

Kent Academic Repository

Full text document (pdf)

Citation for published version

Patel, Liyona and Kilbride, Hannah and Stevens, Paul E and Eaglestone, Gillian and Knight, Sarah and Carter, Joanne and Delaney, Michael and Farmer, Christopher K. and Dalton, Neil and Lamb, Edmund (2018) Symmetric dimethylarginine (SDMA) is a stronger predictor of mortality risk than asymmetric dimethylarginine (ADMA) amongst older people with kidney disease. *Annals*

DOI

<https://doi.org/10.1177/0004563218822655>

Link to record in KAR

<https://kar.kent.ac.uk/70070/>

Document Version

Author's Accepted Manuscript

Copyright & reuse

Content in the Kent Academic Repository is made available for research purposes. Unless otherwise stated all content is protected by copyright and in the absence of an open licence (eg Creative Commons), permissions for further reuse of content should be sought from the publisher, author or other copyright holder.

Versions of research

The version in the Kent Academic Repository may differ from the final published version.

Users are advised to check <http://kar.kent.ac.uk> for the status of the paper. **Users should always cite the published version of record.**

Enquiries

For any further enquiries regarding the licence status of this document, please contact:

researchsupport@kent.ac.uk

If you believe this document infringes copyright then please contact the KAR admin team with the take-down information provided at <http://kar.kent.ac.uk/contact.html>

Annals of Clinical Biochemistry

Symmetric dimethylarginine (SDMA) is a stronger predictor of mortality risk than asymmetric dimethylarginine (ADMA) amongst older people with kidney disease

Journal:	<i>Annals of Clinical Biochemistry</i>
Manuscript ID	ACB-18-185.R1
Manuscript Type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Patel, Liyona; East Kent Hospitals NHS Trust, Clinical Biochemistry Kilbride, Hannah; East Kent Hospitals University NHS Foundation Trust, Renal Medicine Stevens, Paul; Kent and Canterbury Hospital, Renal Medicine Eaglestone, Gillian; East Kent Hospitals University NHS Foundation Trust, Renal Medicine Knight, Sarah; East Kent Hospitals University NHS Foundation Trust, Renal Medicine Carter, Joanne; East Kent Hospitals NHS Trust, Clinical Biochemistry Delaney, Michael; East Kent Hospitals University NHS Foundation Trust, Renal Medicine Farmer, Christopher; East Kent Hospitals University NHS Foundation Trust, Renal Medicine Dalton, R Neil; Evelina London Children's Healthcare Lamb, Edmund; East Kent Hospitals NHS Trust, Clinical Biochemistry;
Keywords:	Renal disease < Clinical studies

SCHOLARONE™
Manuscripts

1
2
3 **Symmetric dimethylarginine (SDMA) is a stronger predictor of mortality risk**
4 **than asymmetric dimethylarginine (ADMA) amongst older people with kidney**
5 **disease**
6
7

8
9
10 Liyona Patel MSc,¹ Hannah S Kilbride BSc MRCP,¹ Paul E Stevens BSc FRCP,¹
11 Gillian Eaglestone BSc,¹ Sarah Knight RGN,¹ Joanne L Carter MSc PhD,¹ Michael P
12 Delaney BSc MD FRCP LLM,¹ Christopher KT Farmer MD FRCP,¹ R Neil Dalton
13 PhD,² Edmund J Lamb PhD FRCPATH.¹
14
15

16
17 ¹East Kent Hospitals University NHS Foundation Trust, Canterbury, Kent, UK and
18

19 ²The Wellchild Laboratory, Evelina London Childrens Hospital, London, UK
20
21

22 Address correspondence to: Dr Edmund Lamb, Consultant Clinical Scientist, Clinical
23 Biochemistry, East Kent Hospitals University NHS Foundation Trust, Kent and
24 Canterbury Hospital, Canterbury, Kent, UK, CT1 3NG. Telephone: 01227 766877,
25 Facsimile: 01227 783077, E-mail: elamb@nhs.net
26
27
28

29
30 Word count (main text): 2560 (excluding abstract, acknowledgements, references,
31 legends)

32
33 Abstract word count: 247

34
35 References: 42

36
37 Short title: Dimethylarginines in older people
38

39 **Declarations/Conflict of interest statement**
40

41 None declared.
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

Background

Circulating asymmetric (ADMA) and symmetric dimethylarginine (SDMA) are increased in patients with kidney disease. SDMA is considered a good marker of glomerular filtration rate (GFR) whilst ADMA is a marker of cardiovascular risk. However, a link between SDMA and all-cause mortality has been reported. In the present study we evaluated both dimethylarginines as risk and GFR markers in a cohort of elderly white individuals, both with and without CKD.

Methods

GFR was measured in 394 individuals aged >74 years using an iohexol clearance method. Plasma ADMA, SDMA and iohexol were measured simultaneously using isotope dilution tandem mass spectrometry.

Results

Plasma ADMA concentrations were increased ($P<0.01$) in people with GFR <60 mL/min/1.73 m² compared to those with GFR ≥ 60 mL/min/1.73 m², but did not differ ($P>0.05$) between those with GFR 30-59 mL/min/1.73 m² and <30 mL/min/1.73 m². Plasma SDMA increased consistently across declining GFR categories ($P<0.0001$). GFR had an independent effect on plasma ADMA concentration whilst GFR, gender, body mass index and haemoglobin had independent effects on plasma SDMA concentration. Participants were followed for a median of 33 months. There were 65 deaths. High plasma ADMA ($P=0.0412$) and SDMA ($P<0.0001$) concentrations were independently associated with reduced survival.

Conclusions

Amongst elderly white individuals with a range of kidney function, SDMA was a better marker of GFR and a stronger predictor of outcome than ADMA. Future studies should further evaluate the role of SDMA as a marker of outcome and assess its potential value as a marker of GFR.

Key words: ADMA, dimethylarginines, kidney disease, older people, SDMA

Introduction

Dimethylarginines are produced in all nucleated cells as a result of methylation of arginine residues in proteins and subsequent release of free methylarginines following proteolysis.¹ It is known that both asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are increased in the blood of patients with kidney failure.^{2, 3} ADMA has structural similarity to the amino acid L-arginine and acts as an endogenous inhibitor of nitric oxide synthesis: SDMA does not have this property.² Consequently increased concentrations of ADMA have been associated with oxidative stress, inflammation, fibrogenesis and endothelial dysfunction and may contribute to both cardiovascular risk^{4, 5} and chronic kidney disease (CKD) progression.⁶⁻¹⁰ Given the strong association between cardiovascular disease and CKD¹¹⁻¹³ there is interest in the use of ADMA as both a risk marker and promoter of cardiovascular disease progression in this setting. It is generally held that SDMA, which is mainly eliminated from the body by renal excretion,¹⁴ is a good marker of glomerular filtration rate (GFR) whilst ADMA is a good marker of cardiovascular risk. However, studies have also shown a strong and independent link between SDMA, all-cause mortality, and cardiovascular events.¹⁵ In the present study we have evaluated both dimethylarginines as risk and GFR markers in a large cohort of elderly white individuals, both with and without CKD, and in whom GFR has been characterised using a reference technique.

Materials and Methods

The study included a cohort of 394 white people aged 74 years and above as previously described.¹⁶ All subjects gave informed consent. The study took place in East Kent, a semirural area of Southern England.

GFR was measured using an iohexol clearance method as previously described.¹⁶ Briefly, following intravenous injection of a 5 mL bolus of Omnipaque 240 (518 g/L iohexol corresponding to 240 g/L of iodine, GE Healthcare www.gelifesciences.com) lithium heparin blood samples were taken before and at 5, 120, 180 and 240 minutes after injection.

Iohexol GFR was calculated using a single compartment model:

$$\text{GFR (mL/min)} = 0.693 \times \text{iohexol volume of distribution (L)} \times 1000 / \text{half-life of iohexol (min)}$$

1
2
3 The GFR (mL/min) was corrected for body surface area and the Brochner-Mortensen
4 correction applied.¹⁷
5
6
7

8 Plasma ADMA, SDMA, creatinine and iohexol were measured simultaneously in
9 lithium heparin plasma using a modified stable isotope dilution electrospray tandem
10 mass spectrometric method reported for creatinine¹⁸ with minor modification.¹⁶ ADMA
11 and SDMA concentrations reported here were measured in blood obtained
12 immediately prior to iohexol injection. Samples were thawed and mixed well, 50 μ L of
13 plasma was mixed with 50 μ L of deionized water containing 50 pmol of [²H₆] ADMA
14 and 50 pmol of [²H₆] SDMA and precipitated with 200 μ L of acetonitrile. The stable
15 isotopes for [²H₆] ADMA and [²H₆] SDMA, were synthesized by Department of
16 Chemistry, King's College London, London, U.K. Following mixing and centrifugation
17 for 3 min at 21800 g, the supernatants were transferred to a 96-deep-well plate.
18 Supernatant (5 μ L) was pipetted using an HTSPAL autosampler into a 250 μ L/min
19 mobile-phase stream of acetonitrile/water (50:50; v/v) with 0.025% (v/v) formic acid.
20 Chromatography was done on a Chirobiotic T 100 mm \times 2.1 mm column with a 2 cm
21 \times 4 mm guard column (Advanced Separation Technologies) and precursor/product
22 ion pairs (m/z 203.1/46.2 and 209.1/52.2 for ADMA and m/z 203.1/172.2 and
23 209.1/175.1 for SDMA) were obtained in positive-ion multiple reaction monitoring
24 method using a Sciex API4000 (Applied Biosystems). Assay standardization was
25 based on aqueous standards at 0.25, 1.0 and 5.0 μ mol/L ADMA/SDMA stored at
26 -80° C. For the internal quality control, pooled and spiked plasma samples were
27 used. Intra-assay coefficients of variation were 2.1% at a concentration of 370 nmol/L
28 for plasma ADMA and 3.5% at a concentration of 440 nmol/L for plasma SDMA.
29 Results were calculated using Analyst version 1.4.1.¹⁹
30
31
32
33
34
35
36
37
38
39
40
41
42
43

44 Statistical analysis was performed using Analyse-it™ (Analyse-it™ Software, Ltd,
45 Leeds, U.K.), InStat® (GraphPad® Software Inc, San Diego, USA) and StatsDirect
46 (StatsDirect Ltd, Cheshire, UK). A *P* value of <0.05 was considered statistically
47 significant. Most data, except haemoglobin, were not normally distributed (*P*<0.001,
48 Shapiro-Wilk test) and all concentrations were expressed as median and interquartile
49 range. Data were studied across GFR groups defined as ≥ 60 , 30-59 and <30
50 mL/min/1.73 m². The Mann-Whitney U-test was used to compare data between two
51 groups and the Kruskal-Wallis test (non-parametric analysis of variance (ANOVA)) to
52 detect trends across more than two groups. Dunn's multiple comparison test was
53 used to undertake pairwise comparisons if a significant effect was observed.
54 Categorical variables were analysed using chi-squared test for trend.
55
56
57
58
59
60

1
2
3
4
5 Spearman rank analysis was used to test for univariate relationships between plasma
6 ADMA and SDMA concentrations and other clinical variables including age, body
7 mass index (BMI), mean arterial blood pressure (MABP), haemoglobin, GFR and
8 plasma creatinine. Multiple linear regression analysis was used to assess the
9 independent effect of clinical variables (age, gender, BMI, MABP, GFR, number of
10 medications, haemoglobin, presence of vascular disease, hypertension and smoking
11 status) on plasma ADMA and SDMA concentrations. Manual backward elimination
12 was performed; clinical variables that were not significant ($P>0.05$) were eliminated
13 from the analysis. Multicollinearity was not detected in any models used.
14
15
16
17
18
19

20 Survival analysis (all-cause mortality) was studied using the Kaplan-Meier method.
21 Significance between risk stratification groups (plasma ADMA and SDMA
22 concentration above and below the median value) was determined using the
23 Wilcoxon log-rank statistical test. Cox proportional hazard ratio was used to
24 determine the association of variables with the risk of all cause death. Unadjusted
25 hazard ratios (HRs) and the 95% confidence interval were calculated for plasma
26 ADMA and SDMA concentration, age, gender, BMI, MABP, GFR, number of
27 medications, haemoglobin concentration, diabetes mellitus, smoking status and
28 hypertension. HRs and 95% confidence intervals were expressed per 1–SD higher
29 value of each variable for continuous variables. Cox regression analysis was
30 performed with adjustment for the significant variables. Manual backward elimination
31 was performed; clinical variables that were not significant ($P>0.05$) were excluded
32 from the analysis. Multicollinearity was not detected in any models used.
33
34
35
36
37
38
39
40
41
42

43 **Results**

44
45
46 The East Kent cohort has been described previously.¹⁶ Briefly, subjects ranged in
47 age from 74 to 97 years and were exclusively white. Approximately equal numbers of
48 men and women were included. Characteristics overall and by GFR category are
49 summarised in Table 1. Age and number of medications increased and haemoglobin
50 concentration decreased with declining GFR. The prevalence of vascular disease,
51 diabetes mellitus and hypertension increased with declining GFR.
52
53
54
55
56

