

Original Article

SDMA is an early marker of change in GFR after living-related kidney donation

Jan T. Kielstein^{1,*}, Hendrik Veldink^{1,*}, Jens Martens-Lobenhoffer², Hermann Haller¹, Michael Burg³, Johan M. Lorenzen¹, Ralf Lichtinghagen⁴, Stefanie M. Bode-Böger^{2,†} and Volker Kliem^{3,†}

¹Department of Internal Medicine, Division of Nephrology and Hypertension, Hannover Medical School Hannover, Germany,

²Institute for Clinical Pharmacology, Otto-von-Guericke University, Magdeburg, Germany, ³Nephrological Centre Lower Saxony, Hannoversch-Münden, Germany and ⁴Institute for Clinical Chemistry, Hannover Medical School Hannover, Germany

Correspondence and offprint requests to: Jan T. Kielstein; E-mail: kielstein@yahoo.com

*J.T.K. and H.V. contributed equally to the manuscript and are both considered first authors.

†S.M.B.-B. and V.K. contributed equally to the manuscript and are both considered last authors.

Abstract

Background. Early detection of changes in the glomerular filtration rate (GFR) is crucial in detecting acute kidney injury. There is burgeoning evidence from preclinical and clinical studies that symmetrical dimethylarginine (SDMA) correlates well with different parameters of renal function. In some studies, SDMA even outperformed creatinine as a marker of GFR. It is however unknown how fast SDMA is increasing after reduction in GFR. The aim of our study was therefore to determine the temporal change of SDMA in comparison with cystatin C after a defined reduction in GFR.

Methods. Blood samples from 24 healthy living-related kidney donors (19 F/5 M), mean age 55.2 ± 8.3 years, were collected prior to donation of the kidney as well as 1, 6, 12, 24, 72 and 168 h after unilateral nephrectomy. SDMA levels were measured using a liquid chromatography–mass spectrometry-based method.

Results. Within 6 h after unilateral nephrectomy, i.e. reduction of GFR by 50%, SDMA rose from 0.571 ± 0.120 to 0.659 ± 0.135 $\mu\text{mol/L}$ ($P < 0.001$). Baseline cystatin C levels increased from 0.87 ± 0.16 to 1.07 ± 0.15 mg/L ($P < 0.001$). Also, serum creatinine rose significantly within 6 h after removal of one kidney from 65.4 ± 8.4 to 88.8 ± 10.2 $\mu\text{mol/L}$ ($P < 0.001$).

Discussion. SDMA might be a valuable and early marker of change in GFR in the clinical and experimental setting. Future studies will have to clarify whether sensitivity, specificity and temporal resolution of SDMA make it an attractive candidate for the assessment of renal function in both the experimental and clinical setting.

Keywords: acute kidney injury; ADMA; renal function; transplantation

Introduction

From 1980 to 2005, the incidence of acute renal failure in hospitalized patients in the USA increased from 1.8 to 36.5/10 000/year [1]. In acute kidney injury (AKI), the early detection of acute changes in glomerular filtration rate (GFR), which is a condition sine qua non for early and effective interventions, is still hampered by the lack of adequate markers. Serum creatinine is an insensitive and late marker for changes in GFR [2]. Therefore, an aggressive search for new markers of renal function has begun. Serum cystatin C (Cys C) has been shown to be an early marker of AKI outperforming serum creatinine at 24 h after cardiac surgery [3]. Symmetrical dimethylarginine (SDMA), the structural isomer of the endogenous nitric oxide synthase inhibitor asymmetrical dimethylarginine (ADMA), also seems to be a promising candidate in this regard. Data from several studies have suggested that SDMA correlates well with parameters of renal function, first shown in children with hypertension [4]. A meta-analysis of 18 studies involving a total of 2131 patients showed a strong correlation between SDMA and different parameters of renal function [5]. Plasma SDMA levels increase in parallel with creatinine and are sometimes even more sensitive to detect renal dysfunction than creatinine itself [6]. Also, in several animal species, a correlation between SDMA and parameters of renal function has been found [7]. Carello *et al.* [8] showed in a total nephrectomy model in rats that SDMA increased rapidly after nephrectomy. After 24 h, SDMA had increased more than an order of magnitude and peaked after 48 h reaching a level about 20 times higher than the baseline SDMA. Comparable data in humans are not available. Therefore, the aim of our study was to compare the temporal resolution of

