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Asymmetrical (ADMA) and symmetrical dimethylarginine (SDMA) as potential risk factors for cardiovascular and renal outcome in chronic kidney disease – possible candidates for paradoxical epidemiology?

M. Busch[1], **C. Fleck**[2], **G. Wolf**[1], and **G. Stein**[1]

[1] Department of Internal Medicine III, University of Jena, Jena, Germany
[2] Institute of Pharmacology and Toxicology, University of Jena, Jena, Germany

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Summary. *Background*: Asymmetrical dimethylarginine (ADMA) is an inhibitor of nitric-oxide synthase. It has been linked to atherosclerotic risk in the general population as well as in end-stage renal disease patients (ESRD), whereas symmetrical dimethylarginine (SDMA) is thought to be biological inactive. Prospective data concerning the role of both dimethylarginines are rare in patients with chronic kidney disease.

Methods: 200 patients with chronic kidney disease (mean age 57.6 ± 13.0 years, 69 female, 131 male); 82 with chronic renal failure (CRF), 81 on maintenance haemodialysis (HD) and 37 renal transplant recipients (RTR) were prospectively followed for 24 months. ADMA and SDMA were measured by HPLC. The relation of plasma levels of ADMA and SDMA together with conventional risk factors for the cardiovascular and renal outcome was investigated with Cox proportional hazards model.

Results: Mean serum levels of SDMA were significantly increased in all groups compared to the control group ($P \leq 0.0005$), ADMA was increased only in HD and RTR ($P \leq 0.004$). Forty-seven cardiovascular events (CVE) occurred during follow-up, 35 patients died, and 39 patients reached ESRD. Multivariate analysis showed diabetes (RR 3.072, $P = 0.01$), ESRD (RR 11.915, $P < 0.0005$), elevated CRP levels (RR 3.916, $P < 0.0005$) and surprisingly a lower ADMA level (RR 0.271, $P = 0.008$) as independent risk factors for CVE. Serum creatinine (RR 11.378, $P = 0.001$), haemoglobin (RR 0.710, $P = 0.038$ for an increment of 1 mmol/l), and SDMA levels (RR 1.633, $P = 0.006$, per 1 µmol/l increment) were predictors for the progression to ESRD.

Conclusions: Data from a heterogeneous group of patients with chronic kidney disease provide evidence that conventional risk factors seem to play a more important role than elevated serum levels of ADMA or SDMA for cardiovascular events. Increasing serum SDMA concentration seems to play an additive role for the renal outcome besides serum creatinine and haemoglobin levels. Whether ADMA might possibly be a candidate for the phenomenon of "paradoxical epidemiology" in chronic kidney disease needs further investigation.

Keywords: Chronic renal failure – Haemodialysis – Renal transplantation – Cardiovascular risk – ADMA – SDMA

Abbreviations: ADMA, asymmetrical dimethylarginine; AMI, acute myocardial infarction; AP, angina pectoris; BMI, body mass index; BP, blood pressure; CI, confidence interval; CRF, chronic renal failure; CRP, C-reactive protein; CV, coefficient of variation; CVE, cardiovascular events; DM, diabetes mellitus; DMA, dimethylarginine; ECG, electrocardiogram; ELISA, enzyme-linked immunosorbent assay; ESRD, end-stage renal disease; GC-MS, gas chromatographic-mass spectrometric assay; HD, haemodialysis patients; HPLC, high-performance liquid chromatography; NO, nitric oxide; PAOD, peripheral arterial occlusive disease; RR, relative risk; RTR, renal transplant recipients; SDMA, symmetrical dimethylarginine; tHcy, total homocysteine.

Introduction

Atherosclerotic vascular complications are the major cause of morbidity and mortality in patients with chronic kidney disease of different stages. The multifactorial origin of the cause is not yet well understood (Parfrey and Foley, 1999). The association between conventional risk factors, together with malnutrition and chronic inflammation, seems to be a major source of oxidative stress (Himmelfarb et al., 2002) playing an important role in the pathogenesis of endothelial dysfunction. Nitric oxide (NO) produced by nitric-oxide synthase (NOS) results in vasodilatation and has an important effect in the regulation of systemic blood pressure and local blood flow (Rees et al., 1989). Reduced bioavailability of NO is, therefore, accepted to play a role in the process of atherosclerotic vascular damage, especially in renal disease (Fliser et al., 2003). Dimethylarginines (DMAs), existing as symmetric and asymmetric molecules, are natural occuring analogues of L-arginine, the substrate for NO synthesis (MacAllister et al., 1994). Asymmetrical dimethylarginine (ADMA) is an endogenous inhibitor of NOS (Vallance et al., 1992), which is mainly metabolised by dimethylaminohydrolase (DDAH) into citrulline and dimethylamine (Ogawa et al., 1989) and

