Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study

Carmine Zoccali, Stefanie M Bode-Böger, Francesca Mallamaci, Frank Antonio Benedetto, Giovanni Tripepi, Lorenzo Salvatore Malatino, Alessandro Cataliotti, Ignazio Bellanuova, Isabella Fermo, Jürgen C Frölich, Rainer H Böger

Summary

Background The plasma concentration of asymmetrical dimethylarginine (ADMA), an inhibitor of nitric-oxide synthase, which has been linked to endothelial dysfunction and atherosclerosis in the general population, is raised in patients with end-stage renal disease and could contribute to the high cardiovascular risk in patients with chronic renal failure. We investigated the relation between cardiovascular risk factors and plasma ADMA concentration in a cohort of haemodialysis patients (n=225), and tested the predictive power of ADMA for mortality and cardiovascular outcomes.

Methods Patients had standard dialysis three times a week. We accurately recorded cardiovascular events over a mean follow-up of 33·4 months (SD 14·6); these events were reviewed by a panel of physicians. We identified correlates of plasma ADMA by univariate and multivariate analyses.

Findings On univariate analysis, ADMA concentration in plasma was directly related to concentrations of fibrinogen and L-arginine in plasma, duration of dialysis treatment, and serum cholesterol concentration, and was inversely related to serum albumin concentration. On multivariate analysis, only plasma fibrinogen (p=0·0001) and serum albumin (p=0·04) concentrations were independently related to plasma ADMA concentration (multiple r=0·44, p=0·0001). 83 patients died, 53 (64%) by cardiovascular causes. In a Cox's proportional-hazards model, plasma ADMA ranked as the second factor predicting overall mortality (hazard ratio 1·26, 95% CI 1·11–1·41, p=0·0001) and cardiovascular events (1·17, 1·04–1·33, p=0·008).

Interpretation In haemodialysis patients, plasma ADMA is a strong and independent predictor of overall mortality and cardiovascular outcome. These findings lend support to the hypothesis that accumulation of ADMA is an important risk factor for cardiovascular disease in chronic renal failure.

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Institute of Clinical Pharmacology, Hannover Medical School, Hannover, Germany (S M Bode-Böger MD, Prof J C Frölich MD); **Clinical Pharmacology Unit, Department of Pharmacology, Institute of Experimental and Clinical Pharmacology and Toxicology, University Hospital Hamburg-Eppendorf, D-20246 Hamburg** (Prof R H Böger MD); **Laboratorio di Cromatografia e Tecniche di Separazione, S Raffaele Hospital, Milan, Italy** (I Fermo MD); **Department of Internal Medicine, Catania University** (L S Malatino MD, A Cataliotti MD, I Bellanuova MD); **and CNR Centre of Clinical Physiology and Renal Unit, Reggio C** (Prof C Zoccali MD, F Mallamaci MD, F A Benedetto MD, G Tripepi)

Correspondence to: Prof Rainer H Böger (e-mail: boeger@uke.uni-hamburg.de)

Introduction

Cardiovascular disease is a major cause of death in patients with end-stage renal disease. The projected life expectancy of patients on dialysis is 20–25% that of the general population.[1] Although patients with chronic renal failure commonly have associated diseases which have a high cardiovascular risk in themselves, such traditional risk factors account for only part of the very high cardiovascular morbidity and mortality in these patients.[1] An expert panel from the USA National Kidney Foundation has recently identified the need for observational studies to ascertain the relation between established cardiovascular risk factors and cardiovascular outcomes and to identify new risk factors as a research priority.[2]

Asymmetrical dimethylarginine (ADMA) is an endogenous inhibitor of nitric-oxide synthase.[3] Concentrations of ADMA are related to endothelial dysfunction in hypercholesterolaemic individuals.[4] ADMA and its biologically inactive stereoisomer—symmetrical dimethylarginine (SDMA)—are not excreted in patients with chronic renal failure, and concentrations of these substances in plasma are two to six times higher in uraemic patients than in healthy control individuals.[3,5,6] ADMA concentrations in plasma are also raised in young hypercholesterolaemic patients with normal renal function,[4] and correlate with intima media thickness in apparently healthy middle-aged individuals.[7] Of note, ADMA concentrations are higher in dialysis patients with clinically manifest atherosclerosis than in those without atherosclerotic disease,[6] which suggests that accumulation of ADMA might be an important cardiovascular risk factor in end-stage renal disease.