57 Plasma ADMA concentrations were increased ($P<0.01$) in people with GFR <60
58 mL/min/1.73 m² compared to those with GFR ≥ 60 mL/min/1.73 m², but did not differ
59 ($P>0.05$) between GFR category 30-59 mL/min/1.73 m² and <30 mL/min/1.73 m².
60

1
2
3 Conversely plasma SDMA increased consistently across declining GFR categories
4 ($P<0.0001$)(Table 1). In univariate analyses both plasma ADMA and SDMA
5 concentrations increased with declining GFR (Table 2, Figure 1). Plasma SDMA but
6 not ADMA concentration was positively correlated ($P<0.0001$) with age (Table 2).
7 Plasma SDMA concentration (median, interquartile range) was significantly higher
8 ($P<0.0001$) in males (762, 627 to 1033 nmol/L) than in females (617, 513 to 823
9 nmol/L): plasma ADMA concentration did not differ between genders ($P>0.05$).
10
11
12
13
14

15
16 GFR was the only variable which had an independent effect on plasma ADMA
17 concentration. The fit (R^2) of the model including GFR as a variable was 0.20. GFR,
18 gender, BMI and haemoglobin had independent effects on plasma SDMA
19 concentration. The overall fit (R^2) of the model including these three variables was
20 0.69 ($P<0.0001$)(Table 3).
21
22
23
24

25 All participants were followed up for a median (interquartile range) period of 33 (26-
26 43) months. During the follow up period, 65 people died. Seventy-two percent of the
27 individuals that died had plasma ADMA concentrations greater than or equal to the
28 median plasma ADMA concentration (≥ 543 nmol/L). When the end point of the follow
29 up period was considered as 48 months the survival chances for people with plasma
30 ADMA concentrations ≥ 543 nmol/L compared to <543 nmol/L were 68% and 89%
31 respectively ($P=0.0009$) (Figure 2). The median plasma SDMA concentration was
32 680 nmol/L: 83% of the individuals that died had plasma SDMA concentrations ≥ 680
33 nmol/L. When the end point of the follow up period was considered as 48 months the
34 survival chances for people with plasma SDMA concentrations ≥ 680 nmol/L
35 compared to <680 nmol/L were 65% and 93% respectively ($P<0.0001$)(Figure 2).
36
37
38
39
40
41
42
43

44 Cox proportional hazard ratios were calculated to determine the significance of
45 variables as predictors of all cause death. In unadjusted analyses ADMA, SDMA,
46 age, GFR, number of medications, haemoglobin, presence of vascular disease and
47 smoking status were significant predictors (Table 4). Manual backward elimination
48 with stepwise elimination of insignificant variables was undertaken in two separate
49 models including ADMA or SDMA. In the final ADMA model age, GFR, presence of
50 vascular disease and smoking status in addition to plasma ADMA concentration
51 remained significant independent predictors of all cause death. In the final SDMA
52 model age and smoking status in addition to plasma SDMA concentration remained
53 significant (Table 4).
54
55
56
57
58
59
60

Discussion

To our knowledge this is the first study to evaluate ADMA and SDMA as markers of both GFR and outcome in an exclusively elderly cohort across a range of kidney function in conjunction with a reference GFR measurement. In this study both ADMA and SDMA were inversely related to GFR. This relationship was stronger for SDMA than ADMA. Whilst ADMA concentration was influenced by GFR only, SDMA concentration was also affected by gender, BMI and haemoglobin concentration. ADMA predicted mortality risk, but this effect only just achieved significance with age, GFR, presence of vascular disease and smoking history all contributing strongly and independently to risk. Conversely, SDMA was a highly significant predictor of death in this cohort, with age and smoking history but not GFR contributing to risk in this model.

The strength of the relationship we observed between GFR and ADMA ($r_s -0.42$) is broadly similar to that observed in other cohorts ($r -0.24$,⁹ $r -0.29$,⁵ $r -0.26$ ²⁰), and not of sufficient strength to suggest that ADMA could be a useful marker on its own of GFR. Whilst SDMA is mainly eliminated through the kidneys,¹⁴ ADMA is mainly metabolised through enzymatic degradation in both the liver and kidney involving dimethylarginine dimethylaminohydrolase (DDAH). Consequently the relationship between ADMA and GFR may be confounded by hepatic function.²¹⁻²³ In a retrospective analysis of participants in the Modification of Diet in Renal Disease Study, Young et al also found GFR to be the only variable tested to be significantly associated with ADMA concentration, but their model only explained 5% of the variability in ADMA concentration.²⁴

There has been extensive study of the relationship between SDMA and GFR.²⁵ SDMA shows some of the properties of an ideal glomerular filtration marker, including being produced at a constant rate and being almost completely eliminated from the body by renal excretion.¹⁴ The strong relationship we have observed between SDMA and GFR ($r_s -0.87$) is consistent with that from other studies,^{20, 25, 26} particularly those that also used a reference GFR technique where observed correlation coefficients ranged from 0.78 to 0.90.²⁵ The SDMA-GFR relationship was also similar to that we observed between serum creatinine and GFR ($r_s -0.87$) and for the proposed GFR marker cystatin C ($r_s -0.90$, data not shown) in this group. SDMA has also been shown to be an early and sensitive marker of abrupt change in kidney function following kidney donation.²⁷ In addition to being related to GFR,

1
2
3 plasma SDMA concentration is also strongly affected by gender, raising the
4 possibility that GFR prediction from SDMA could be improved by models that also
5 take gender into account.
6
7

8
9 Given the clear pathophysiological construct linking ADMA and vascular dysfunction,
10 many studies have addressed the relationship between increased circulating ADMA
11 concentration and mortality risk across a variety of populations,^{22, 28-30} including
12 amongst older people.³¹ In the setting of kidney disease, an early study in dialysis
13 patients showed that ADMA, but not SDMA, predicted mortality.³² In several cohorts
14 of CKD patients, and amongst renal transplant recipients,³³ high ADMA concentration
15 was an independent predictor of all-cause mortality.^{5, 9} Conversely, in the study of
16 Young et al amongst patients with moderate to severe CKD, ADMA did not reach
17 significance as an independent predictor of all-cause mortality, although it was a
18 significant predictor of cardiovascular mortality.²⁴
19
20
21
22
23
24
25

26
27 Fewer studies have explored the relationship between SDMA and mortality risk but
28 increasing evidence suggests a role. In a German study of individuals receiving
29 coronary angiography following an ischaemic event, SDMA demonstrated a J-shaped
30 relationship with all-cause and cardiovascular mortality whereas the ADMA-mortality
31 relationship was more linear.²⁶ SDMA, but not ADMA, was independently associated
32 with survival after stroke,³⁴ non-ST elevation myocardial infarction²⁰ and amongst
33 individuals admitted to an intensive care unit.³⁵
34
35
36
37
38

39 Part of the association between SDMA and risk of mortality may reflect the strong
40 relationship between GFR itself and risk. Indeed, the strong relationship between
41 SDMA and GFR probably explains why only one of these factors remained significant
42 in our adjusted model. A relationship between SDMA and mortality independent of
43 GFR has also been reported in renal transplant recipients.³⁶ Recent evidence
44 suggests potential direct pathophysiological links between SDMA and cardiovascular
45 disease through indirect inhibition of nitric oxide synthesis.¹⁵ The proposed
46 mechanism is through competition with L-arginine for transport, hence limiting the
47 availability of L-arginine to nitric oxide synthase.³⁷ In turn, reactive oxygen species
48 may further promote intracellular ADMA formation.³⁸ Dose-dependent inhibition of
49 nitric oxide synthesis by SDMA has been observed in cultured endothelial cells,
50 coupled with increased production of reactive oxygen species.¹⁵ In vitro SDMA, but
51 not ADMA, was shown to cause release of proinflammatory cytokines (interleukin-6,
52 IL-6; tumour necrosis factor- α , TNF- α) from monocytes: release of cytokines was
53
54
55
56
57
58
59
60

1
2
3 linked to activation of nuclear factor-Kappa B.³⁹ In vivo, plasma SDMA concentration
4 was correlated to IL-6 and TNF- α concentrations amongst patients with CKD, with
5 much weaker relationships observed for ADMA and cytokine concentrations.³⁹
6 SDMA, but not ADMA, concentrations have previously been observed to be
7 independently increased in patients with coronary vascular disease and related to
8 extent of such disease.¹⁵ There is therefore increasing evidence suggesting that
9 SDMA could contribute to endothelial dysfunction and the chronic inflammatory state
10 characteristic of CKD, with attendant increase in cardiovascular risk.
11
12
13
14
15
16

17 The strengths of this study include the simultaneous measurement of ADMA and
18 SDMA across a well-characterised, large number of subjects covering a spectrum of
19 kidney disease assessed using a reference GFR technique. The study has some
20 limitations. Only single baseline measures of the dimethylarginines were available.
21 Only all-cause mortality data was collected: pathophysiologically one would
22 anticipate the role of dimethylarginines to be more closely linked to cardiovascular
23 mortality. However, although cardiovascular disease is the major cause of mortality
24 amongst people with CKD,¹² other pathology including malignancy and infection⁴⁰
25 could be contributing to the relationship between mortality and SDMA due to
26 increased SDMA production as a result of increased cell turnover. The study was
27 exclusively white and findings may therefore not hold in other ethnic groups.⁴¹ In
28 common with most studies in this field, plasma concentrations of dimethylarginines
29 were measured. Any effects in vivo are likely to be related to intracellular
30 dimethylarginine concentrations. Plasma concentrations of dimethylarginines may not
31 reflect their intracellular concentration, potentially obscuring their true relationship
32 with mortality.⁴²
33
34
35
36
37
38
39
40
41
42
43

44 In conclusion we have shown that amongst elderly white individuals and across a
45 range of kidney function, SDMA appears to be both a better marker of GFR and a
46 stronger predictor of outcome than ADMA. The prognostic power of SDMA may be
47 related to its more recently described roles in stimulating the pro-inflammatory state.
48 Future studies should further evaluate the role of SDMA as a marker of outcome and
49 assess its potential value as a marker of GFR.
50
51
52
53
54
55

56 **Funding**

57 This paper presents independent research commissioned by the National Institute for
58 Health Research (NIHR) under the Research for Patient Benefit Programme
59
60

1
2
3 (reference PB-PG-0107-12073). The views expressed are those of the authors and
4 not necessarily those of the NHS, the NIHR or the Department of Health.
5
6
7

8 **Ethical approval**

9 The study had full ethical approval from the East Kent Research Ethics Committee
10 (07/Q1803/37).
11
12
13

14 **Guarantor**

15 EJJ
16
17
18

19 **Contributorship**

20 All authors participated sufficiently in the work to take public responsibility for the
21 content as described in the Journal's instructions to authors.
22
23
24

25 **Acknowledgements**

26 We are grateful to the staff of Clinical Biochemistry and The Kent Kidney Care
27 Centre, East Kent Hospitals University NHS Foundation Trust for their co-operation
28 and help. Dr S O'Riordan helped with recruitment to the study and Ms J Irving helped
29 with data analysis. The study received statistical advice from Mr Paul Bassett,
30 Statsconsultancy Ltd., Amersham, Buckinghamshire, UK. The research team
31 acknowledge the support of the National Institute for Health Research (NIHR),
32 through the Comprehensive Clinical Research Network (Research for Patient Benefit
33 Programme, grant reference PB-PG-0107-12073).
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