additionally eliminated by the kidney (Nijveldt et al., 2002). Symmetric dimethylarginine (SDMA) is eliminated only via renal excretion (Nijveldt et al., 2002). Therefore SDMA is more closely related to the glomerular filtration rate compared to ADMA (MacAllister et al., 1996). Both DMAs accumulate in chronic renal insufficiency (MacAllister et al., 1996). Although there is growing evidence that ADMA is a predictor of cardiovascular events in the general population (Valkonen et al., 2001) as well as in patients with chronic kidney disease (Zoccali et al., 2001), the role of dimethylarginines for the progression of renal disease remains still a matter of debate.

The aim of this prospective study was to investigate whether increased levels of ADMA and SDMA in relation to conventional risk factors are linked to a higher risk for atherothrombotic events and renal outcome in patients with chronic renal disease.

Material and methods

Study population

A total of 200 patients (69 female, 131 male patients) with chronic kidney diseases were included in the study. The patients were part of a study presented previously (Busch et al., 2004), preliminary data belonging dimethylarginine concentrations were already published (Fleck et al., 2003). The group underwent ADMA testing, comprised 82 patients with chronic renal failure before starting dialysis treatment (CRF), 81 patients on maintenance hemodialysis treatment (HD) and 37 patients after renal transplantation (renal transplant recipients: RTR). Fifty-eight patients suffered from diabetes mellitus, mainly from type 2 diabetes ($n = 56$). The patients were representative of the population treated in our dialysis unit or the outpatient department (including renal transplant recipients). Each participant provided written informed consent. Patients were classified as having CRF when the serum creatinine level was higher than 150 µmol/l; the mean duration of CRF was 54.5 ± 57.8 months. Dialysis treatment was performed thrice weekly for 3.5–5 hours. Low-flux cellulosic membranes were used in 47 patients whereas 34 patients were dialysed with high-flux synthetic membranes (polycarbonate or polyamide). The mean time on dialysis was 38.0 ± 30.4 months. In the RTR, the mean time after transplantation at baseline was 41.1 ± 48.6 months. Renal diseases in the different groups were classified for glomerulonephritis (26% in CRF/27% in HD/60% in RTR), diabetic nephropathy (13%/31%/5%), interstitial nephritis (26%/25%/3%), polycystic kidney disease (2%/11%/8%) and other causes (33%/6%/24%). Reference ranges for ADMA and SDMA were calculated using the sera of 22 healthy volunteers (14 women, 8 men, mean age 46.4 ± 11.6 years) as controls. All of them had normal renal function as reflected by creatinine Cockroft-clearance within the normal range. The control group showed mean serum levels of 0.73 ± 0.26 µmol/l for ADMA, 0.50 ± 0.19 µmol/l for SDMA and 74.79 ± 27.51 µmol/l for arginine corresponding with current data from the literature (Teerlink et al., 2002; Ravani et al., 2005).

History of cardiovascular events (CVE)

The presence of cardiovascular disease at baseline was defined if symptoms or events typical for coronary heart disease (i.e. myocardial infarction, angina pectoris), cerebrovascular disease (i.e. stroke or transitory ischemia) or peripheral arterial occlusive disease (PAOD) were present before the time of entry to the study. The exact definition of events is described elsewhere (Busch et al., 2004).

Follow-up

Follow-up ended with the occurrence of CVE, initiation of dialysis treatment (excepting HD group), renal transplantation (only HD group), a change of treatment location, or death. The presence of CVE was defined if any event of acute myocardial infarcion (AMI), angina pectoris (AP) or cerebrovascular disease occurred, according to the criteria already described. Independently of clinical signs, all participants had at least one ECG measurement per year. The endpoint of PAOD was estimated if PAOD was diagnosed for the first time or if a preexisting stage of PAOD had to be upgraded. One case of death following fatal cardiac failure in the HD group was classified as AMI, because the autopsy revealed the total occlusion of at least one of the main coronary arteries.