With this background in mind, we aimed to study prospectively the association between ADMA concentration in plasma, survival, and cardiovascular outcomes in a cohort of patients on chronic haemodialysis.

Methods

Patients

225 haemodialysis patients with end-stage renal disease (123 men, 102 women) who had undergone regular dialysis treatment for at least 6 months (median 42 months, IQR 21–109) without clinical evidence of circulatory congestion (defined as dyspnoea in addition to two of the following conditions: raised jugular venous pressure; bibasilar crackles; pulmonary venous hypertension or interstitial oedema on chest radiograph requiring admission to hospital or extra ultrafiltration; and ejection fraction <35%) were judged eligible for the study. These patients represented about 70% of the total number of patients undergoing dialysis at the four participating dialysis units.

Patients were being treated three times a week with standard bicarbonate dialysis (Na^+ 138 mmol/L, HCO_3^- 35 mmol/L, K^+ 1·5 mmol/L, Ca^{2+} 1·25 mmol/L, Mg^{2+} 0·75 mmol/L) by 1·1–1·7 m^2 dialysers with either

cuprophan or semisynthetic membranes. Dry weight was targeted in every patient to achieve a normotensive oedema-free state. 82 patients were habitual smokers (mean 22 cigarettes/day, SD 16, range 1–80), and an equal number were on antihypertensive treatment—58 on monotherapy with angiotensin-converting-enzyme inhibitors, angiotensin I antagonists, calcium-channel blockers, alfa-blockers, and beta-blockers, and 24 on double or triple therapy with various combinations of these drugs.

The protocol adhered to the ethical guidelines of our institutions, and oral informed consent was obtained from each participant. All studies were done on a midweek non-dialysis day, between 0800 h and 1300 h.

Procedures

Patients were followed up for a mean of 33·4 months (SD 14·6). During follow-up, cardiovascular events and deaths were accurately recorded and reviewed by a panel of five physicians. As part of the review process, all available medical information was obtained, and this included study and hospital records. In the case of death out of hospital, family members were interviewed by telephone to ascertain the circumstances surrounding death.

Blood sampling was done after 20–30 min of quiet resting in a semirecumbent position. Samples were stored in prechilled vacutainers containing edetic acid, placed immediately on ice, and centrifuged within 30 min at 4°C; plasma was stored at –80°C until required. Blood pressure was estimated by averaging all predialysis arterial pressure recordings taken the month before the study (total of 12 measurements—ie, three per week).[8]

Concentrations of lipids, albumin, calcium, and phosphate in serum, and concentrations of fibrinogen and haemoglobin in plasma, were measured by standard methods in the clinical laboratory. C-reactive protein concentration was measured with a commercially available kit (Behring, Scoppito, L'Aquila, Italy). Homocysteine concentrations in plasma were measured by high-performance liquid chromatography.

Concentrations of L-arginine and dimethylarginine in plasma were measured by high-performance liquid chromatography, by precolumn derivatisation with *o*-phthalaldehyde,[4] after removal of plasma samples with carboxylic acid solid-phase extraction cartridges (Varian, Harbor City, CA, USA). The coefficients of variation were 5·2% within-assay and 5·5% between-assay; the detection limit of the assay was 0·1 μmol/L. The concentration of ADMA in the plasma of normal individuals has a positively skewed distribution, with a median value of 0·95 μmol/L (IQR 0·76–1·53) and a 90th percentile of 2·2 μmol/L,[6] which we judged to be the upper limit of the normal range.

Statistical analyses

Correlates of plasma ADMA were identified by univariate and multivariate analyses. The relations between ADMA concentration in plasma, all-cause mortality, and cardiovascular events (fatal and non-fatal) were analysed with the multivariate Cox's proportional-hazards model. Variables included concentrations of L-arginine and SDMA in plasma, and traditional risk factors (age, sex, previous cardiovascular events, systolic blood pressure, smoking, diabetes, total cholesterol and LDL in serum, and fibrinogen concentration), emerging risk factors (C-reactive protein and homocysteine), and factors exclusive to end-stage renal disease (haemoglobin, calcium phosphate product [$Ca \times PO_4$], albumin, duration of dialysis treatment, and fractional urea clearance). For patients who had multiple events, survival analysis was restricted to the first event. All calculations were done with a standard statistical package (SPSS for Windows, version 9.0.1).