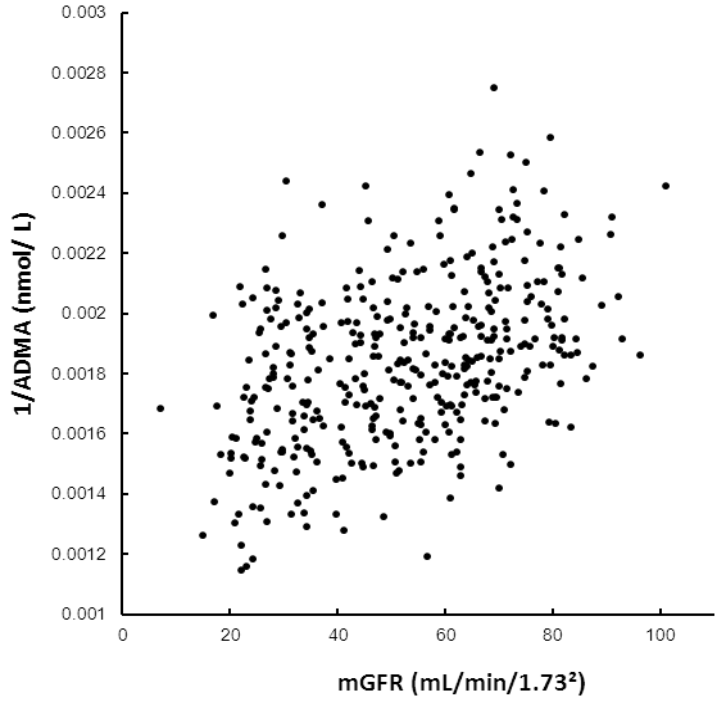
1. Teerlink T. ADMA metabolism and clearance. *Vasc Med* 2005; 10 Suppl 1: S73-81.
2. Vallance P, Leone A, Calver A, et al. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. *J Cardiovasc Pharmacol* 1992; 20 Suppl 12: S60-62.
3. Wahbi N, Dalton RN, Turner C, et al. Dimethylarginines in chronic renal failure. *J Clin Pathol* 2001; 54: 470-473.
4. Boger RH. Asymmetric dimethylarginine (ADMA) and cardiovascular disease: insights from prospective clinical trials. *Vasc Med* 2005; 10 Suppl 1: S19-25.
5. Lu TM, Chung MY, Lin CC, et al. Asymmetric dimethylarginine and clinical outcomes in chronic kidney disease. *Clin J Am Soc Nephrol* 2011; 6: 1566-1572.
6. Fliser D, Kronenberg F, Kielstein JT, et al. Asymmetric dimethylarginine and progression of chronic kidney disease: the mild to moderate kidney disease study. *J Am Soc Nephrol* 2005; 16: 2456-2461.
7. Mihout F, Shweke N, Bige N, et al. Asymmetric dimethylarginine (ADMA) induces chronic kidney disease through a mechanism involving collagen and TGF-beta1 synthesis. *J Pathol* 2011; 223: 37-45.
8. Aldamiz-Echevarria L and Andrade F. Asymmetric dimethylarginine, endothelial dysfunction and renal disease. *Int J Mol Sci* 2012; 13: 11288-11311.
9. Ravani P, Tripepi G, Malberti F, et al. Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. *J Am Soc Nephrol* 2005; 16: 2449-2455.
10. Mookerjee RP, Dalton RN, Davies NA, et al. Inflammation is an important determinant of levels of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) in acute liver failure. *Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society* 2007; 13: 400-405. 2007/02/24. DOI: 10.1002/lt.21053.
11. Baigent C, Burbury K and Wheeler D. Premature cardiovascular disease in chronic renal failure. *Lancet* 2000; 356: 147-152.
12. Foley RN, Parfrey PS and Sarnak MJ. Epidemiology of cardiovascular disease in chronic renal disease. *J Am Soc Nephrol* 1998; 9: S16-23.
13. Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure

- 1
2
3 Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation* 2003;
4 108: 2154-2169.
5
6 14. McDermott JR. Studies on the catabolism of Ng-methylarginine, Ng, Ng-
7 dimethylarginine and Ng, Ng-dimethylarginine in the rabbit. *Biochem J* 1976; 154:
8 179-184.
9
10 15. Bode-Boger SM, Scalera F, Kielstein JT, et al. Symmetrical dimethylarginine:
11 a new combined parameter for renal function and extent of coronary artery disease. *J*
12 *Am Soc Nephrol* 2006; 17: 1128-1134.
13
14 16. Kilbride HS, Stevens PE, Eaglestone G, et al. Accuracy of the MDRD
15 (Modification of Diet in Renal Disease) study and CKD-EPI (CKD Epidemiology
16 Collaboration) equations for estimation of GFR in the elderly. *Am J Kidney Dis* 2013;
17 61: 57-66.
18
19 17. Brochner-Mortensen J. A simple method for the determination of glomerular
20 filtration rate. *Scand J Clin Lab Invest* 1972; 30: 271-274.
21
22 18. Greenberg N, Roberts WL, Bachmann LM, et al. Specificity characteristics of
23 7 commercial creatinine measurement procedures by enzymatic and jaffe method
24 principles. *Clin Chem* 2012; 58: 391-401.
25
26 19. Marcovecchio ML, Widmer B, Dunger DB, et al. Effect of acute variations of
27 insulin and glucose on plasma concentrations of asymmetric dimethylarginine in
28 young people with Type 1 diabetes. *Clin Sci (Lond)* 2008; 115: 361-369.
29
30 20. Cavalca V, Veglia F, Squellerio I, et al. Circulating levels of dimethylarginines,
31 chronic kidney disease and long-term clinical outcome in non-ST-elevation
32 myocardial infarction. *PLoS One* 2012; 7: e48499.
33
34 21. Pilz S, Putz-Bankuti C, Meinitzer A, et al. Association of homoarginine and
35 methylarginines with liver dysfunction and mortality in chronic liver disease. *Amino*
36 *Acids* 2015; 47: 1817-1826. 2015/05/09. DOI: 10.1007/s00726-015-2000-7.
37
38 22. Koch A, Weiskirchen R, Kunze J, et al. Elevated asymmetric dimethylarginine
39 levels predict short- and long-term mortality risk in critically ill patients. *J Crit Care*
40 2013; 28: 947-953. 2013/08/21. DOI: 10.1016/j.jcrc.2013.05.016.
41
42 23. Czarnecka A, Milewski K and Zielinska M. Asymmetric dimethylarginine and
43 hepatic encephalopathy: cause, effect or association? *Neurochem Res* 2017; 42:
44 750-761. 2016/11/26. DOI: 10.1007/s11064-016-2111-x.
45
46 24. Young JM, Terrin N, Wang X, et al. Asymmetric dimethylarginine and
47 mortality in stages 3 to 4 chronic kidney disease. *Clin J Am Soc Nephrol* 2009; 4:
48 1115-1120.
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 25. Kielstein JT, Salpeter SR, Bode-Boeger SM, et al. Symmetric
4 dimethylarginine (SDMA) as endogenous marker of renal function--a meta-analysis.
5 *Nephrol Dial Transplant* 2006; 21: 2446-2451.
6
7
- 8 26. Meinitzer A, Kielstein JT, Pilz S, et al. Symmetrical and asymmetrical
9 dimethylarginine as predictors for mortality in patients referred for coronary
10 angiography: the Ludwigshafen Risk and Cardiovascular Health study. *Clin Chem*
11 2011; 57: 112-121.
12
13
- 14 27. Kielstein JT, Veldink H, Martens-Lobenhoffer J, et al. SDMA is an early
15 marker of change in GFR after living-related kidney donation. *Nephrol Dial*
16 *Transplant* 2011; 26: 324-328.
17
18
- 19 28. Meinitzer A, Seelhorst U, Wellnitz B, et al. Asymmetrical dimethylarginine
20 independently predicts total and cardiovascular mortality in individuals with
21 angiographic coronary artery disease (the Ludwigshafen Risk and Cardiovascular
22 Health study). *Clin Chem* 2007; 53: 273-283.
23
24
- 25 29. Lu TM, Chung MY, Lin MW, et al. Plasma asymmetric dimethylarginine
26 predicts death and major adverse cardiovascular events in individuals referred for
27 coronary angiography. *Int J Cardiol* 2011; 153: 135-140.
28
29
- 30 30. Boger RH, Maas R, Schulze F, et al. Asymmetric dimethylarginine (ADMA) as
31 a prospective marker of cardiovascular disease and mortality--an update on patient
32 populations with a wide range of cardiovascular risk. *Pharmacol Res* 2009; 60: 481-
33 487. 2009/07/15. DOI: 10.1016/j.phrs.2009.07.001.
34
35
- 36 31. Pizzarelli F, Maas R, Dattolo P, et al. Asymmetric dimethylarginine predicts
37 survival in the elderly. *Age (Dordr)* 2013; 35: 2465-2475. 2013/04/16. DOI:
38 10.1007/s11357-013-9523-1.
39
40
- 41 32. Zoccali C, Bode-Boger S, Mallamaci F, et al. Plasma concentration of
42 asymmetrical dimethylarginine and mortality in patients with end-stage renal disease:
43 a prospective study. *Lancet* 2001; 358: 2113-2117.
44
45
- 46 33. Frenay AR, van den Berg E, de Borst MH, et al. Plasma ADMA associates
47 with all-cause mortality in renal transplant recipients. *Amino Acids* 2015; 47: 1941-
48 1949. 2015/06/17. DOI: 10.1007/s00726-015-2023-0.
49
50
- 51 34. Schulze F, Carter AM, Schwedhelm E, et al. Symmetric dimethylarginine
52 predicts all-cause mortality following ischemic stroke. *Atherosclerosis* 2010; 208:
53 518-523.
54
- 55 35. Koch A, Weiskirchen R, Bruensing J, et al. Regulation and prognostic
56 relevance of symmetric dimethylarginine serum concentrations in critical illness and
57 sepsis. *Mediators Inflamm* 2013; 2013: 413826.
58
59
60

- 1
2
3 36. Pihlstrom H, Mjoen G, Dahle DO, et al. Symmetric dimethylarginine as
4 predictor of graft loss and all-cause mortality in renal transplant recipients.
5
6 *Transplantation* 2014; 98: 1219-1225. 2014/07/08. DOI:
7
8 10.1097/TP.0000000000000205.
- 9 37. Closs EI, Basha FZ, Habermeier A, et al. Interference of L-arginine analogues
10 with L-arginine transport mediated by the y+ carrier hCAT-2B. *Nitric Oxide* 1997; 1:
11
12 65-73.
- 13 38. Wilcox CS. Asymmetric dimethylarginine and reactive oxygen species:
14 unwelcome twin visitors to the cardiovascular and kidney disease tables.
15
16 *Hypertension* 2012; 59: 375-381.
- 17 39. Schepers E, Barreto DV, Liabeuf S, et al. Symmetric dimethylarginine as a
18 proinflammatory agent in chronic kidney disease. *Clin J Am Soc Nephrol* 2011; 6:
19
20 2374-2383.
- 21 40. Mortensen KM, Itenov TS, Haase N, et al. High levels of methylarginines
22 were associated with increased mortality in patients with severe sepsis. *Shock* 2016;
23
24 46: 365-372. 2016/05/21. DOI: 10.1097/SHK.0000000000000649.
- 25 41. Schutte AE, Schutte R, Huisman HW, et al. Dimethylarginines: their vascular
26 and metabolic roles in Africans and Caucasians. *Eur J Endocrinol* 2010; 162: 525-
27
28 533. 2009/12/10. DOI: 10.1530/EJE-09-0865.
- 29 42. Davids M and Teerlink T. Plasma concentrations of arginine and asymmetric
30 dimethylarginine do not reflect their intracellular concentrations in peripheral blood
31
32 mononuclear cells. *Metabolism* 2013; 62: 1455-1461. 2013/07/31. DOI:
33
34 10.1016/j.metabol.2013.05.017.
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 1A. Scatter plot of 1/plasma ADMA in association with measured glomerular filtration rate (mGFR). $r_s = 0.42, p < 0.0001$



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 1B. Scatter plot of 1/plasma SDMA in association with measured glomerular filtration rate (mGFR). $r_s = 0.87$, $p < 0.0001$

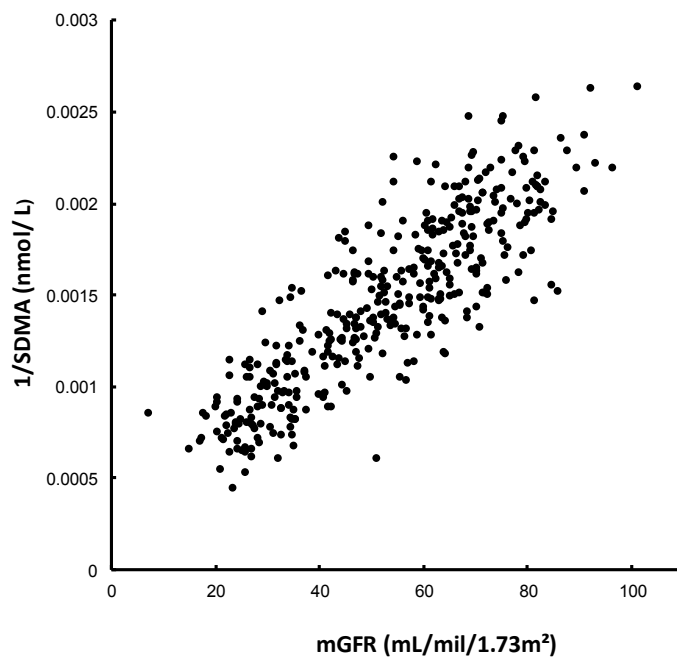


Figure 2a. Kaplan-Meier survival curve by median ADMA concentration (543 nmol/L); $P=0.0009$.

