health Study. Clin Chem, 2010
15) Schulze F, Carter AM, Schwedhelm E, Ajjan R, Maas R, von Holten RA, Atzler D, Grant PJ, Boger RH: Symmetric dimethylarginine predicts all-cause mortality following ischemic stroke. Atherosclerosis, 2010; 208: 518-523
16) Brouns R, Marescau B, Possemiers I, Sheorajpanday R, De Deyn PP: Dimethylarginine levels in cerebrospinal fluid of hyperacute ischemic stroke patients are associated with stroke severity. Neurochem Res, 2009; 34: 1642-1649
17) McColl BW, Allan SM, Rothwell NJ: Systemic infection, inflammation and acute ischemic stroke. Neuroscience, 2009; 158: 1049-1061
18) The national institute of neurological disorders and stroke rt-pa stroke study group: Tissue plasminogen activator for acute ischemic stroke. N Engl J Med, 1995; 333: 1581-1587
19) Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE, 3rd: Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. Toast. Trial of org 10172 in acute stroke treatment. Stroke, 1993; 24: 35-41
20) Martens-Lobenhoffer J, Bode-Boger SM: Fast and efficient determination of arginine, symmetric dimethylarginine, and asymmetric dimethylarginine in biological fluids by hydrophilic-interaction liquid chromatography-electrospray tandem mass spectrometry. Clin Chem, 2006; 52: 488-493
21) Cooke JP: Asymmetrical Dimethylarginine The Über Marker? Circulation, 2004, 109: 1813-1819
22) Jin JS, D'Alecy LG: Central and peripheral effects of asymmetric dimethylarginine, an endogenous nitric oxide synthetase inhibitor. J Cardiovasc Pharmacol, 1996; 28: 439-446
23) Faraci FM, Brian JE Jr, Heistad DD: Response of cerebral blood vessels to an endogenous inhibitor of nitric oxide synthase. Am J Physiol, 1995; 269: H1522-H1527
24) Kielstein JT, Donnerstag F, Gasper S, Menne J, Kielstein A, Martens-Lobenhoffer J, Scalera F, Cooke JP, Fliser D, Bode-Boger SM: Adma increases arterial stiffness and decreases cerebral blood flow in humans. Stroke, 2006; 37: 2024-2029
25) Jung CS, Iuliano BA, Harvey-White J, Espey MG, Oldfield EH, Pluta RM: Association between cerebrospinal fluid levels of asymmetric dimethyl-L-arginine, an endogenous inhibitor of endothelial nitric oxide synthase, and cerebral vasospasm in a primate model of subarachnoid hemorrhage. J Neurosurg, 2004; 101: 836-884
26) Kielstein JT, Veldink H, Martens-Lobenhoffer J, Haller H, Burg M, Lorenzen JM, Lichtinghagen R, Bode-Böger SM, Kliem V: SDMA is an early marker of change in GFR after living-related kidney donation. Nephrol Dial Transplant, 2011; 26: 324-328. Epub 2010 Jul 9
27) Zacharia BE, Ducruet AF, Hickman ZL, Grobelny BT,

Fernandez L, Schmidt JM, Narula R, Ko LN, Cohen ME, Mayer SA, Connolly ES Jr: Renal dysfunction as an independent predictor of outcome after aneurysmal subarachnoid hemorrhage: a single-center cohort study. Stroke, 2009; 40: 2375-2381
28) Tsagalis G, Akrivos T, Alevizaki M, Manios E, Theodorakis M, Laggouranis A, Vemmos KN: Long-term prognosis of acute kidney injury after first acute stroke. Clin J Am Soc Nephrol, 2009; 4: 616-622
29) Closs EI, Basha FZ, Habermeier A, Forstermann U: Interference of l-arginine analogues with l-arginine transport mediated by the y+ carrier hcat-2b. Nitric Oxide, 1997; 1: 65-73
30) Bode-Boger SM, Scalera F, Kielstein JT, Martens-Lobenhoffer J, Breithardt G, Fobker M, Reinecke H: Symmetrical dimethylarginine: A new combined parameter for renal function and extent of coronary artery disease. J Am Soc Nephrol, 2006; 17: 1128-1134
31) Schepers E, Glorieux G, Dhondt A, Leybaert L, Vanholder R: Role of symmetric dimethylarginine in vascular damage by increasing ros via store-operated calcium influx in monocytes. Nephrol Dial Transplant, 2009; 24: 1429-1435
32) Jin R, Yang G, Li G: Inflammatory mechanisms in ischemic stroke: Role of inflammatory cells. J Leukoc Biol, 2010; 87: 779-789
33) Cardounel AJ, Xia Y, Zweier JL: Endogenous methylarginines modulate superoxide as well as nitric oxide generation from neuronal nitric-oxide synthase: Differences in the effects of monomethyl- and dimethylarginines in the presence and absence of tetrahydrobiopterin. J Biol Chem, 2005; 280: 7540-7549
34) Won SJ, Kim DY, Gwag BJ: Cellular and molecular pathways of ischemic neuronal death. J Biochem Mol Biol, 2002; 35: 67-86