Table 1. Patient characteristics

Mean age (years)	55.2 ± 8.3
Male/female	5/19
Height (cm)	165.0 ± 7.1
Weight (kg)	75.0 ± 12.5
Baseline creatinine (µmol/L)	61.88 ± 9.72
Baseline haemoglobin (g/dL)	14.3 ± 1.1
BMI (kg/m ²)	27.9 ± 4.0
Cholesterol (mg/dL)	199.0 ± 29.1
Triglycerides (mg/dL)	93.0 ± 56.9
Renal function side distribution (R/L)	48 ± 3%/52 ± 3%

SDMA and cystatin C in a setting of well-defined reduction in GFR, i.e. living-related kidney donation, for up to 7 days after reduction in GFR of 50%.

Materials and methods

The study was approved by the local ethics committee of Hannover Medical School, Hannover, Germany. All patients gave written informed consent. We studied 24 Caucasian living-related kidney donors (5 M/19 F). Table 1 shows the clinical characteristics of the study population. Blood samples for measurement of plasma SDMA, cystatin C and routine chemistry were drawn before as well as 1,6,12,24,72 and 168 h after unilateral nephrectomy. Blood samples were immediately cooled on ice, centrifuged at 1500 g and 4°C for 10 min. Supernatants were stored in 1-mL aliquots at -80°C until further use. Side distribution of renal function was analysed by a renal scintigraphy with isotope technique using ^{99m}Tc-MAG3 as part of the routine workup for living kidney donation and is reported as percentage mean values of the renal clearance (Table 1).

Measurements and calculation

Plasma concentrations of SDMA were measured applying a recently developed liquid chromatography–mass spectrometry method described elsewhere [9]. Plasma levels of cystatin C were measured by particle-enhanced immunonephelometry on a Behring nephelometer system (Siemens Healthcare, Eschborn, Germany), and CRP was measured by a latex-enhanced immunoturbidimetric assay on a Hitachi 917 analyser (Invicon, Munich, Germany).

All other measurements were done with routine laboratory tests using certified assay methods.

Statistical analysis

We used GraphPad Prism 5 for statistical analysis. The normality of data distribution was confirmed with the Shapiro–Wilk test. ANOVA was used to compare the biochemical parameters at the different time points. The significance level was set at $P < 0.05$.

Results

Living kidney donation was well tolerated in all subjects. Six hours after nephrectomy, SDMA was significantly elevated as compared with baseline (0.659 ± 0.135 µmol/L vs 0.571 ± 0.120 µmol/L, $P < 0.001$). This difference became even more marked 24 h after the operation (0.901 ± 0.165 µmol/L vs. baseline, $P < 0.001$) and persisted up to the end of the observation period (Figure 1). The changes in SDMA were paralleled by the changes in cystatin C. Baseline cystatin C levels (0.87 ± 0.16 mg/L) were not significantly different from cystatin C 1 h after unilateral nephrectomy (0.89 ± 0.15 mg/L, $P = 0.52$). Six hours after nephrectomy, cystatin C was significantly elevated as compared with baseline (1.07 ± 0.15 mg/L vs. baseline, $P < 0.001$). This difference became the most marked 24 h after the operation (1.21 ± 0.22 mg/L vs. baseline, $P < 0.001$) and persisted up to the end of the observation period (Figure 2). Also, creatinine increased from 65.4 ± 8.4 µmol/L at baseline to 74.2 ± 13.4 µmol/L after 1 h ($P < 0.01$ vs. baseline) and became even more significant after 6 h (88.2 ± 10.2 µmol/L vs. baseline, $P < 0.001$) (Figure 3). The inflammatory marker CRP, which was normal at baseline, peaked at 24 h and was still elevated at the end of the observation period (Figure 4).