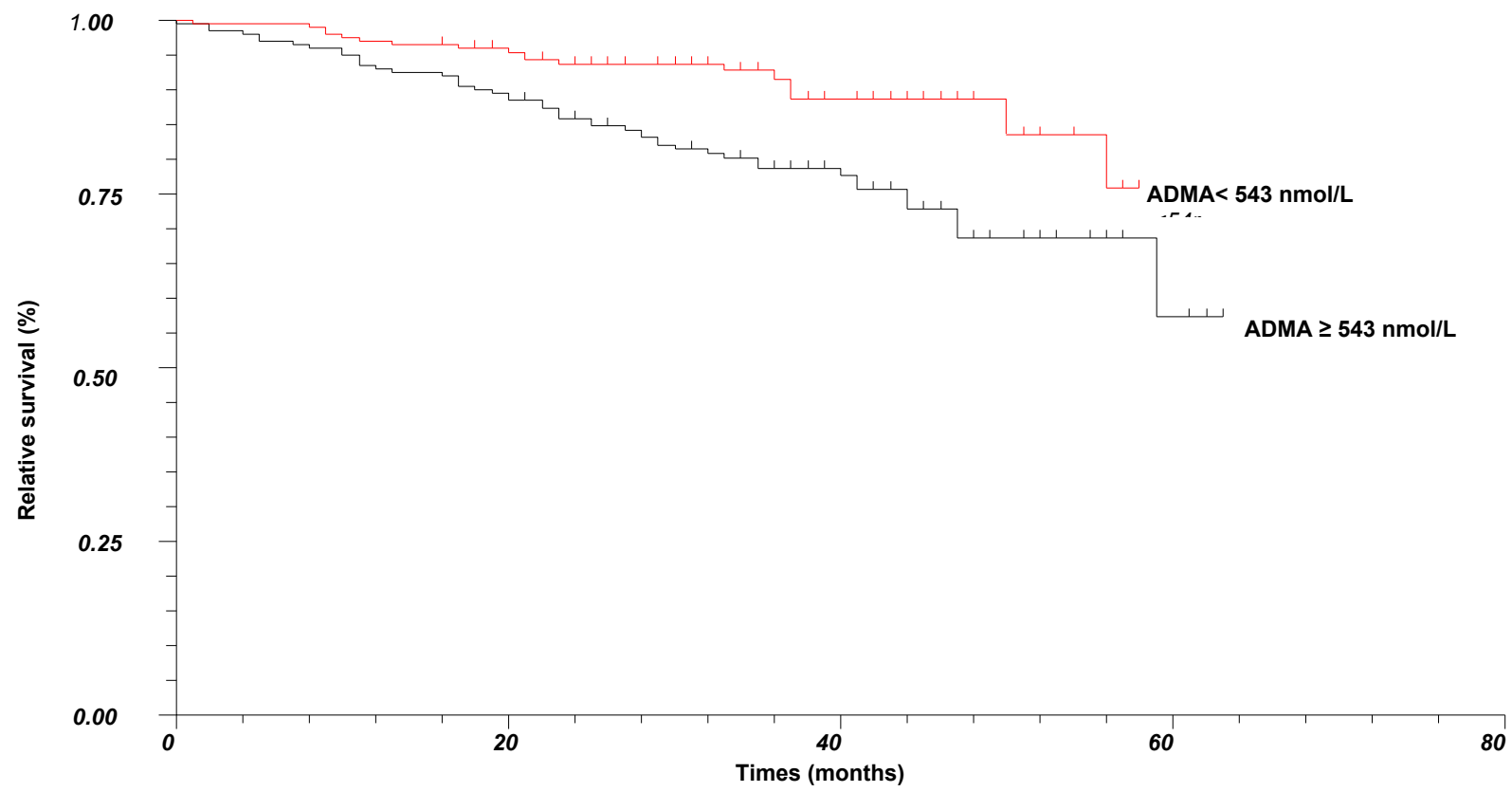


Figure 2b. Kaplan-Meier survival curve by median SDMA concentration (680 nmol/L); $P < 0.0001$.

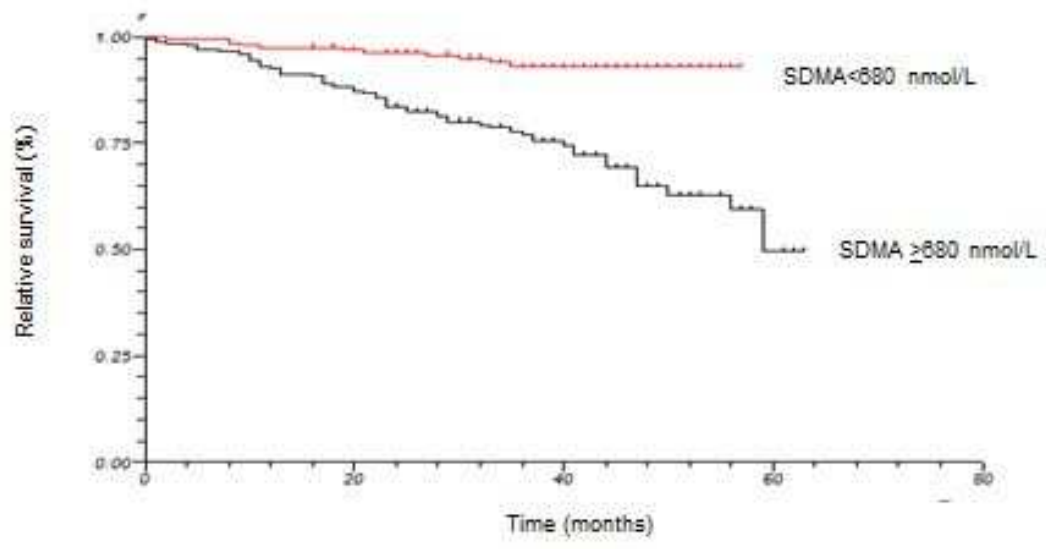


Table 1. Subject characteristics by GFR category. Values for continuous variables are expressed as median (interquartile range) unless stated otherwise. *P*<0.05 was considered significant.

	Total cohort	≥60 mL/min/1.73 m ²	30–59 mL/min/1.73 m ²	<30 mL/min/1.73 m ²	<i>P</i> for trend
n	394	163	171	60	-
Male:female, n (% male)	189:205	67:96 (41)	90:81 (53)	33:28 (55)	0.0617
Age, y	80 (77-83)	79 (76-81) ^{a, b}	80 (77-84)	81 (77-86)	<0.0001
BMI, weight(kg)/height(m) ²	26.1 (23.6-29.3)	25.8 (23.1-28.6)	26.2 (24.1-29.7)	27.3 (23.9-30.0)	0.0998
MABP, mm Hg*	96.0 (89.3-103.6)	94.3 (87.3-104.4)	96.6 (91.4-104.8)	96.3 (87.1-100.9)	0.4100
Number of medications, n	5 (3-8)	4 (1-6) ^{a, b}	6 (4-8)	7 (5-10)	<0.0001
Vascular disease, n (%)	172 (44)	49 (31) ^a	90 (53)	33 (54)	<0.0001
Diabetes, n (%)	77 (20)	21 (13) ^b	36 (21)	20 (33)	0.0032
Hypertension, n (%)	251 (55)	71 (44) ^{a, b}	104 (61)	40 (66)	0.0014
Smoker, n (%)	86 (22)	26 (16) ^a	51 (30) ^c	9 (15)	0.0034
Haemoglobin, g/L	130 (120-139)	136 (129-142) ^{a, b}	126 (117-136) ^c	118 (118-129)	<0.0001
Serum creatinine, μmol/L	95 (73-144)	71 (61-84)	111 (88-145)	191 (157-247)	-
GFR, mL/min/1.73 m ²	53 (35-68)	69 (64-78)	45 (36-52)	25 (22-27)	-
ADMA, nmol/L	543 (498- 608)	521 (472-559) ^{a, b}	565 (513-624)	595 (526-673)	<0.0001
SDMA, nmol/L	680 (542-927)	530 (480-610) ^{a, b}	775 (663-934) ^c	1220 (1067-1390)	<0.0001

*MABP data was obtained for 374 patients in total and 154, 163 and 57 patients in the ≥60, 30-59 and <30 mL/min/1.73 m² groups respectively.

Abbreviations: ADMA, asymmetric dimethylarginine; BMI, Body mass index; GFR, measured glomerular filtration rate (using iohexol); MABP, mean arterial blood pressure; SDMA, symmetric dimethylarginine;

Dunn's multiple comparison test for pairwise comparisons of the effect of GFR category on plasma ADMA, SDMA and serum cystatin C concentration are denoted as shown below.

^a *P*<0.05 for ≥60 mL/min/1.73 m² vs. 30-59 mL/min/1.73 m²

^b *P*<0.05 for ≥60 mL/min/1.73 m² vs. <30 mL/min/1.73 m²

^c *P*<0.05 for 30-59 mL/min/1.73 m² vs. <30 mL/min/1.73 m²

Table 2. Correlation (r_s) between plasma ADMA and SDMA concentrations and clinical variables.

Variable	ADMA	SDMA
Age	0.10	0.25†
BMI	0.14	0.08
MABP*	0.07	0.01
Haemoglobin	-0.20†	-0.41†
GFR	-0.42†	-0.87†
Serum creatinine	0.34†	0.87†

Note. Values expressed as correlation coefficients obtained using Spearman's rank statistic. Statistical significance is shown as † ($P < 0.0001$ in each case); the remaining correlations were not statistically significant.

Abbreviations: ADMA, asymmetric dimethylarginine; BMI, body mass index; GFR, measured glomerular filtration rate (using iohexol); MABP, mean arterial blood pressure; SDMA, symmetric dimethylarginine;

Table 3. Effect of clinical variables on plasma ADMA and SDMA concentrations. Values shown are the beta coefficients (95% confidence intervals) for the variables that remained significant in the multiple regression model.

	ADMA	SDMA
Constant	664.1 (641.5 to 686.6), <i>P</i> <0.0001	1828.1 (1654.1 to 2002.0), <i>P</i> <0.0001
Glomerular filtration rate	-2.032 (-2.436 to -1.628), <i>P</i> <0.0001	-11.779 (-12.791 to -10.768), <i>P</i> <0.0001
Haemoglobin	-	-2.723 (-3.968 to -1.478), <i>P</i> <0.0001
Gender	-	98.416 (63.281 to 133.55), <i>P</i> <0.0001
Body mass index	-	-4.907 (-8.699 to -1.116), <i>P</i> =0.0116

Table 4. Unadjusted and adjusted hazard ratios for death. For continuous variables, the hazard ratios are expressed as the increased risk associated with a one standard deviation increase (ADMA, age, BMI, MABP, medication no.) or decrease (GFR, haemoglobin). For categorical variables, the hazard ratios are expressed as risk if conditions present (vascular disease, hypertension, smoker and diabetes mellitus) or if female rather than male, compared to risk in the absence of condition.

Variable	Unadjusted hazard ratio (95% CI) per standard deviation	<i>P</i>	ADMA model: adjusted hazard ratio (95% CI) per standard deviation	<i>P</i>	SDMA model: adjusted hazard ratio (95% CI) per standard deviation	<i>P</i>
ADMA, nmol/ L	1.67 (1.32 - 2.10)	<0.0001	1.30 (1.01-1.68)	0.0412	-	-
SDMA, nmol/L	2.01 (1.66 - 2.44)	<0.0001	-	-	2.43 (1.80 – 3.28)	<0.0001
Age, years	1.65 (1.36 – 2.00)	<0.0001	1.43 (1.17 – 1.75)	0.0005	1.47 (1.20 - 1.81)	0.0003
Gender (f/m)	1.42 (0.87 - 2.32)	0.1651 ^a	-	-	-	-
BMI, weight(kg)/height(m) ²	0.95 (0.74 - 1.21)	0.6593	-	-	-	-
MABP, mmHg	0.99 (0.77 - 1.27)	0.9265	-	-	-	-
GFR, mL/min/1.73 m ²	0.44 (0.33 - 0.59)	<0.0001	0.54 (0.39 – 0.76)	0.0003	-	-
Number of medications, n	1.39 (1.11 - 1.75)	0.0045	-	-	-	-
Haemoglobin, g/L	0.63 (0.46 - 0.85)	0.0032	-	-	-	-
Vascular Disease (y/n)	2.61 (1.56 - 4.37)	0.0162^a	1.91 (1.14 – 3.21) ^a	0.0145	-	-
Diabetes (y/n)	1.42 (0.82 - 2.48)	0.2143 ^a	-	-	-	-
Smoker (y/n)	2.14 (1.30 - 3.53)	0.0029^a	1.87 (1.13 – 3.10) ^a	0.0155	1.99 (1.20 - 3.29) ^a	0.0074
Hypertension (y/n)	1.38 (0.84 - 2.29)	0.2061 ^a	-	-	-	-

Abbreviations: ADMA, asymmetric dimethylarginine; BMI, Body mass index; CI, confidence interval; GFR, glomerular filtration rate (using iohexol); MABP, mean arterial blood pressure; SDMA, symmetric dimethylarginine

^a adjusted hazard ratio (95% CI) for presence compared to absence or female versus male

Pathology Department,
Kent and Canterbury Hospital,
Ethelbert Road,
Canterbury, Kent,
CT1 3NG.
Tel: 01227 864112
Email: elamb@nhs.net

31st October 2018

Prof Maurice O’Kane,
Deputy Editor
Annals of Clinical Biochemistry

Dear Maurice,

Re: Manuscript ID ACB-18-185 entitled "Symmetric dimethylarginine (SDMA) is a stronger predictor of mortality risk than asymmetric dimethylarginine (ADMA) amongst older people with kidney disease"

Thank you for returning our manuscript. We were pleased with the generally encouraging responses from the referees.

Specifically in response to their points (our responses in bold):

Referee: 1

1. The data in table 1 are stated in to be presented as median (interquartile range), but the range in brackets in fact appears to be the absolute range, which is consistent with what is stated in the methods (page 4, final paragraph). Presentation as median (IQR) would be preferable. Similarly the data presented textually for sex differences in SDMA (page 6, first paragraph) could be presented in this way.

We apologise for this. Interquartile range data has now been included.