Laboratory analysis

Blood samples were drawn after an overnight fasting; the serum samples were immediately centrifuged (3000 rpm, 4 °C) and cooled within 1 h and stored at −80 °C until analysis was performed. Measurements of dimethylarginines were performed in accordance with MacAllister et al. (1996) modified by Teerlink et al. (2002). In brief, we used equilibrated CBA columns (Bondelut CBA, Varian, U.S.A.) for 3-fold washing of serum samples with methanol (Roth, Karlsruhe, Germany) and distilled water. Thereafter the samples were eluted with 10% ammonia (Merck, Darmstadt, Germany) and dried at 130 °C. The sediment was dissolved in water, centrifuged and the supernatant was measured by HPLC using Spherisorb ODS columns (Jasco, Groß-Umstadt, Germany). L-monomethyl-arginine (Sigma, Deisenhofen, Germany) was used as internal standard. In aqueous calibration runs the recovery rates of ADMA and SDMA (Sigma, Deisenhofen, Germany) were approximately 100%, after administration of the three methylarginines into the sera of rats, the recovery rates were distinctly lower, but in the same range. A constant amount of 4 µmol/l L-monomethyl-arginine was given into the plasma samples and the concentrations of ADMA and SDMA were calculated on the basis of the recovery rates for L-monomethyl-arginine. Parameters such as lipid levels, creatinine, and albumin were determined using automated standardized laboratory techniques.

Statistical methods

The results are given as means with standard deviations (mean ± S.D.) and medians. The Mann-Whitney U-test was used to compare differences between two independent groups, the Spearman rank correlation test for estimating relationships between variables. A P value of ≤ 0.05 was considered as statistically significant. The associations between the potential risk factors and the endpoints are described by hazard ratios (denoted as relative risk, RR) and the corresponding 95% confidence intervals. The categorization of some of the continuous variables was necessary due to a non-linear log hazard in the covariates of interest (Hosmer and Lemeshow, 1999). Smokers, DM patients, and patients with a past history of CVE were compared to those without these risk factors. Relative risk was calculated by univariate following stepwise multivariate analysis with an additive followed by a stepwise subtraction model. Due to the limited number of patients, only variables on a significance level of $P < 0.20$ in the univariate analysis were entered in the initial multivariate model. Covariates with $P < 0.10$ were included in the final multivariate model. Statistics were done using the software Statistical Package of Social Science (SPSS 11.5, 2003; SPSS Inc., Chicago, IL, USA).

Results

ADMA, SDMA, and relationships between selected parameters at baseline

Table 1 shows the clinical characteristics of all groups. At baseline, the mean serum levels of SDMA were found to be significantly increased in all three groups as compared with normal subjects ($P \leq 0.0005$) (Fig. 1). ADMA was significantly increased in HD and RTR compared to controls ($P \leq 0.004$). The mean ADMA level in the CRF group (mean creatinine $385 \pm 223\,\mu mol/l$) was not significantly different from that in the control group,

Table 1. Baseline clinical and laboratory data in 200 patients with chronic kidney diseases: 82 patients with chronic renal failure (CRF), 81 haemodialysis patients (HD) and 37 patients after renal transplantation (RTR)