Both phosphate and serum urea increased after unilateral nephrectomy with a peak at 12/24 h after the procedure, respectively. While phosphate increased significantly from 1.2 ± 0.1 to 1.6 ± 0.2 mmol/L at 6 h ($P < 0.001$), it was not different from baseline (1.1 ± 0.2 mmol/L) 168 h after nephrectomy. In contrast to phosphate, urea did not significantly change during our observation period.

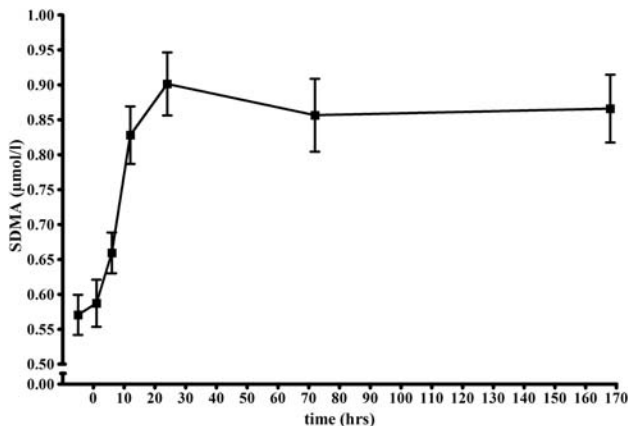


Fig. 1. Time course of SDMA levels after unilateral nephrectomy in 24 kidney donors (mean ± SEM).

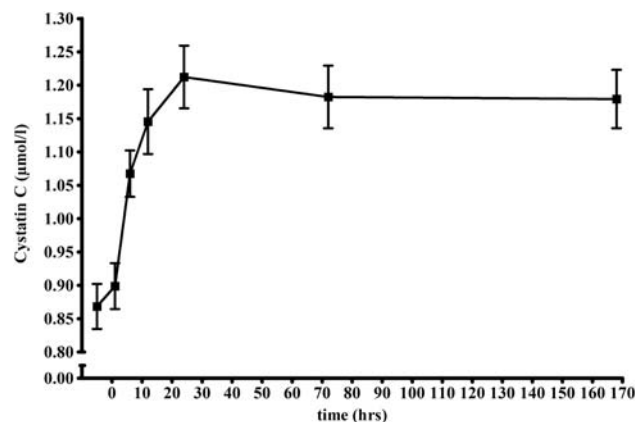


Fig. 2. Time course of cystatin C levels after unilateral nephrectomy in 24 kidney donors (mean ± SEM).

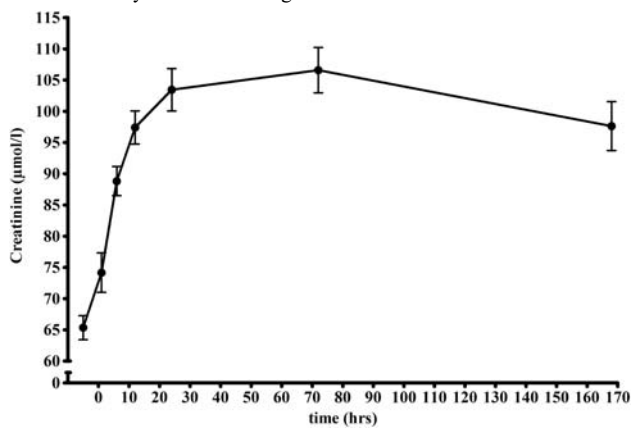


Fig. 3. Time course of creatinine levels after unilateral nephrectomy in 24 kidney donors (mean \pm SEM).