2. In the discussion (page 7, 2nd paragraph), the final r value presented in brackets should presumably read "r-0.26" rather than "r=0.26".

Thank you for spotting this error. It has been amended to -0.26.

3. I would like to clarify whether the SDMA model which reports an adjusted HR of 2.43 per SD is adjusted for GFR, i.e. whether it could be said that SDMA adds anything over GFR for risk prediction, or whether SDMA is merely a marker of GFR, given the strong correlation between GFR and SDMA. This possibility is indeed acknowledged by the authors in the discussion (page 8); the cited study by Pihlstrøm (36) shows an independent effect of SDMA on all-cause mortality only in quartile 4 vs quartile 1, and the SDMA concentrations in the present study are lower than those in Pihlstrøm's.

GFR was excluded from the adjusted SDMA model and we feel we have made this clear in the manuscript. In the results section we state that "In the final SDMA model age and smoking status in addition to plasma SDMA concentration remained significant (Table 4). In the Discussion we expand on this further: "Part of the association between SDMA and risk of mortality may reflect the strong relationship between GFR itself and risk. Indeed, the strong relationship between SDMA and GFR probably explains why only one of these factors remained significant in our adjusted model." What is interesting is that SDMA pushes GFR out of the model, suggesting that it is indeed closely related to GFR but is also contributing something else in terms of risk assessment.

1
2
3
4 **We do not fully understand the comment regarding the Pihlstrom et al study. Reported SDMA**
5 **concentrations will be affected by the method used: we used IDMS, Pihlstrom et al used HPLC.**
6 **Nevertheless, the results are broadly comparable e.g. Pihlstrom's Q4 (mean eGFR 35 mL/min)**
7 **had SDMA concentrations ranging from 1380 to 4410 nmol/L, compared to the group with**
8 **GFR<30 in the present study (median mGFR 25 mL/min) where SDMA concentrations ranged**
9 **from 708 to 2240 nmol/L.**
10

11 4. Data are presented for all-cause mortality. Is it possible to refine this by cause of death to see
12 whether CV death is specifically associated with SDMA/ADMA in this population? Given the
13 hypothesised effects of dimethylarginines CV death in particular would be of interest.
14

15 **We agree with the reviewer on this point but unfortunately we did not collect cause of death**
16 **data. We acknowledge this as a limitation of the study in the Discussion, whilst also discussing**
17 **that increased dimethylarginine concentrations may reflect other non-cardiac pathology that**
18 **contributes to mortality in CKD.**
19

20
21 Referee: 2
22

23 Comments to the Author

24 I was impressed with this paper. It uses clear methodology to establish a link between the molecules
25 studied and clinical outcomes. The statistics used seem appropriate. It adds to the evidence base with
26 regard to the increased risk of adverse outcomes associated with Chronic Kidney Disease. As well as
27 a marker for outcomes this may lead to potential therapeutic targets. I would have liked to have seen if
28 there was a correlation with a measure of proteinuria as a clinical variable, particularly as this is an
29 established risk factor for cardiovascular disease. Similarly I would have liked to have seen whether
30 there is a correlation between the primary renal diagnosis and ADMA and SDMA concentrations. It
31 might be worthwhile for the authors to consider adding these variables to future research but overall I
32 don't think these omissions detract from the paper.
33

34 **We are grateful to reviewer 2 for his encouraging comments. Unfortunately we are unable to**
35 **undertake the analyses he has suggested in this cohort.**
36

37
38
39 I hope that we have sufficiently addressed the points raised. I look forward to hearing from you further
40 regarding our paper.
41

42 With kind regards
43

44
45 Yours sincerely
46

47
48
49
50
51
52
53
54
55
56
57
58
59
60



52 Dr Edmund Lamb PhD FRCPATH
53 Clinical Director of Pathology and Consultant Clinical Scientist (Biochemistry)
54
55
56
57
58
59
60

1
2
3 **Symmetric dimethylarginine (SDMA) is a stronger predictor of mortality risk**
4 **than asymmetric dimethylarginine (ADMA) amongst older people with kidney**
5 **disease**
6
7

8
9
10 Liyona Patel MSc,¹ Hannah S Kilbride BSc MRCP,¹ Paul E Stevens BSc FRCP,¹
11 Gillian Eaglestone BSc,¹ Sarah Knight RGN,¹ Joanne L Carter MSc PhD,¹ Michael P
12 Delaney BSc MD FRCP LLM,¹ Christopher KT Farmer MD FRCP,¹ R Neil Dalton
13 PhD,² Edmund J Lamb PhD FRCPATH.¹
14
15

16
17 ¹East Kent Hospitals University NHS Foundation Trust, Canterbury, Kent, UK and

18
19 ²The Wellchild Laboratory, Evelina London Childrens Hospital, London, UK
20
21

22 Address correspondence to: Dr Edmund Lamb, Consultant Clinical Scientist, Clinical
23 Biochemistry, East Kent Hospitals University NHS Foundation Trust, Kent and
24 Canterbury Hospital, Canterbury, Kent, UK, CT1 3NG. Telephone: 01227 766877,
25 Facsimile: 01227 783077, E-mail: elamb@nhs.net
26
27
28

29
30 Word count (main text): 2560 (excluding abstract, acknowledgements, references,
31 legends)

32
33 Abstract word count: 247

34
35 References: 42

36
37 Short title: Dimethylarginines in older people
38

39 **Declarations/Conflict of interest statement**
40

41 None declared.
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

Background

Circulating asymmetric (ADMA) and symmetric dimethylarginine (SDMA) are increased in patients with kidney disease. SDMA is considered a good marker of glomerular filtration rate (GFR) whilst ADMA is a marker of cardiovascular risk. However, a link between SDMA and all-cause mortality has been reported. In the present study we evaluated both dimethylarginines as risk and GFR markers in a cohort of elderly white individuals, both with and without CKD.

Methods

GFR was measured in 394 individuals aged >74 years using an iohexol clearance method. Plasma ADMA, SDMA and iohexol were measured simultaneously using isotope dilution tandem mass spectrometry.

Results

Plasma ADMA concentrations were increased ($P<0.01$) in people with GFR <60 mL/min/1.73 m² compared to those with GFR ≥ 60 mL/min/1.73 m², but did not differ ($P>0.05$) between those with GFR 30-59 mL/min/1.73 m² and <30 mL/min/1.73 m². Plasma SDMA increased consistently across declining GFR categories ($P<0.0001$). GFR had an independent effect on plasma ADMA concentration whilst GFR, gender, body mass index and haemoglobin had independent effects on plasma SDMA concentration. Participants were followed for a median of 33 months. There were 65 deaths. High plasma ADMA ($P=0.0412$) and SDMA ($P<0.0001$) concentrations were independently associated with reduced survival.

Conclusions

Amongst elderly white individuals with a range of kidney function, SDMA was a better marker of GFR and a stronger predictor of outcome than ADMA. Future studies should further evaluate the role of SDMA as a marker of outcome and assess its potential value as a marker of GFR.

Key words: ADMA, dimethylarginines, kidney disease, older people, SDMA

Introduction

Dimethylarginines are produced in all nucleated cells as a result of methylation of arginine residues in proteins and subsequent release of free methylarginines following proteolysis.¹ It is known that both asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are increased in the blood of patients with kidney failure.^{2, 3} ADMA has structural similarity to the amino acid L-arginine and acts as an endogenous inhibitor of nitric oxide synthesis: SDMA does not have this property.² Consequently increased concentrations of ADMA have been associated with oxidative stress, inflammation, fibrogenesis and endothelial dysfunction and may contribute to both cardiovascular risk^{4, 5} and chronic kidney disease (CKD) progression.⁶⁻¹⁰ Given the strong association between cardiovascular disease and CKD¹¹⁻¹³ there is interest in the use of ADMA as both a risk marker and promoter of cardiovascular disease progression in this setting. It is generally held that SDMA, which is mainly eliminated from the body by renal excretion,¹⁴ is a good marker of glomerular filtration rate (GFR) whilst ADMA is a good marker of cardiovascular risk. However, studies have also shown a strong and independent link between SDMA, all-cause mortality, and cardiovascular events.¹⁵ In the present study we have evaluated both dimethylarginines as risk and GFR markers in a large cohort of elderly white individuals, both with and without CKD, and in whom GFR has been characterised using a reference technique.

Materials and Methods

The study included a cohort of 394 white people aged 74 years and above as previously described.¹⁶ All subjects gave informed consent. The study took place in East Kent, a semirural area of Southern England.

GFR was measured using an iohexol clearance method as previously described.¹⁶ Briefly, following intravenous injection of a 5 mL bolus of Omnipaque 240 (518 g/L iohexol corresponding to 240 g/L of iodine, GE Healthcare www.gelifesciences.com) lithium heparin blood samples were taken before and at 5, 120, 180 and 240 minutes after injection.

Iohexol GFR was calculated using a single compartment model:

$$\text{GFR (mL/min)} = 0.693 \times \text{iohexol volume of distribution (L)} \times 1000 / \text{half-life of iohexol (min)}$$

1
2
3 The GFR (mL/min) was corrected for body surface area and the Brochner-Mortensen
4 correction applied.¹⁷
5
6
7

8 Plasma ADMA, SDMA, creatinine and iohexol were measured simultaneously in
9 lithium heparin plasma using a modified stable isotope dilution electrospray tandem
10 mass spectrometric method reported for creatinine¹⁸ with minor modification.¹⁶ ADMA
11 and SDMA concentrations reported here were measured in blood obtained
12 immediately prior to iohexol injection. Samples were thawed and mixed well, 50 μ L of
13 plasma was mixed with 50 μ L of deionized water containing 50 pmol of [²H₆] ADMA
14 and 50 pmol of [²H₆] SDMA and precipitated with 200 μ L of acetonitrile. The stable
15 isotopes for [²H₆] ADMA and [²H₆] SDMA, were synthesized by Department of
16 Chemistry, King's College London, London, U.K. Following mixing and centrifugation
17 for 3 min at 21800 g, the supernatants were transferred to a 96-deep-well plate.
18 Supernatant (5 μ L) was pipetted using an HTSPAL autosampler into a 250 μ L/min
19 mobile-phase stream of acetonitrile/water (50:50; v/v) with 0.025% (v/v) formic acid.
20 Chromatography was done on a Chirobiotic T 100 mm \times 2.1 mm column with a 2 cm
21 \times 4 mm guard column (Advanced Separation Technologies) and precursor/product
22 ion pairs (m/z 203.1/46.2 and 209.1/52.2 for ADMA and m/z 203.1/172.2 and
23 209.1/175.1 for SDMA) were obtained in positive-ion multiple reaction monitoring
24 method using a Sciex API4000 (Applied Biosystems). Assay standardization was
25 based on aqueous standards at 0.25, 1.0 and 5.0 μ mol/L ADMA/SDMA stored at
26 -80° C. For the internal quality control, pooled and spiked plasma samples were
27 used. Intra-assay coefficients of variation were 2.1% at a concentration of 370 nmol/L
28 for plasma ADMA and 3.5% at a concentration of 440 nmol/L for plasma SDMA.
29 Results were calculated using Analyst version 1.4.1.¹⁹
30
31
32
33
34
35
36
37
38
39
40
41
42
43

44 Statistical analysis was performed using Analyse-it™ (Analyse-it™ Software, Ltd,
45 Leeds, U.K.), InStat® (GraphPad® Software Inc, San Diego, USA) and StatsDirect
46 (StatsDirect Ltd, Cheshire, UK). A *P* value of <0.05 was considered statistically
47 significant. Most data, except haemoglobin, were not normally distributed (*P*<0.001,
48 Shapiro-Wilk test) and all concentrations were expressed as median and interquartile
49 range. Data were studied across GFR groups defined as ≥ 60 , 30-59 and <30
50 mL/min/1.73 m². The Mann-Whitney U-test was used to compare data between two
51 groups and the Kruskal-Wallis test (non-parametric analysis of variance (ANOVA)) to
52 detect trends across more than two groups. Dunn's multiple comparison test was
53 used to undertake pairwise comparisons if a significant effect was observed.
54 Categorical variables were analysed using chi-squared test for trend.
55
56
57
58
59
60

1
2
3
4
5 Spearman rank analysis was used to test for univariate relationships between plasma
6 ADMA and SDMA concentrations and other clinical variables including age, body
7 mass index (BMI), mean arterial blood pressure (MABP), haemoglobin, GFR and
8 plasma creatinine. Multiple linear regression analysis was used to assess the
9 independent effect of clinical variables (age, gender, BMI, MABP, GFR, number of
10 medications, haemoglobin, presence of vascular disease, hypertension and smoking
11 status) on plasma ADMA and SDMA concentrations. Manual backward elimination
12 was performed; clinical variables that were not significant ($P>0.05$) were eliminated
13 from the analysis. Multicollinearity was not detected in any models used.
14
15
16
17
18
19