	Total population (n=225)
Demographic	
Age (years)	59·9 (15·1)
Sex (men/women)	123/102
Anthropometric	
Bodyweight (kg)	64·4 (12·0)
Height (cm)	162·2 (9·9)
Cardiovascular risk factors	
Systolic blood pressure (mm Hg)	139·4 (24·9)
Diastolic blood pressure (mm Hg)	75·9 (12·9)
Diabetes	15%
Smokers	36%
Hypercholesterolaemia	51%
Treatment with erythropoietin	54%
Biochemical	
Plasma homocysteine (μmol/L)	27·0 (19·4–42·7)
Plasma fibrinogen (mg/dL)	379·5 (257·5–526·7)
Serum total cholesterol (mmol/L)	5·4 (1·5)
Serum LDL cholesterol (mmol/L)	3·4 (1·3)
Serum triglycerides (mmol/L)	2·0 (1·0)
Serum calcium (mmol/L)	2·3 (0·5)
Serum phosphate (mmol/L)	2·0 (1·3)
Serum C-reactive protein (mg/L)	7·4 (3·4–16·4)
Serum albumin (g/L)	42·0 (5·0)
Haemoglobin (g/dL)	10·8 (1·9)
Fractional urea clearance	1·21 (0·26)

Data are mean (SD), median (IQR), or percentage frequency, as appropriate.

Table 1: **Patients' characteristics**

Results

Table 1 shows the baseline characteristics of the patients. Median concentration of ADMA in plasma was

	Number of patients (n=225)
Cardiovascular events	
Stroke	25 (11%)
Arrhythmia	21 (9%)
Myocardial infarction	18 (8%)
Heart failure	17 (8%)
Angina	15 (7%)
Transient ischaemic attack	8 (4%)
Mesenteric infarction	5 (2%)
Sudden death	4 (2%)
Major episodes of venous thrombosis	3 (1%)
Pulmonary embolism	2 (1%)
Retinal-artery thrombosis	1 (0·4%)
Peripheral-artery disease	1 (0·4%)
Total	120 (53%)
Causes of death	
Cardiovascular	
Stroke	16 (7%)
Myocardial infarction	13 (6%)
Heart failure	9 (4%)
Arrhythmia	4 (2%)
Sudden death	4 (2%)
Pulmonary embolism	2 (1%)
Mesenteric infarction	5 (2%)
Subtotal	53 (24%)
Other causes	
Sepsis/infection	8 (4%)
Cachexia	8 (4%)
Neoplasia	4 (2%)
Hyperkalaemia	4 (2%)
Gastrointestinal haemorrhage	3 (1%)
Chronic-obstructive pulmonary disease	1 (0·4%)
Diabetes, hyperosmolar coma	1 (0·4%)
Treatment withdrawal	1 (0·4%)
Subtotal	30 (13%)
Total	83 (37%)

Table 2: **Cardiovascular events (fatal and non-fatal) and causes of death**

Concentration of ADMA (percentile)	Number of patients*	Hazard ratio† (95% CI)	p
All-cause mortality			
<50th	33/113 (29%)	1·00	
50–75th	22/56 (39%)	1·72 (1·00–2·97)	0·05
>75th	28/56 (50%)	3·11 (1·83–5·27)	<0·0001
Fatal and non-fatal cardiovascular events			
<50th	29/113 (26%)	1·00	
50–75th	25/56 (45%)	2·13 (1·24–3·65)	0·006
>75th	27/56 (48%)	2·80 (1·63–4·81)	0·0002

ADMA=asymmetrical dimethylarginine. *Denominator represents number of patients at risk. †Adjusted for age and sex.

Table 3: **Hazard ratios by plasma ADMA concentration**

2·52 μmol/L (IQR 1·58–3·85). 133 (59%) patients had ADMA concentrations above the upper limit of the normal range (>2·2 μmol/L). The median concentration of L-arginine and SDMA in plasma was 70·0 μmol/L (57·6–78·8) and 3·01 μmol/L (2·40–4·52), respectively.

On univariate analysis, concentration of ADMA in plasma correlated with the concentrations of fibrinogen (r=0·50, p=0·0001) and L-arginine (r=0·17, p=0·004) in plasma, duration of regular dialysis treatment (r=0·13, p=0·02), and concentration of cholesterol in serum (r=0·12, p=0·04), and was inversely related to concentration of albumin in serum (r=–0·12, p=0·04). In a multiple regression model, only concentrations of fibrinogen in plasma (r=0·29, p=0·0001) and albumin in serum (r=–0·16, p=0·04) were independently related to concentration of ADMA in plasma (multiple r=0·44, p=0·0001).