		Total	CRF	HD	RTR	Normal range*
Gender (m/f)		131/69	55/27	48/33	9/28	
Diabetes		58 (29%)	24 (29%)	30 (37%)	4 (11%)	
Smokers		37 (19%)	18 (22%)	9 (11%)	10 (27%)	
History of CVE		70 (35%)	16 (20%)	41 (51%)	13 (35%)	
Age (years)	mean ± S.D.	57.6 ± 13.0	57.5 ± 12.8	61.3 ± 12.4	49.6 ± 11.6	
	median	58.8	58.9	62.0	54.0	
	(min–max)	(24.0–87.0)	(33.0–84.0)	(24.0–87.0)	(25.0–67.0)	
BMI (kg/m^2)	mean ± S.D.	26 ± 4	28 ± 5	25 ± 3	25 ± 3	20–25
	median	26	27	25	25	
	(min–max)	(15–44)	(19–44)	(15–32)	(18–32)	
Creatinine (μmol/l)	mean ± S.D.	507 ± 316	385 ± 223	789 ± 209	157 ± 70	80–95
	median	521	316	745	142	
	(min–max)	(64–1284)	(129–1107)	(397–1284)	(64–371)	
CRP (mg/l)	mean ± S.D.	18.3 ± 29.9	18.2 ± 22.3	16.7 ± 22.4	7.0 ± 13.7	<5.0
	median	6.8	8.1	5.3	3.3	
	(min–max)	(1.2–236.9)	(5.0–95.3)	(2.0–96.0)	(1.2–84.5)	
Albumin (g/l)	mean ± S.D.	38.9 ± 4.9	38.6 ± 4.7	38.0 ± 5.1	41.6 ± 3.9	35–55
	median	38.9	39.6	38.3	41.8	
	(min–max)	(20.1–54.2)	(20.1–46.2)	(25.3–54.2)	(31.5–48.2)	
Hemoglobin (mmol/l)	mean ± S.D.	7.3 ± 1.4	7.5 ± 1.4	6.7 ± 1.1	8.1 ± 1.4	35–55
	median	7.1	7.5	6.6	7.8	
	(min–max)	(3.9–13.0)	(4.1–11.5)	(3.9–13.0)	(5.5–10.9)	
LDL (mmol/l)	mean ± S.D.	3.5 ± 1.2	3.7 ± 1.3	3.4 ± 1.1	3.2 ± 1.2	<3.5
	median	3.4	3.5	3.4	3.2	
	(min–max)	(0.88–8.11)	(0.9–8.1)	(0.9–7.6)	(0.9–5.2)	
HDL (mmol/l)	mean ± S.D.	1.3 ± 0.8	1.2 ± 0.4	1.1 ± 0.4	1.7 ± 1.8	>1.1
	median	1.2	1.2	1.0	1.3	
	(min–max)	(0.36–10.8)	(0.5–2.7)	(0.4–2.3)	(0.5–10.8)	
Triglycerides (mmol/l)	mean ± S.D.	2.2 ± 1.4	2.3 ± 1.3	2.1 ± 1.2	2.4 ± 1.9	<2.29
	median	1.9	2.1	1.7	1.9	
	(min–max)	(0.3–11.1)	(0.3–8.2)	(0.5–6.7)	(0.5–11.1)	
BP syst. (mmHg)	mean ± S.D.	139 ± 20	140 ± 19	140 ± 22	136 ± 17	<140
	median	140	140	143	135	
	(min–max)	(88–187)	(100–187)	(88–186)	(90–176)	
BP diast. (mmHg)	mean ± S.D.	81 ± 12	84 ± 11	77 ± 11	82 ± 12	<90
	median	80	85	77	83	
	(min–max)	(38–109)	(54–105)	(38–100)	(49–109)	
ADMA (μmol/l)	mean ± S.D.	0.97 ± 0.37	0.88 ± 0.37	1.06 ± 0.35	0.98 ± 0.36	
	median	0.93	0.85	0.99	0.96	
	(min–max)	(0.07–2.37)	(0.07–2.37)	(0.33–2.37)	(0.42–1.75)	
SDMA (μmol/l)	mean ± S.D.	2.12 ± 1.12	2.00 ± 1.11	2.96 ± 1.14	1.12 ± 0.71	
	median	1.89	1.75	2.54	1.02	
	(min–max)	(0.33–7.13)	(0.39–6.62)	(0.40–7.13)	(0.33–4.32)	
Arginine (μmol/l)	mean ± S.D.	60.4 ± 25.6	55.6 ± 28.6	58.7 ± 19.6	72.6 ± 28.1	
	median	59.1	53.4	58.4	72.0	
	(min–max)	(7.6–168.3)	(7.6–168.3)	(18.1–125.2)	(35.6–155.3)	

BMI body mass index, *BP* blood pressure, *CVE* cardiovascular events, *CRP* C-reactive protein. * Normal ranges referred to healthy, middle aged men and women *without* cardiovascular risk profile

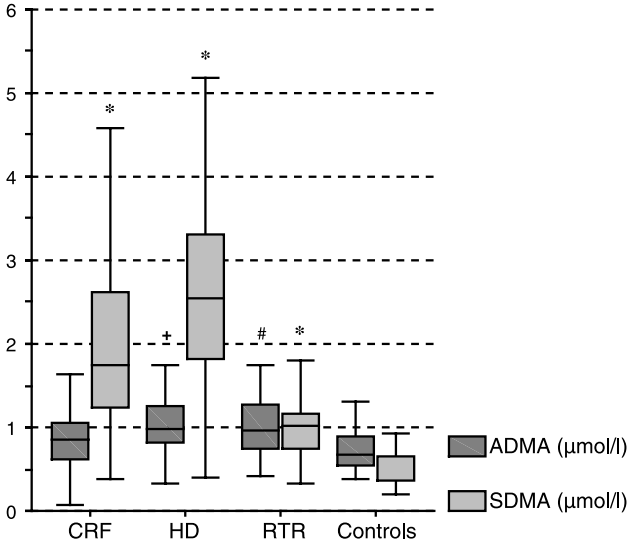

Fig. 1. Boxplots of serum ADMA and SDMA concentrations in 82 patients with chronic renal failure (CRF), 81 haemodialysis patients (HD), 37 patients after renal transplantation (RTR) and 22 healthy volunteers (Controls). $^{*}P < 0.0005$ compared to each other group, $^{\#}P = 0.004$ compared to controls, $^{+}P \leq 0.002$ compared to controls and CRF