20 Survival analysis (all-cause mortality) was studied using the Kaplan-Meier method.
21 Significance between risk stratification groups (plasma ADMA and SDMA
22 concentration above and below the median value) was determined using the
23 Wilcoxon log-rank statistical test. Cox proportional hazard ratio was used to
24 determine the association of variables with the risk of all cause death. Unadjusted
25 hazard ratios (HRs) and the 95% confidence interval were calculated for plasma
26 ADMA and SDMA concentration, age, gender, BMI, MABP, GFR, number of
27 medications, haemoglobin concentration, diabetes mellitus, smoking status and
28 hypertension. HRs and 95% confidence intervals were expressed per 1–SD higher
29 value of each variable for continuous variables. Cox regression analysis was
30 performed with adjustment for the significant variables. Manual backward elimination
31 was performed; clinical variables that were not significant ($P>0.05$) were excluded
32 from the analysis. Multicollinearity was not detected in any models used.
33
34
35
36
37
38
39
40
41

42 **Results**

43
44
45 The East Kent cohort has been described previously.¹⁶ Briefly, subjects ranged in
46 age from 74 to 97 years and were exclusively white. Approximately equal numbers of
47 men and women were included. Characteristics overall and by GFR category are
48 summarised in Table 1. Age and number of medications increased and haemoglobin
49 concentration decreased with declining GFR. The prevalence of vascular disease,
50 diabetes mellitus and hypertension increased with declining GFR.
51
52
53
54
55

56 Plasma ADMA concentrations were increased ($P<0.01$) in people with GFR <60
57 mL/min/1.73 m² compared to those with GFR ≥ 60 mL/min/1.73 m², but did not differ
58 ($P>0.05$) between GFR category 30-59 mL/min/1.73 m² and <30 mL/min/1.73 m².
59
60

Conversely plasma SDMA increased consistently across declining GFR categories ($P < 0.0001$) (Table 1). In univariate analyses both plasma ADMA and SDMA concentrations increased with declining GFR (Table 2, Figure 1). Plasma SDMA but not ADMA concentration was positively correlated ($P < 0.0001$) with age (Table 2). Plasma SDMA concentration (median, interquartile range) was significantly higher ($P < 0.0001$) in males (median 762, range 379-627 to 2240-1033 nmol/L) than in females (median 617, range 104-513 to 1870-823 nmol/L); plasma ADMA concentration did not differ between genders ($P > 0.05$).

GFR was the only variable which had an independent effect on plasma ADMA concentration. The fit (R^2) of the model including GFR as a variable was 0.20. GFR, gender, BMI and haemoglobin had independent effects on plasma SDMA concentration. The overall fit (R^2) of the model including these three variables was 0.69 ($P < 0.0001$) (Table 3).

All participants were followed up for a median (interquartile range) period of 33 (26-43) months (IQR = 26-43 months). During the follow up period, 65 people died. Seventy-two percent of the individuals that died had plasma ADMA concentrations greater than or equal to the median plasma ADMA concentration (≥ 543 nmol/L). When the end point of the follow up period was considered as 48 months the survival chances for people with plasma ADMA concentrations ≥ 543 nmol/L compared to < 543 nmol/L were 68% and 89% respectively ($P = 0.0009$) (Figure 2). The median plasma SDMA concentration was 680 nmol/L: 83% of the individuals that died had plasma SDMA concentrations ≥ 680 nmol/L. When the end point of the follow up period was considered as 48 months the survival chances for people with plasma SDMA concentrations ≥ 680 nmol/L compared to < 680 nmol/L were 65% and 93% respectively ($P < 0.0001$) (Figure 2).

Cox proportional hazard ratios were calculated to determine the significance of variables as predictors of all cause death. In unadjusted analyses ADMA, SDMA, age, GFR, number of medications, haemoglobin, presence of vascular disease and smoking status were significant predictors (Table 4). Manual backward elimination with stepwise elimination of insignificant variables was undertaken in two separate models including ADMA or SDMA. In the final ADMA model age, GFR, presence of vascular disease and smoking status in addition to plasma ADMA concentration remained significant independent predictors of all cause death. In the final SDMA

1
2
3 model age and smoking status in addition to plasma SDMA concentration remained
4 significant (Table 4).
5
6
7

8 **Discussion**

9

10
11 To our knowledge this is the first study to evaluate ADMA and SDMA as markers of
12 both GFR and outcome in an exclusively elderly cohort across a range of kidney
13 function in conjunction with a reference GFR measurement. In this study both ADMA
14 and SDMA were inversely related to GFR. This relationship was stronger for SDMA
15 than ADMA. Whilst ADMA concentration was influenced by GFR only, SDMA
16 concentration was also affected by gender, BMI and haemoglobin concentration.
17 ADMA predicted mortality risk, but this effect only just achieved significance with age,
18 GFR, presence of vascular disease and smoking history all contributing strongly and
19 independently to risk. Conversely, SDMA was a highly significant predictor of death
20 in this cohort, with age and smoking history but not GFR contributing to risk in this
21 model.
22
23
24
25
26
27
28
29

30 The **strength of the** relationship we observed between GFR and ADMA (**$rs=-0.42$**) is
31 broadly similar to that observed in other cohorts ($r=0.24$,⁹ $r=0.29$,⁵ $r=-0.26$ ²⁰), and not
32 of sufficient strength to suggest that ADMA could be a useful marker on its own of
33 GFR. Whilst SDMA is mainly eliminated through the kidneys,¹⁴ ADMA is mainly
34 metabolised through enzymatic degradation in both the liver and kidney involving
35 dimethylarginine dimethylaminohydrolase (DDAH). Consequently the relationship
36 between ADMA and GFR may be confounded by hepatic function.²¹⁻²³ In a
37 retrospective analysis of participants in the Modification of Diet in Renal Disease
38 Study, Young et al also found GFR to be the only variable tested to be significantly
39 associated with ADMA concentration, but their model only explained 5% of the
40 variability in ADMA concentration.²⁴
41
42
43
44
45
46
47
48

49 There has been extensive study of the relationship between SDMA and GFR.²⁵
50 SDMA shows some of the properties of an ideal glomerular filtration marker,
51 including being produced at a constant rate and being almost completely eliminated
52 from the body by renal excretion.¹⁴ The strong relationship we have observed
53 **between SDMA and GFR** (**$rs=-0.87$**) is consistent with that from other studies,^{20, 25, 26}
54 particularly those that also used a reference GFR technique where observed
55 correlation coefficients ranged from 0.78 to 0.90.²⁵ The SDMA-GFR relationship was
56 also similar to that we observed between serum creatinine and GFR (**$rs=-0.87$**) and
57
58
59
60

1
2
3 for the proposed GFR marker cystatin C ($rs=-0.90$, data not shown) in this group.
4 SDMA has also been shown to be an early and sensitive marker of abrupt change in
5 kidney function following kidney donation.²⁷ In addition to being related to GFR,
6 plasma SDMA concentration is also strongly affected by gender, raising the
7 possibility that GFR prediction from SDMA could be improved by models that also
8 take gender into account.
9
10
11
12

13
14 Given the clear pathophysiological construct linking ADMA and vascular dysfunction,
15 many studies have addressed the relationship between increased circulating ADMA
16 concentration and mortality risk across a variety of populations,^{22, 28-30} including
17 amongst older people.³¹ In the setting of kidney disease, an early study in dialysis
18 patients showed that ADMA, but not SDMA, predicted mortality.³² In several cohorts
19 of CKD patients, and amongst renal transplant recipients,³³ high ADMA concentration
20 was an independent predictor of all-cause mortality.^{5, 9} Conversely, in the study of
21 Young et al amongst patients with moderate to severe CKD, ADMA did not reach
22 significance as an independent predictor of all-cause mortality, although it was a
23 significant predictor of cardiovascular mortality.²⁴
24
25
26
27
28
29
30

31
32 Fewer studies have explored the relationship between SDMA and mortality risk but
33 increasing evidence suggests a role. In a German study of individuals receiving
34 coronary angiography following an ischaemic event, SDMA demonstrated a J-shaped
35 relationship with all-cause and cardiovascular mortality whereas the ADMA-mortality
36 relationship was more linear.²⁶ SDMA, but not ADMA, was independently associated
37 with survival after stroke,³⁴ non-ST elevation myocardial infarction²⁰ and amongst
38 individuals admitted to an intensive care unit.³⁵
39
40
41
42
43

44 Part of the association between SDMA and risk of mortality may reflect the strong
45 relationship between GFR itself and risk. Indeed, the strong relationship between
46 SDMA and GFR probably explains why only one of these factors remained significant
47 in our adjusted model. A relationship between SDMA and mortality independent of
48 GFR has also been reported in renal transplant recipients.³⁶ Recent evidence
49 suggests potential direct pathophysiological links between SDMA and cardiovascular
50 disease through indirect inhibition of nitric oxide synthesis.¹⁵ The proposed
51 mechanism is through competition with L-arginine for transport, hence limiting the
52 availability of L-arginine to nitric oxide synthase.³⁷ In turn, reactive oxygen species
53 may further promote intracellular ADMA formation.³⁸ Dose-dependent inhibition of
54 nitric oxide synthesis by SDMA has been observed in cultured endothelial cells,
55
56
57
58
59
60

1
2
3 coupled with increased production of reactive oxygen species.¹⁵ In vitro SDMA, but
4 not ADMA, was shown to cause release of proinflammatory cytokines (interleukin-6,
5 IL-6; tumour necrosis factor- α , TNF- α) from monocytes: release of cytokines was
6 linked to activation of nuclear factor-Kappa B.³⁹ In vivo, plasma SDMA concentration
7 was correlated to IL-6 and TNF- α concentrations amongst patients with CKD, with
8 much weaker relationships observed for ADMA and cytokine concentrations.³⁹
9 SDMA, but not ADMA, concentrations have previously been observed to be
10 independently increased in patients with coronary vascular disease and related to
11 extent of such disease.¹⁵ There is therefore increasing evidence suggesting that
12 SDMA could contribute to endothelial dysfunction and the chronic inflammatory state
13 characteristic of CKD, with attendant increase in cardiovascular risk.
14
15
16
17
18
19
20
21

22 The strengths of this study include the simultaneous measurement of ADMA and
23 SDMA across a well-characterised, large number of subjects covering a spectrum of
24 kidney disease assessed using a reference GFR technique. The study has some
25 limitations. Only single baseline measures of the dimethylarginines were available.
26 Only all-cause mortality data was collected: pathophysiologically one would
27 anticipate the role of dimethylarginines to be more closely linked to cardiovascular
28 mortality. However, although cardiovascular disease is the major cause of mortality
29 amongst people with CKD,¹² other pathology including malignancy and infection⁴⁰
30 could be contributing to the relationship between mortality and SDMA due to
31 increased SDMA production as a result of increased cell turnover. The study was
32 exclusively white and findings may therefore not hold in other ethnic groups.⁴¹ In
33 common with most studies in this field, plasma concentrations of dimethylarginines
34 were measured. Any effects in vivo are likely to be related to intracellular
35 dimethylarginine concentrations. Plasma concentrations of dimethylarginines may not
36 reflect their intracellular concentration, potentially obscuring their true relationship
37 with mortality.⁴²
38
39
40
41
42
43
44
45
46
47
48

49 In conclusion we have shown that amongst elderly white individuals and across a
50 range of kidney function, SDMA appears to be both a better marker of GFR and a
51 stronger predictor of outcome than ADMA. The prognostic power of SDMA may be
52 related to its more recently described roles in stimulating the pro-inflammatory state.
53 Future studies should further evaluate the role of SDMA as a marker of outcome and
54 assess its potential value as a marker of GFR.
55
56
57
58
59
60

Funding

This paper presents independent research commissioned by the National Institute for Health Research (NIHR) under the Research for Patient Benefit Programme (reference PB-PG-0107-12073). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Ethical approval

The study had full ethical approval from the East Kent Research Ethics Committee (07/Q1803/37).

Guarantor

EJL

Contributorship

All authors participated sufficiently in the work to take public responsibility for the content as described in the Journal's instructions to authors.

Acknowledgements

We are grateful to the staff of Clinical Biochemistry and The Kent Kidney Care Centre, East Kent Hospitals University NHS Foundation Trust for their co-operation and help. Dr S O'Riordan helped with recruitment to the study and Ms J Irving helped with data analysis. The study received statistical advice from Mr Paul Bassett, Statsconsultancy Ltd., Amersham, Buckinghamshire, UK. The research team acknowledge the support of the National Institute for Health Research (NIHR), through the Comprehensive Clinical Research Network (Research for Patient Benefit Programme, grant reference PB-PG-0107-12073).