120 cardiovascular events (fatal and non-fatal) in 81 patients occurred during mean follow-up of 33·4 months (SD 14·6, table 2). Overall, 83 patients died, 53 (64%) from cardiovascular causes (table 2). Concentration of ADMA in plasma was higher in patients who died during follow-up (median 3·02 μmol/L, IQR 1·77–4·23) than in those who survived (2·26 μmol/L, 1·47–3·55, p=0·006). Similarly, concentration of ADMA in plasma was significantly higher in patients with cardiovascular events during follow-up (3·21 μmol/L, 1·87–4·47) than in those with no such events (2·21 μmol/L, 1·44–3·41, p=0·001). The overall risk of death and fatal and non-fatal cardiovascular events (adjusted for age and sex) was progressively higher from the 50th percentile onwards (table 3). In a Cox's regression model, concentration of ADMA in plasma ranked as the second factor predicting all-cause mortality and cardiovascular outcome (table 4).

Discussion

Our findings show that ADMA is a stronger independent predictor of all-cause mortality and cardiovascular outcome in patients with chronic renal failure than some traditional risk factors. We observed a high occurrence of cardiovascular complications, with 81 (36%) patients having at least one cardiovascular event during mean follow-up of 33·4 months. This observation is in agreement with findings of other studies.[9] Patients who died from cardiovascular complications had higher baseline ADMA concentrations than those who had no cardiovascular events. Our findings confirm the hypothesis from an echo-colour doppler study,[7] that there is a relation between ADMA concentration and cardiovascular disease. Independent predictors for death in that study included high ADMA concentration, increasing age, male sex, diabetes mellitus, concentration of albumin in serum, and C-reactive protein. An increase by 1 μmol/L in concentration of ADMA in plasma was independently associated with a 26% increase in risk of all-cause mortality.

There is a high frequency of traditional cardiovascular risk factors, such as hypertension, hypercholesterolaemia, diabetes, and smoking, among patients with end-stage

	Unit of increase	All-cause mortality				Fatal and non-fatal cardiovascular events			
		Hazard ratio* (95% CI)	p	Fully adjusted hazard ratio (95% CI)	p	Hazard ratio* (95% CI)	p	Fully adjusted hazard ratio (95% CI)	p
ADMA	1 μmol/L	1·28 (1·16–1·41)	<0·0001	1·26 (1·11–1·41)	0·0001	1·21 (1·10–1·32)	0·0001	1·17 (1·04–1·33)	0·008
SDMA	1 μmol/L	1·02 (0·93–1·11)	0·73	1·06 (0·94–1·18)	0·34	0·97 (0·88–1·07)	0·61	1·00 (0·88–1·14)	0·98
L-arginine	10 mmol/L	1·01 (0·89–1·14)	0·92	0·92 (0·80–1·05)	0·22	1·06 (0·94–1·19)	0·37	1·00 (0·87–1·15)	0·97
Previous cardiovascular events	··	1·39 (0·83–2·34)	0·21	1·51 (0·86–2·64)	0·15	1·92 (1·15–3·19)	0·01	1·87 (1·07–3·28)	0·03
Age	1 year	1·05 (1·03–1·07)	<0·0001	1·06 (1·03–1·08)	<0·0001	1·03 (1·01–1·05)	0·0002	1·04 (1·02–1·06)	0·0003
Male sex	··	2·22 (1·40–3·53)	0·0007	2·30 (1·29–4·10)	0·005	1·53 (0·97–2·41)	0·07	1·49 (0·82–2·72)	0·19
Systolic pressure	10 mm Hg	1·03 (0·94–1·12)	0·57	1·00 (0·90–1·11)	0·97	1·07 (0·98–1·17)	0·15	1·10 (0·99–1·22)	0·09
Diabetes	··	1·79 (1·09–2·95)	0·02	2·24 (1·22–4·09)	0·009	1·80 (1·06–3·06)	0·03	2·03 (1·08–3·81)	0·03
Smoking	10 cigarettes/day	0·97 (0·83–1·12)	0·65	0·95 (0·81–1·11)	0·52	1·08 (0·93–1·25)	0·31	1·10 (0·94–1·29)	0·23
Homocysteine	10 mmol/L	1·01 (0·93–1·09)	0·86	1·07 (0·99–1·17)	0·10	1·04 (0·97–1·12)	0·25	1·09 (1·01–1·18)	0·03
Fibrinogen	10 mg/dL	1·02 (1·01–1·03)	0·0001	1·01 (1·00–1·03)	0·12	1·02 (1·01–1·03)	0·0001	1·02 (1·00–1·04)	0·02
Total cholesterol	(0·026 mmol/L)	1·00 (1·00–1·01)	0·41	1·01 (0·99–1·02)	0·39	1·00 (1·00–1·00)	0·64	1·01 (0·99–1·02)	0·18
LDL cholesterol	(0·026 mmol/L)	1·00 (1·00–1·00)	0·92	0·99 (0·98–1·01)	0·44	1·00 (0·99–1·00)	0·80	0·99 (0·97–1·00)	0·09
Calcium phosphate product	1 mmol/L	1·11 (0·91–1·37)	0·31	1·00 (0·79–1·27)	0·98	1·11(0·90–1·36)	0·33	1·11 (0·87–1·41)	0·40
C-reactive protein	1 mg/L	1·01 (1·00–1·01)	0·03	1·01 (1·00–1·02)	0·06	1·00 (0·99–1·01)	0·53	0·99 (0·98–1·00)	0·09
Albumin	10 g/L	0·66 (0·42–1·04)	0·07	0·56 (0·31–1·01)	0·06	0·88 (0·55–1·43)	0·61	0·88 (0·47–1·65)	0·69
Haemoglobin	10 g/L	1·00 (0·89–1·14)	0·96	1·04 (0·90–1·20)	0·60	0·99 (0·87–1·12)	0·84	0·95 (0·82–1·10)	0·50
Fractional urea clearance	··	0·59 (0·23–1·56)	0·29	0·40 (0·13–1·17)	0·09	1·19 (0·50–2·82)	0·70	0·89 (0·32–2·45)	0·82
Duration of regular dialysis treatment	1 year	1·02 (0·98–1·06)	0·25	1·00 (0·96–1·05)	0·87	1·05 (1·02–1·09)	0·004	1·06 (1·01–1·10)	0·02