whereas RTR patients showed a higher ADMA concentration despite a better renal function (mean creatinine $157 \pm 70\,\mu mol/l$). The HD group showed the highest levels for dimethylarginines (ADMA: $1.06 \pm 0.35\,\mu mol/l$, l/l, SDMA: $2.69 \pm 1.14\,\mu mol/l$). Arginine levels in the CRF and HD group were significantly lower compared to controls ($55.6 \pm 28.6\,\mu mol/l$ and $58.7 \pm 19.6\,\mu mol/l$ *vs.* $74.8 \pm 27.5\,\mu mol/l$ in controls, $P \leq 0.011$), whereas no difference was found between RTR and controls.

In the total group, there was a significant positive correlation between SDMA and creatinine ($R = 0.635$, $P \leq 0.0005$) whereas ADMA showed no relationship to the serum creatinine level. SDMA correlated with ADMA ($R = 0.389$, $P \leq 0.0005$). Only SDMA showed a weak negative relation to the albumin level ($R = -0.176$, $P \leq 0.013$) and to that of HDL cholesterol ($R = -0.156$, $P \leq 0.032$). SDMA was positively correlated to the CRP level ($R = 0.190$, $P \leq 0.007$).

In the HD group, no significant differences in ADMA, SDMA, and arginine levels were found between low-flux (n = 47) and high-flux membrane dialysed patients. Patients with a prior history of CVE (total group) showed no significant differences in dimethylarginine and arginine levels compared to patients without a prior CVE. Furthermore, the levels of SDMA, ADMA, and arginine in diabetics were not different from those of non-diabetics.

Mortality and cardiovascular events during follow-up

Median follow-up time was 24 (1–52) months. A total of 35 patients died during the follow-up period, 21 patients died from AMI, mainly in the HD group (n = 19, each one in CRF and RTR), 7 from cerebrovascular causes (two in CRF, five in HD). Seven deaths were classified as death from other causes (four in CRF, two in HD and one in RTR), two outside of the hospital without a known cause. Two patients in the HD group were lost for follow-up because of the change of the dialysis centre. Seven patients in the HD group received a renal transplantation and were no further eligible for the study.

Table 2. Unadjusted hazard ratios for cardiovascular events (n = 47) significantly associated with traditional and non-traditional risk factors in 200 patients with chronic kidney disease

Variable	Units of increase or categories	RR	95% CI	*P* value
Haemodialysis	HD *vs.* CRF and RTR	8.225	3.838–17.625	<0.0005
Diabetes	diabetes *vs.* non-diabetes	3.157	1.779–5.603	<0.0005
Age	1 year	1.046	1.022–1.072	<0.0005
BMI	$1\,kg/m^2$	0.926	0.857–1.001	0.052
CV history	yes *vs.* no	3.333	1.849–6.007	<0.0005
Albumin	1 g/l	0.914	0.865–0.966	0.002
CRP	highest quartile (>17.6 mg/l) *vs.* lowest quartile (≤4.9 mg/l)	3.383	1.694–6.755	0.001
Haemoglobin	1 mmol/l	0.555	0.438–0.704	<0.0005
Creatinine	1 µmol/l	1.002	1.001–1.003	<0.0005
SDMA	highest quartile (>2.82 µmol/l) *vs.* lowest quartile (≤1.17 µmol/l)	4.392	1.578–12.225	0.005

Forty-seven cases of cardiovascular events (CVE) including cardiovascular death occurred during the follow-up (total group), 24 cases of AMI (each one in CRF and RTR, 22 in HD), five cases of angina pectoris (all in HD), 10 cases of cerebrovascular insufficiency (each five in CRF and HD) and 8 patients with PAOD (one in CRF, seven in HD).

Table 3. Adjusted relative risk estimates for the combined cardiovascular endpoint (n = 47) and the renal endpoint (n = 39) during a median follow-up of two years in 200 patients with chronic kidney disease resulting from final multivariate Cox proportional hazards model

Categories of risk factors at baseline	Hazard ratio (95% confidence interval)[a]	*P* value
Combined cardiovascular endpoint		
Diabetes mellitus	3.072 (1.603–5.889)	0.001
End-stage renal disease[b]	11.915 (4.679–30.341)	<0.0005
C-reactive protein[c]	3.916 (1.833–5.889)	<0.0005
ADMA[c,d]	0.271 (0.103–0.709)	0.008
Renal endpoint		
Creatinine[c]	11.378 (2.801–46.227)	0.001
Haemoglobin (per 1 mmol/l increase)	0.710 (0.513–0.981)	0.038
SDMA (per 1 µmol/l increase)	1.633 (1.152–2.316)	0.006