References

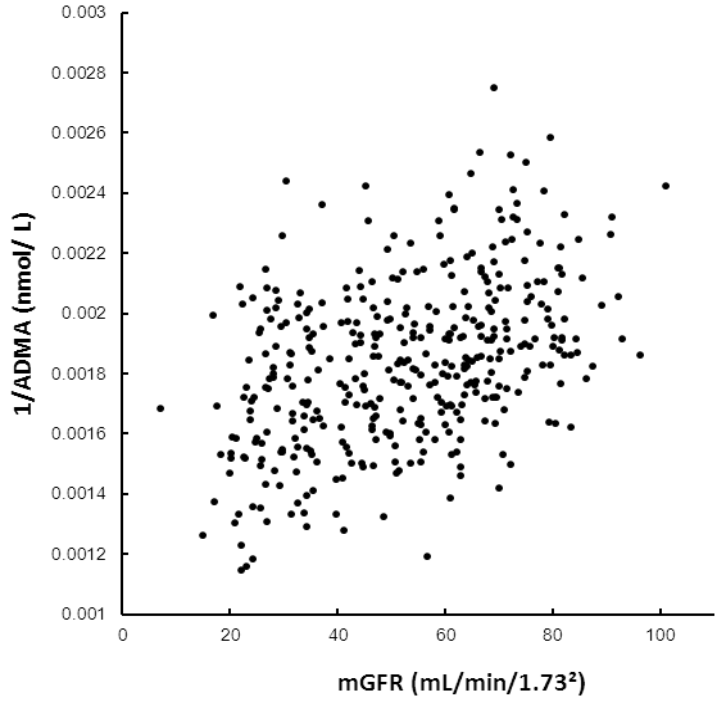
1. Teerlink T. ADMA metabolism and clearance. *Vasc Med* 2005; 10 Suppl 1: S73-81.
2. Vallance P, Leone A, Calver A, et al. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. *J Cardiovasc Pharmacol* 1992; 20 Suppl 12: S60-62.
3. Wahbi N, Dalton RN, Turner C, et al. Dimethylarginines in chronic renal failure. *J Clin Pathol* 2001; 54: 470-473.
4. Boger RH. Asymmetric dimethylarginine (ADMA) and cardiovascular disease: insights from prospective clinical trials. *Vasc Med* 2005; 10 Suppl 1: S19-25.
5. Lu TM, Chung MY, Lin CC, et al. Asymmetric dimethylarginine and clinical outcomes in chronic kidney disease. *Clin J Am Soc Nephrol* 2011; 6: 1566-1572.
6. Fliser D, Kronenberg F, Kielstein JT, et al. Asymmetric dimethylarginine and progression of chronic kidney disease: the mild to moderate kidney disease study. *J Am Soc Nephrol* 2005; 16: 2456-2461.
7. Mihout F, Shweke N, Bige N, et al. Asymmetric dimethylarginine (ADMA) induces chronic kidney disease through a mechanism involving collagen and TGF-beta1 synthesis. *J Pathol* 2011; 223: 37-45.
8. Aldamiz-Echevarria L and Andrade F. Asymmetric dimethylarginine, endothelial dysfunction and renal disease. *Int J Mol Sci* 2012; 13: 11288-11311.
9. Ravani P, Tripepi G, Malberti F, et al. Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. *J Am Soc Nephrol* 2005; 16: 2449-2455.
10. Mookerjee RP, Dalton RN, Davies NA, et al. Inflammation is an important determinant of levels of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) in acute liver failure. *Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society* 2007; 13: 400-405. 2007/02/24. DOI: 10.1002/lt.21053.
11. Baigent C, Burbury K and Wheeler D. Premature cardiovascular disease in chronic renal failure. *Lancet* 2000; 356: 147-152.
12. Foley RN, Parfrey PS and Sarnak MJ. Epidemiology of cardiovascular disease in chronic renal disease. *J Am Soc Nephrol* 1998; 9: S16-23.
13. Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure

- 1
2
3 Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation* 2003;
4 108: 2154-2169.
5
6 14. McDermott JR. Studies on the catabolism of Ng-methylarginine, Ng, Ng-
7 dimethylarginine and Ng, Ng-dimethylarginine in the rabbit. *Biochem J* 1976; 154:
8 179-184.
9
10 15. Bode-Boger SM, Scalera F, Kielstein JT, et al. Symmetrical dimethylarginine:
11 a new combined parameter for renal function and extent of coronary artery disease. *J*
12 *Am Soc Nephrol* 2006; 17: 1128-1134.
13
14 16. Kilbride HS, Stevens PE, Eaglestone G, et al. Accuracy of the MDRD
15 (Modification of Diet in Renal Disease) study and CKD-EPI (CKD Epidemiology
16 Collaboration) equations for estimation of GFR in the elderly. *Am J Kidney Dis* 2013;
17 61: 57-66.
18
19 17. Brochner-Mortensen J. A simple method for the determination of glomerular
20 filtration rate. *Scand J Clin Lab Invest* 1972; 30: 271-274.
21
22 18. Greenberg N, Roberts WL, Bachmann LM, et al. Specificity characteristics of
23 7 commercial creatinine measurement procedures by enzymatic and jaffe method
24 principles. *Clin Chem* 2012; 58: 391-401.
25
26 19. Marcovecchio ML, Widmer B, Dunger DB, et al. Effect of acute variations of
27 insulin and glucose on plasma concentrations of asymmetric dimethylarginine in
28 young people with Type 1 diabetes. *Clin Sci (Lond)* 2008; 115: 361-369.
29
30 20. Cavalca V, Veglia F, Squellerio I, et al. Circulating levels of dimethylarginines,
31 chronic kidney disease and long-term clinical outcome in non-ST-elevation
32 myocardial infarction. *PLoS One* 2012; 7: e48499.
33
34 21. Pilz S, Putz-Bankuti C, Meinitzer A, et al. Association of homoarginine and
35 methylarginines with liver dysfunction and mortality in chronic liver disease. *Amino*
36 *Acids* 2015; 47: 1817-1826. 2015/05/09. DOI: 10.1007/s00726-015-2000-7.
37
38 22. Koch A, Weiskirchen R, Kunze J, et al. Elevated asymmetric dimethylarginine
39 levels predict short- and long-term mortality risk in critically ill patients. *J Crit Care*
40 2013; 28: 947-953. 2013/08/21. DOI: 10.1016/j.jcrc.2013.05.016.
41
42 23. Czarnecka A, Milewski K and Zielinska M. Asymmetric dimethylarginine and
43 hepatic encephalopathy: cause, effect or association? *Neurochem Res* 2017; 42:
44 750-761. 2016/11/26. DOI: 10.1007/s11064-016-2111-x.
45
46 24. Young JM, Terrin N, Wang X, et al. Asymmetric dimethylarginine and
47 mortality in stages 3 to 4 chronic kidney disease. *Clin J Am Soc Nephrol* 2009; 4:
48 1115-1120.
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 25. Kielstein JT, Salpeter SR, Bode-Boeger SM, et al. Symmetric
4 dimethylarginine (SDMA) as endogenous marker of renal function--a meta-analysis.
5 *Nephrol Dial Transplant* 2006; 21: 2446-2451.
6
7
8 26. Meinitzer A, Kielstein JT, Pilz S, et al. Symmetrical and asymmetrical
9 dimethylarginine as predictors for mortality in patients referred for coronary
10 angiography: the Ludwigshafen Risk and Cardiovascular Health study. *Clin Chem*
11 2011; 57: 112-121.
12
13
14 27. Kielstein JT, Veldink H, Martens-Lobenhoffer J, et al. SDMA is an early
15 marker of change in GFR after living-related kidney donation. *Nephrol Dial*
16 *Transplant* 2011; 26: 324-328.
17
18
19 28. Meinitzer A, Seelhorst U, Wellnitz B, et al. Asymmetrical dimethylarginine
20 independently predicts total and cardiovascular mortality in individuals with
21 angiographic coronary artery disease (the Ludwigshafen Risk and Cardiovascular
22 Health study). *Clin Chem* 2007; 53: 273-283.
23
24
25 29. Lu TM, Chung MY, Lin MW, et al. Plasma asymmetric dimethylarginine
26 predicts death and major adverse cardiovascular events in individuals referred for
27 coronary angiography. *Int J Cardiol* 2011; 153: 135-140.
28
29
30 30. Boger RH, Maas R, Schulze F, et al. Asymmetric dimethylarginine (ADMA) as
31 a prospective marker of cardiovascular disease and mortality--an update on patient
32 populations with a wide range of cardiovascular risk. *Pharmacol Res* 2009; 60: 481-
33 487. 2009/07/15. DOI: 10.1016/j.phrs.2009.07.001.
34
35
36 31. Pizzarelli F, Maas R, Dattolo P, et al. Asymmetric dimethylarginine predicts
37 survival in the elderly. *Age (Dordr)* 2013; 35: 2465-2475. 2013/04/16. DOI:
38 10.1007/s11357-013-9523-1.
39
40
41 32. Zoccali C, Bode-Boger S, Mallamaci F, et al. Plasma concentration of
42 asymmetrical dimethylarginine and mortality in patients with end-stage renal disease:
43 a prospective study. *Lancet* 2001; 358: 2113-2117.
44
45
46 33. Frenay AR, van den Berg E, de Borst MH, et al. Plasma ADMA associates
47 with all-cause mortality in renal transplant recipients. *Amino Acids* 2015; 47: 1941-
48 1949. 2015/06/17. DOI: 10.1007/s00726-015-2023-0.
49
50
51 34. Schulze F, Carter AM, Schwedhelm E, et al. Symmetric dimethylarginine
52 predicts all-cause mortality following ischemic stroke. *Atherosclerosis* 2010; 208:
53 518-523.
54
55
56 35. Koch A, Weiskirchen R, Bruensing J, et al. Regulation and prognostic
57 relevance of symmetric dimethylarginine serum concentrations in critical illness and
58 sepsis. *Mediators Inflamm* 2013; 2013: 413826.
59
60

- 1
2
3 36. Pihlstrom H, Mjoen G, Dahle DO, et al. Symmetric dimethylarginine as
4 predictor of graft loss and all-cause mortality in renal transplant recipients.
5
6 *Transplantation* 2014; 98: 1219-1225. 2014/07/08. DOI:
7
8 10.1097/TP.0000000000000205.
- 9 37. Closs EI, Basha FZ, Habermeier A, et al. Interference of L-arginine analogues
10 with L-arginine transport mediated by the y+ carrier hCAT-2B. *Nitric Oxide* 1997; 1:
11
12 65-73.
- 13 38. Wilcox CS. Asymmetric dimethylarginine and reactive oxygen species:
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
39. Schepers E, Barreto DV, Liabeuf S, et al. Symmetric dimethylarginine as a
proinflammatory agent in chronic kidney disease. *Clin J Am Soc Nephrol* 2011; 6:
2374-2383.
40. Mortensen KM, Itenov TS, Haase N, et al. High levels of methylarginines
were associated with increased mortality in patients with severe sepsis. *Shock* 2016;
46: 365-372. 2016/05/21. DOI: 10.1097/SHK.0000000000000649.
41. Schutte AE, Schutte R, Huisman HW, et al. Dimethylarginines: their vascular
and metabolic roles in Africans and Caucasians. *Eur J Endocrinol* 2010; 162: 525-
533. 2009/12/10. DOI: 10.1530/EJE-09-0865.
42. Davids M and Teerlink T. Plasma concentrations of arginine and asymmetric
dimethylarginine do not reflect their intracellular concentrations in peripheral blood
mononuclear cells. *Metabolism* 2013; 62: 1455-1461. 2013/07/31. DOI:
10.1016/j.metabol.2013.05.017.

Figure 1A. Scatter plot of 1/plasma ADMA in association with measured glomerular filtration rate (mGFR). $r_s = 0.42, p < 0.0001$



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 1B. Scatter plot of 1/plasma SDMA in association with measured glomerular filtration rate (mGFR). $r_s = 0.87$, $p < 0.0001$