ADMA=asymmetrical dimethylarginine; SDMA=symmetrical dimethylarginine. *Adjusted for age and sex.

Table 4: **Cox's proportional-hazard models for all-cause mortality and fatal and non-fatal cardiovascular events**

renal disease.[1] However, hyperhomocysteinemia, which has recently been identified as a common and independent risk factor in the general population and in patients with end-stage renal disease,[10] only partly accounts for increased cardiovascular risk. Conventional risk factors are associated with dysfunction of the endothelial L-arginine/nitric-oxide pathway.[11] Endothelial dysfunction occurs before the onset of overt vascular disease, suggesting that impaired availability of biologically active nitric oxide, which has an important role in the regulation of renal function in health and disease,[11] contributes to progression of cardiovascular disease.[4] The endothelium in patients with end-stage renal disease is dysfunctional, as suggested by findings of several clinical studies,[12,13] but the mechanism underlying this defect is not fully understood. Kari and colleagues[12] found that endothelial dysfunction in uraemic children was related to concentration of ADMA in plasma. Hand and colleagues[13] showed that endothelial dysfunction was reversed after haemodialysis sessions. A similar time course was reported for concentration of ADMA in plasma, since ADMA is eliminated during haemodialysis.[6,14] Therefore, accumulation of ADMA might explain endothelial dysfunction in patients with end-stage renal disease.

ADMA at pathophysiologically high concentrations—similar to those seen in our study (3–15 μmol/L)—significantly inhibits vascular nitric-oxide elaboration.[3,15] In individuals at high risk of cardiovascular disease or with overt atherosclerotic vascular disease, ADMA concentration is associated with degree of endothelial dysfunction and with the reduction in nitric-oxide elaboration.[4,16] Kielstein and colleagues[6] observed that mean concentration of ADMA in plasma was higher in patients with end-stage renal disease and atherosclerotic vascular disease than in those without vascular complications. These workers suggested that end-stage renal disease and atherosclerotic vascular disease could independently cause ADMA concentration to rise, which might predict atherosclerosis in people with normal renal function and in patients with end-stage renal disease. This conclusion was recently corroborated by Miyazaki and colleagues[7] who showed that ADMA concentrations were significantly correlated to intima-media thickness of the carotid artery in 116 individuals without vascular or renal disease. ADMA might therefore be a useful marker for atherosclerosis in patients with end-stage renal disease and in the general population.