[a] Resulting from the final forward model
[b] Compared to CRF patients together with renal transplant recipients
[c] For the highest quartile compared to the lowest (see text and Tables 2 and 3)
[d] See Fig. 2

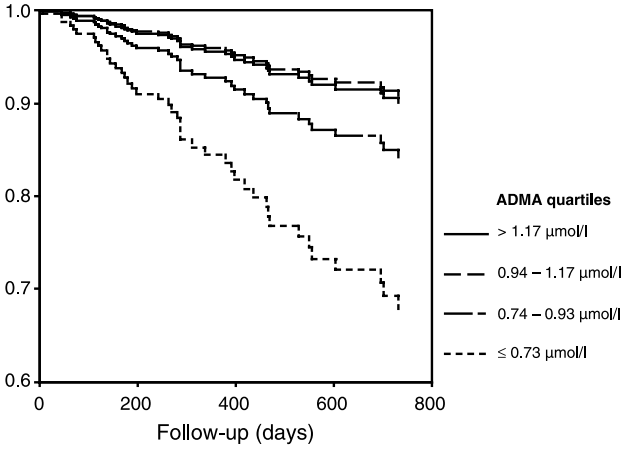

Fig. 2. Survivor functions (Cox regression analysis) of the final forward multivariate model for a first cardiovascular event during follow-up in patients with ADMA levels of different quartiles. In patients with infra-median ADMA levels (≤0.93 µmol/l), progression was significantly faster ($P \leq 0.008$)

In univariate analysis, the need for dialysis treatment (HD patients *vs.* RTR and CRF), the presence of diabetes, higher age, lower BMI, prior cardiovascular events, a lower serum concentration of albumin, CRP levels in the highest quartile, lower haemoglobin levels, as well as higher creatinine and SDMA levels were associated with the subsequent development of cardiovascular events ($P \leq 0.05$) (Table 2).

Results of multivariate analysis are shown in Table 3. Final multivariate Cox regression analysis showed diabetes, CRP levels in the highest quartile (>17.6 mg/l) compared to the lowest (≤4.9 mg/l), haemodialysis treatment and ADMA levels as predictors for CVE (forward and backward model). Surprisingly, in our study population, higher ADMA levels lead to a significant decrease in risk for a subsequent CV event for ADMA levels in the third (RR, 0.248; 95% CI, 0.095–0.649, $P = 0.005$) and the highest quartile compared to the lowest, respectively (Fig. 2, Table 3).

Renal endpoints

The CRF and the RTR group were additional followed for renal endpoints as were the initiation (CRF group) or the re-initiation (RTR group) of dialysis treatment. Thirty-nine patients reached the renal endpoint (35 in CRF, 4 in RTR). In the four patients after renal transplantation, the mean time after transplantation was 74 months.

The univariate analysis showed higher age, stage of kidney disease (RTR *vs.* CRF), hypertension, albumin levels in the lowest quartile, CRP in the highest quartile, lower haemoglobin concentration, higher creatinine levels, higher LDL cholesterol levels, and increased SDMA concentration as predictive for the progression of loss of renal function followed by the need for dialysis treatment (Table 4).

As Table 3 shows, the forward model of the multivariate analysis revealed the concentration of haemoglobin, creatinine levels in the highest quartile (>445 µmol/l) compared to the lowest (≤152 µmol/l) and the serum concentration of SDMA as independent predictors for the renal endpoint. In the backward model, albumin concentrations in the lowest quartile (≤36.7 g/l) compared to the highest (>43.2 g/l) were additionally associated with the reach of end-stage renal disease (RR 5.768, 95% CI 1.531–21.730, $P = 0.01$).

Table 4. Unadjusted hazard ratios for the renal endpoint (n = 39) significantly associated with traditional and non-traditional risk factors in 82 patients with chronic renal failure and 37 patients after renal transplantation

Variable	Units of increase or categories	RR	95% CI	*P* value
RTR	RTR *vs.* CRF	0.203	0.072–0.575	0.003
Age	1 year	1.044	1.016–1.073	0.002
Hypertension	$\geq$140/90 mmHg *vs.* below	2.550	1.268–5.125	0.009
Albumin	lowest quartile ($\leq$36.7 g/l) *vs.* highest quartile (>43.2 g/l)	4.853	1.578–14.920	0.006
CRP	highest quartile (>12.1 mg/l) *vs.* lowest quartile ($\leq$5.0 mg/l)	3.994	1.513–10.545	0.005
Hemoglobin	1 mmol/l	0.516	0.398–0.670	<0.0005
Creatinine	highest quartile (>445 µmol/l) *vs.* lowest quartile ($\leq$152 µmol/l)	41.083	11.742–143.745	<0.0005
LDL	1 mmol/l	1.410	1.132–1.757	0.002
SDMA	1 µmol/l	2.566	1.994–3.301	<0.0005