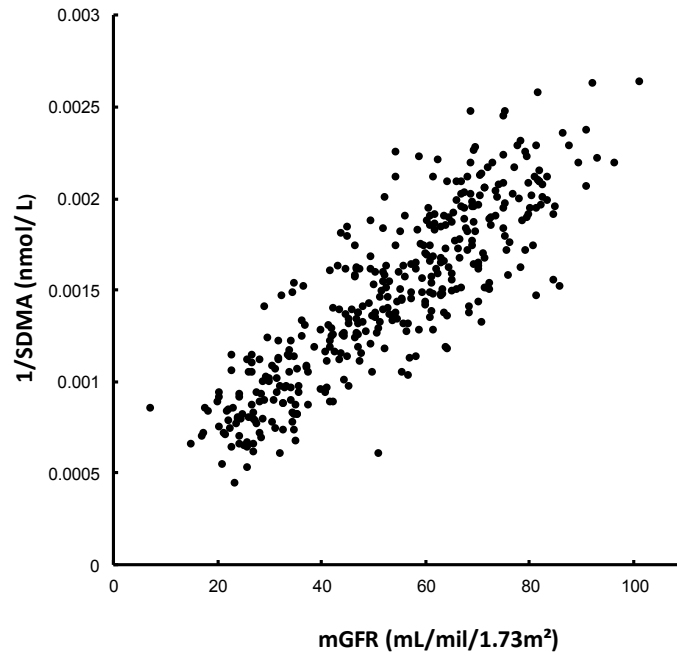
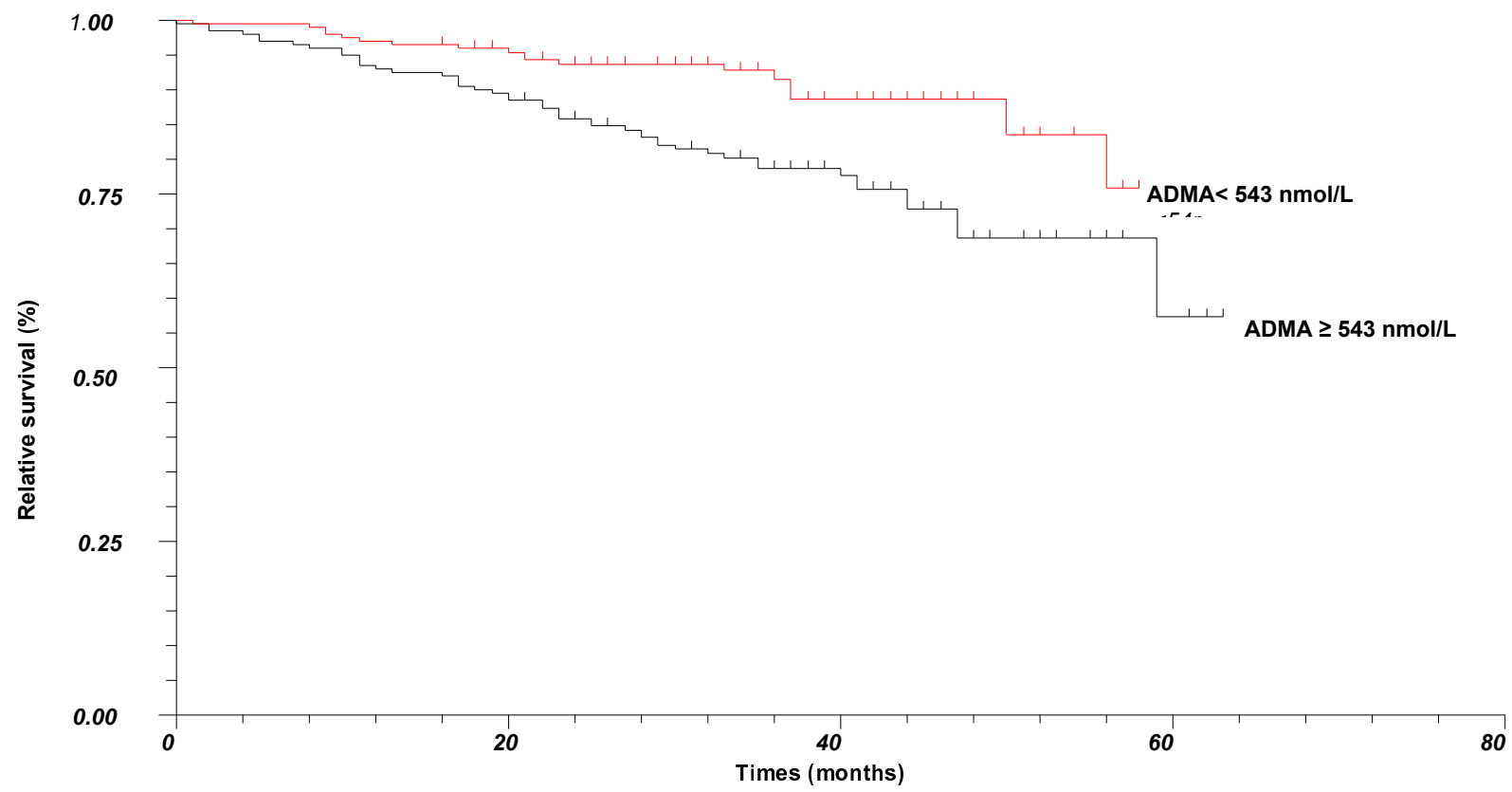


Figure 2a. Kaplan-Meier survival curve by median ADMA concentration (543 nmol/L); $P=0.0009$.



1
2
3
4
5 **Figure 2b. Kaplan-Meier survival curve by median SDMA concentration (680 nmol/L); $P < 0.0001$.**
6
7
8
9
10
11
12
13

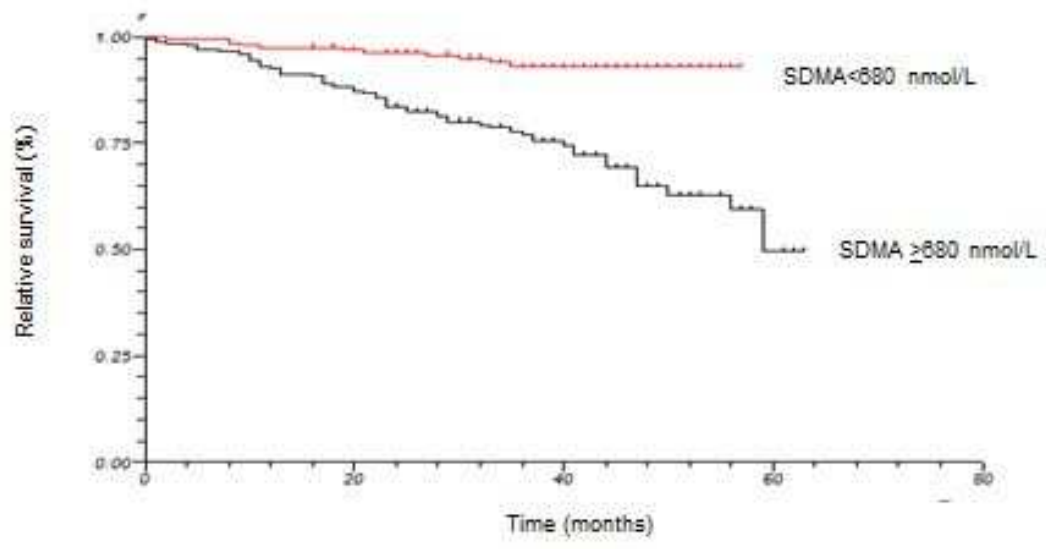


Table 1. Subject characteristics by GFR category. Values for continuous variables are expressed as median (interquartile interquartile range) unless stated otherwise. *P*<0.05 was considered significant.

	Total cohort	≥60 mL/min/1.73 m ²	30–59 mL/min/1.73 m ²	<30 mL/min/1.73 m ²	<i>P</i> for trend
n	394	163	171	60	-
Male:female, n (% male)	189:205	67:96 (41)	90:81 (53)	33:28 (55)	0.0617
Age, y	80 (774 - 8397)	79 (764 - 819) ^{a, b}	80 (774 - 8497)	81 (775 - 8697)	<0.0001
BMI, weight(kg)/height(m) ²	26.1 (13.723.6 - 47.629.3)	25.8 (13.723.1 - 42.228.6)	26.2 (16.424.1 - 47.629.7)	27.3 (17.323.9 - 39.330.0)	0.0998
MABP, mm Hg*	96.05.7 (89.360.0 - 144.3103.6)	94.3 (6087.3 - 144.3104.4)	96.6 (74.391.4 - 133.3104.8)	96.3 (72.087.1 - 128.6100.9)	0.4100
Number of medications, n	5 (30 - 18)	4 (0.1 - 176) ^{a, b}	6 (0.4 - 18)	7 (50 - 105)	<0.0001
Vascular disease, n (%)	172 (44)	49 (31) ^a	90 (53)	33 (54)	<0.0001
Diabetes, n (%)	77 (20)	21 (13) ^b	36 (21)	20 (33)	0.0032
Hypertension, n (%)	251 (55)	71 (44) ^{a, b}	104 (61)	40 (66)	0.0014
Smoker, n (%)	86 (22)	26 (16) ^a	51 (30) ^c	9 (15)	0.0034
Haemoglobin, g/L	130 (12077-139168)	136 (93129-14268) ^{a, b}	126 (11777-164136) ^c	118 (90118-54129)	<0.0001
Serum creatinine, µmol/L	94.5 (34.873 - 382.0144)	71.4 (34.861 - 306.884)	1110.5 (53.788 - 282.4145)	191.0 (91.1157 - 382247.0)	-
GFR, mL/min/1.73 m ²	53 (357-68104)	69 (6064-10178)	45 (360-529)	25 (227-279)	-
ADMA, nmol/L	543 (49893- 874608)	521 (364472-723559) ^{a, b}	565 (51393-624841)	595 (443526-874673)	<0.0001
SDMA, nmol/L	680 (104542-2240927)	530 (379480-1170610) ^{a, b}	775 (104663-1650934) ^c	1220 (7081067-22401390)	<0.0001

*MABP data was obtained for 374 patients in total and 154, 163 and 57 patients in the >60, 30-59 and <30 mL/min/1.73 m² groups respectively.

Abbreviations: ADMA, asymmetric dimethylarginine; BMI, Body mass index; GFR, measured glomerular filtration rate (using iohexol); MABP, mean arterial blood pressure; SDMA, symmetric dimethylarginine;

Dunn's multiple comparison test for pairwise comparisons of the effect of GFR category on plasma ADMA, SDMA and serum cystatin C concentration are denoted as shown below.

^a $P < 0.05$ for ≥ 60 mL/min/1.73 m² vs. 30-59 mL/min/1.73 m²

^b $P < 0.05$ for ≥ 60 mL/min/1.73 m² vs. < 30 mL/min/1.73 m²

^c $P < 0.05$ for 30-59 mL/min/1.73 m² vs. < 30 mL/min/1.73 m²

Table 2. Correlation (r_s) between plasma ADMA and SDMA concentrations and clinical variables.

Variable	ADMA	SDMA
Age	0.10	0.25†
BMI	0.14	0.08
MABP*	0.07	0.01
Haemoglobin	-0.20†	-0.41†
GFR	-0.42†	-0.87†
Serum creatinine	0.34†	0.87†

Note. Values expressed as correlation coefficients obtained using Spearman's rank statistic. Statistical significance is shown as † ($P < 0.0001$ in each case); the remaining correlations were not statistically significant.

1
2
3
4
5 Abbreviations: ADMA, asymmetric dimethylarginine; BMI, body mass index; GFR, measured glomerular filtration rate (using iohexol); MABP, mean arterial
6 blood pressure; SDMA, symmetric dimethylarginine;
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

Table 3. Effect of clinical variables on plasma ADMA and SDMA concentrations. Values shown are the beta coefficients (95% confidence intervals) for the variables that remained significant in the multiple regression model.

	ADMA	SDMA
Constant	664.1 (641.5 to 686.6), $P < 0.0001$	1828.1 (1654.1 to 2002.0), $P < 0.0001$
Glomerular filtration rate	-2.032 (-2.436 to -1.628), $P < 0.0001$	-11.779 (-12.791 to -10.768), $P < 0.0001$
Haemoglobin	-	-2.723 (-3.968 to -1.478), $P < 0.0001$
Gender	-	98.416 (63.281 to 133.55), $P < 0.0001$
Body mass index	-	-4.907 (-8.699 to -1.116), $P = 0.0116$

Table 4. Unadjusted and adjusted hazard ratios for death. For continuous variables, the hazard ratios are expressed as the increased risk associated with a one standard deviation increase (ADMA, age, BMI, MABP, medication no.) or decrease (GFR, haemoglobin). For categorical variables, the hazard ratios are expressed as risk if conditions present (vascular disease, hypertension, smoker and diabetes mellitus) or if female rather than male, compared to risk in the absence of condition.

Variable	Unadjusted hazard ratio (95% CI) per standard deviation	<i>P</i>	ADMA model: adjusted hazard ratio (95% CI) per standard deviation	<i>P</i>	SDMA model: adjusted hazard ratio (95% CI) per standard deviation	<i>P</i>
ADMA, nmol/ L	1.67 (1.32 - 2.10)	<0.0001	1.30 (1.01-1.68)	0.0412	-	-
SDMA, nmol/L	2.01 (1.66 - 2.44)	<0.0001	-	-	2.43 (1.80 – 3.28)	<0.0001
Age, years	1.65 (1.36 – 2.00)	<0.0001	1.43 (1.17 – 1.75)	0.0005	1.47 (1.20 - 1.81)	0.0003
Gender (f/m)	1.42 (0.87 - 2.32)	0.1651 ^a	-	-	-	-
BMI, weight(kg)/height(m) ²	0.95 (0.74 - 1.21)	0.6593	-	-	-	-
MABP, mmHg	0.99 (0.77 - 1.27)	0.9265	-	-	-	-
GFR, mL/min/1.73 m ²	0.44 (0.33 - 0.59)	<0.0001	0.54 (0.39 – 0.76)	0.0003	-	-
Number of medications, n	1.39 (1.11 - 1.75)	0.0045	-	-	-	-
Haemoglobin, g/L	0.63 (0.46 - 0.85)	0.0032	-	-	-	-
Vascular Disease (y/n)	2.61 (1.56 - 4.37)	0.0162^a	1.91 (1.14 – 3.21) ^a	0.0145	-	-
Diabetes (y/n)	1.42 (0.82 - 2.48)	0.2143 ^a	-	-	-	-
Smoker (y/n)	2.14 (1.30 - 3.53)	0.0029^a	1.87 (1.13 – 3.10) ^a	0.0155	1.99 (1.20 - 3.29) ^a	0.0074
Hypertension (y/n)	1.38 (0.84 - 2.29)	0.2061 ^a	-	-	-	-

Abbreviations: ADMA, asymmetric dimethylarginine; BMI, Body mass index; CI, confidence interval; GFR, glomerular filtration rate (using iohexol); MABP, mean arterial blood pressure; SDMA, symmetric dimethylarginine

^a adjusted hazard ratio (95% CI) for presence compared to absence or female versus male