The pathophysiology of raised ADMA concentrations in end-stage renal disease could be multifactorial. In healthy human beings, ADMA is eliminated from the body by renal excretion and by metabolism to L-citrulline by dimethylarginine dimethylaminohydrolase (DDAH).[3,17] Retention of DDAH in the body of patients with end-stage renal disease might therefore be a logical consequence of reduced excretion via the urine. However, concentrations of ADMA are also high among patients with hypercholesterolaemia, hypertension, and atherosclerotic vascular disease independently of changes in renal function.[4,16,18] We have previously presented evidence that atherosclerosis and chronic renal failure independently contribute to a rise in concentration of ADMA in plasma.[6] Reduced activity of DDAH has been shown to account for high ADMA concentrations in the presence of oxidised LDL or tumour necrosis factor-α in cultured endothelial cells.[19] Inhibition of DDAH causes vasoconstriction in vascular segments, suggesting that changes in ADMA concentrations via this mechanism are relevant to vascular tone.[20] ADMA concentration is also raised in hyperhomocysteinaemia, which is probably attributable to increased methylation of L-arginine residues.[21,22] Despite these multiple interactions of ADMA with other risk factors, we have found that concentrations of ADMA were independently correlated with fibrinogen concentrations and serum albumin only. These data suggest that ADMA might be a risk factor for atherosclerosis, and that mechanisms leading to raised ADMA concentrations in patients with end-stage renal disease could be independent of other cardiovascular risk factors.

The mechanism by which ADMA increases the risk of atherosclerosis is probably by inhibition of endothelial nitric-oxide elaboration. High ADMA concentrations block nitric-oxide synthase activity in vitro[3,22] and inhibit endothelium-dependent vasodilation in animals[15,21] and in human beings.[4] Importantly, only ADMA (not SDMA) exerts this biological activity. Therefore, analytical assays are required that differentiate ADMA and SDMA with high specificity. High-performance liquid chromatography is a suitable assay;[4] however, other workers have reported "total dimethylarginine" concentrations rather than ADMA.[23] In our study, variability in SDMA concentration was moderate between patients, and there was no correlation with mortality. These observations are attributable to renal excretion (the only known process modulating SDMA concentration in plasma)[6,14] and the absence of biological activity as seen in vitro.[3]

Regulation of endothelial nitric-oxide-synthase activity by ADMA is likely to modulate the progression of atherosclerosis. In animals, chronic inhibition of nitric-oxide elaboration accelerates the progression of vascular lesions, whereas long-term supplementation with L-arginine, the precursor of nitric oxide, inhibits progression.[24–26] Absence of biologically active nitric oxide is also associated with platelet aggregation and leucocyte adhesion, mechanisms that contribute to acute atherothrombotic events that might increase cardiovascular mortality rates.[11] Therefore, the relation between increased ADMA concentration and cardiovascular events in our study might extend to the general population, although no prospective data exist to confirm this hypothesis.

Increased ADMA concentrations prospectively determine cardiovascular and overall mortality in patients with end-stage renal disease. Since ADMA competes with L-arginine to bind to nitric-oxide synthase, supplementation with L-arginine in patients with end-stage renal disease could be useful for reduction of cardiovascular events. Findings of interventional studies suggest that acute L-arginine supplementation improves endothelial dysfunction in patients with end-stage renal disease[13] and in patients with coronary-artery disease.[27] Longer term L-arginine treatment has been shown to improve symptoms of cardiovascular disease in patients with peripheral atherosclerosis[28] and in patients with coronary-artery disease.[29] Case-control and prospective trials are needed to assess such treatment in larger groups of patients, and to assess the relation between ADMA and cardiovascular disease in the general population.

Contributors

C Zoccali and R Böger conceived the study and wrote the report. S Bode-Böger and J C Frölich were responsible for L-arginine, ADMA, and SDMA measurements and data analysis. F Mallamaci supervised data collection and management. F A Benedetto did all echo colour doppler studies. L S Malatino, I Bellanuova, and A Cataliotti did data collection and followed up patients. G Tripepi did the statistical data analysis with C Zoccali. I Fermo was responsible for homocysteine measurements. All investigators revised the report and agreed to its final form.

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