Discussion

In patients with chronic renal failure, risk of cardiovascular morbidity and mortality is substantially increased (Parfrey and Foley, 1999). In addition to the well-known cardiovascular risk factors such as diabetes mellitus or ESRD, parameters such as elevated serum levels of CRP, fibrinogen, and homocysteine have been newly defined as cardiovascular risk factors (Arici and Walls 2001; Suliman et al., 2000; Massy, 2000). Evidence increasingly suggests that ADMA is also a potential candidate enhancing vascular complications in renal disease patients (Fliser et al., 2003; Vallance et al., 1992; Valkonen et al., 2001; Zoccali et al., 2001).

Our study in a heterogeneous population of patients with chronic renal failure of different stages confirmed previous findings demonstrating a substantial increase of both DMAs in patients with renal insufficiency (Vallance et al., 1992; MacAllister et al., 1996; Zoccali et al., 2001) compared to healthy controls. The increase was more pronounced for SDMA indicating its strong relation to renal excretion as confirmed in the correlation tests. Neither in the total group nor in any of the subgroups, any correlation between ADMA and creatinine was found. In accordance with Nijveldt et al. (2002) it can be assumed that ADMA is more depending on metabolism by DDAH (Ogawa et al., 1989) than on the glomerular filtration rate. Although information on the impact of haemodialysis on ADMA plasma levels is controversial (Kielstein et al., 1999, 2004), haemodialysis seems not suitable for a long-lasting removal of DMAs as confirmed in our HD group. Moreover, no differences in the DMA levels between low-flux and high-flux dialysed patients were found.

Our main findings were that several cardiovascular risk factors as diabetes, end-stage renal disease and elevated CRP levels were associated with increased morbidity for cardiovascular events as previously shown in nearly the same study population and discussed in deep by our group (Parfrey and Foley, 1999; Himmelfarb et al., 2002; Busch et al., 2004; Arici and Walls 2001). As expected, the creatinine level was confirmed to be the strongest predictor for the renal outcome. In addition, increasing haemoglobin values were shown to be protective against the decline of renal function with a nearly thirty percent risk reduction for the need of dialysis treatment during follow-up per an increase in the serum haemoglobin level of 1 mmol/l. This supports previous findings leading to the strategy of early erythropoietin therapy in chronic renal failure (Gouva et al., 2004).

Surprisingly, one finding of our study was that patients with ADMA levels in the both upper quartiles had a decrease in their risk for cardiovascular events of more than seventy percent compared with ADMA levels in the lowest quartile. This result of the multivariate analysis was also a consistent finding if continuous statistical models were used (data not shown). Although it is difficult to explain this new aspect, it shows that increased ADMA levels are not constantly linked to the occurrence of subsequent cardiovascular events as the existing evidence suggests in the general population (Valkonen et al., 2001) as well as in patients suffering from chronic renal insufficiency (Fliser et al., 2003; Zoccali et al., 2001; Kielstein et al., 1999). It seems to be rather that certain circumstances may lead to unexpected findings referred to as ''paradoxical epidemiology'' in chronic kidney disease patients, particularly in this study. The phenomenon of ''paradoxical'' or ''reverse epidemiology'' is currently discussed belonging the different impact of several traditional and non-traditional cardiovascular risk factors in renal disease patients, often contradict to the existing

evidence in the general population (Shoji and Nishizawa, 2005). For example, increased serum levels of total homocysteine (tHcy) and of advanced glycated end products (AGEs) failed to show expected relationships to cardiovascular endpoints in several studies undertaken in chronic renal failure patients (Busch et al., 2004; Suliman et al., 2000; Schwedler et al., 2002). In two of these trials, decreasing concentrations of tHcy (Suliman et al., 2000) and of the AGE compound N^{ε}-carboxymethyllysine (Schwedler et al., 2002) were even associated with a poor outcome, respectively. These findings were also contradicting to previous findings concerning the role of hyperhomocysteinaemia for cardiovascular outcome in end-stage renal disease (Moustapha et al., 1998) comparable to the unexpected results presented here. Contradictory findings in ESRD patients were mostly explained by a diminished nutritional status and an increased state of inflammation together with oxidative stress in ESRD patients on dialysis treatment (Himmelfarb et al., 2002; Busch et al., 2004; Arici and Walls, 2001; Suliman et al., 2000; Massy, 2000; Shoji and Nishizawa, 2005; Schwedler et al., 2002). In healthy subjects, relationships between nutritional parameters and ADMA levels exist. In subjects with mild hypercholesterolemia a high amount of dietary carbohydrates was strongly associated with low levels of plasma ADMA (Paiva et al., 2004) and ADMA levels were elevated in young hypercholesterolemic individuals (Böger et al., 1998). Zoccali et al. (2001) could show a positive correlation of ADMA in plasma with the concentration of fibrinogen and an inverse relation of ADMA to the serum concentration of albumin in a cohort of 225 dialysis patients, demonstrating an influence of nutrition and inflammation on serum levels of ADMA even in ESRD. Although, in our patients, no relationships between BMI, serum lipid-, albumin- or CRP levels with ADMA were found, malnutrition together with chronic inflammation might have played yet a pronounced role for the cardiovascular outcome in our study. The low arginine levels in the CRF and HD group might be an indicator for that. In the same manner it is noteworthy that most of the CVE occurred in the HD group showing only a slight increase in ADMA levels compared to CRF and RTR whereas the impact of several other risk factors including malnutrition and inflammation might have been greater under the circumstances of chronic HD treatment (Parfrey and Foley, 1999; Himmelfarb et al., 2002; Busch et al., 2004; Arici and Walls, 2001; Suliman et al., 2000; Massy, 2000).

The fact that an increasing SDMA level could be identified as a predictor for renal outcome whereas ADMA level could not, is just as well a contradict finding since SDMA is described to be a biologically inactive substance (MacAllister et al., 1994; Nijveldt et al., 2002). It failed to show any relationship with cardiovascular or renal endpoints as yet, whereas ADMA could be confirmed recently as an independent renal risk factor (Ravani et al., 2005; Fliser et al., 2005). It lead to a twenty percent increase in the risk for the progression to dialysis and death by each 0.1 μmol/l increment in 131 patients with chronic kidney disease (Ravani et al., 2005), especially in nondiabetic kidney diseases (Fliser et al., 2005). However, in both studies, plasma ADMA levels were inversely related to the GFR contradicting previous findings showing no relationship between ADMA levels and GFR (Zoccali et al., 2001; Böger et al., 1998). Comparable to these findings, in our patients, only SDMA was correlated with the serum creatinine level. However, although SDMA does not inhibit NO synthase directly, it does compete with the cationic amino acid transporter in the endothelial cell membrane possibly accentuating intracellular arginine deficiency leading to a decreased NO production (Bogle et al., 1995). The high SDMA concentrations determined in the CRF patients, mainly followed for the renal endpoint, could indicate such effects influencing the renal outcome. Since a substantial increase of SDMA already in patients with mild renal impairment or even in patients with normal renal function showing ADMA levels in the normal range could be found, the determination of both dimethylarginines in the design of clinical trials has been suggested (Paroni et al., 2005).

Nevertheless, it should be noted that there are some limitations to our study: above all the limited number of patients recruited in this single centre study, furthermore for statistical reasons stated above, it was necessary to use the categorization of selected continuous variables. Another problem might be that all parameters were measured only at baseline levels and might have been influenced by short term changes in metabolism. Although our study could not confirm the existing evidence belonging the role of ADMA and SDMA in chronic kidney disease, it confirms numerous previous findings of cardiovascular risk factors (Parfrey and Foley, 1999; Busch et al., 2004; Arici and Walls, 2001; Suliman et al., 2000; Schwedler et al., 2002; Shlipak et al., 2005) and predictors for the renal outcome (Ravani et al., 2005; Gouva et al., 2004; Fliser et al., 2005), which could be valued as a factor for the statistical validity of our investigation.

In conclusion, our data support the existing evidence that risk factors such as elevated CRP levels, diabetes, and the degree of kidney impairment seem to play a more

important role for the cardiovascular outcome of patients with chronic kidney disease than elevated serum levels of ADMA and SDMA. Moreover, in our study decreasing rather than increasing ADMA levels were predictive of cardiovascular events, which could hint to ADMA as a new candidate for the current debate of "paradoxical epidemiology" in chronic kidney disease. Besides well-known markers for the progression of kidney disease to ESRD such as creatinine and haemoglobin, our data show for the first time a predictive role also for increasing SDMA levels.

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Authors' address: Dr. med. Martin Busch, Department of Internal Medicine III, Friedrich-Schiller-University of Jena, D-07740 Jena, Germany, Fax: +49-3641-9325832, E-mail: martin.busch@med.uni-jena.de