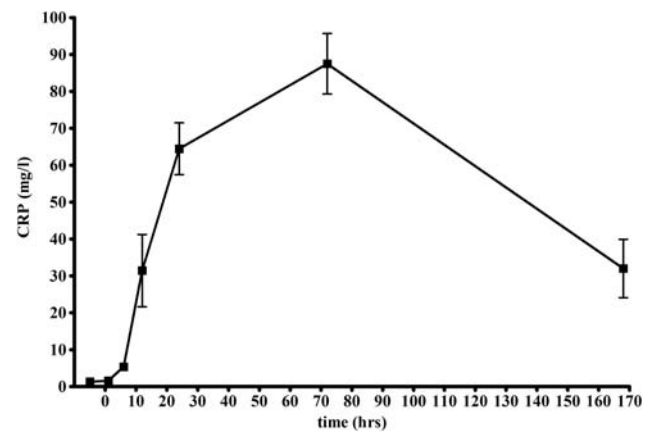


Fig. 4. Time course of CRP levels after unilateral nephrectomy in 24 kidney donors (mean \pm SEM).

Discussion

The pertinent findings of our study were (i) SDMA as well as cystatin C increased as early as 6 h after a reduction of GFR by 50%, as did creatinine, and (ii) the increase of these parameters peaked at 24 h after unilateral nephrectomy, (iii) persisted for up to 7 days after kidney donation and (iv) was independent from the temporary increase in CRP.

SDMA as an early marker of change in GFR

Our study shows for the first time that SDMA is an early indicator of change in GFR in men. SDMA stems from protein methylation by protein-methyltransferase (PRMT) type 2 [10]. It is supposedly produced at a constant rate. Already in 1970, Kakimoto and co-workers provided evidence that SDMA is almost completely eliminated by renal excretion [11] which is in line with recent data on concentration of SDMA in the renal artery and vein in men [12]. SDMA correlates well with different parameters of renal function in cross-sectional analysis. This holds true for both laboratory animals [7] and humans [5]. Plasma SDMA levels increase in parallel with creatinine and are sometimes even more sensitive to detect renal dysfunction than creatinine itself [6]. Carello *et al.* [8] showed in a rat model that SDMA increased rapidly after total nephrectomy. After 24 h, SDMA had increased more than an order of magnitude and peaked after 48 h reaching a level \sim 20 times higher than the baseline SDMA. Six- and 12-h data in their animal study were not reported. We showed that SDMA was already significantly increased by 1.15 times after 6 h and 1.43 times after 12 h. Peak SDMA levels were reached at 24 h. Although not investigated in our study, it is worth mentioning potentially important differences between SDMA and cystatin C. While cystatin C is influenced by steroid treatment [13,14], we know from a study in humans with IgA nephropathy that SDMA is not influenced even by high doses of steroids [15]. Moreover, SDMA is not affected by acute inflammation [16], while cystatin C can be influenced by systemic inflammation [17]. Some authors do however suggest that SDMA might be also elevated by cer-

tain disease states, independently of renal function [18,19]. In our patients, the increase in SDMA was unrelated to CRP as a marker of systemic inflammation. Despite the decrease in CRP at Day 7, SDMA remained high supporting the idea that SDMA in this setting is indeed more related to renal function than to inflammation.

Whether the sustained elevation of SDMA will have any long-term deleterious effects on the kidney donor remains to be elucidated. Pathophysiological reasoning for this assumption stems from the fact that SDMA is known to interfere (indirectly) with NO synthesis (for review see [7]). Also, there is recent preclinical evidence that SDMA stimulates production of reactive oxygen species in monocytes [20].

Cystatin C

Cystatin C is synthesized and released into the blood at a relatively constant rate by all nucleated cells. Also, it is freely filtered by the glomerulus, completely reabsorbed by the proximal tubule, completely catabolized during the reabsorption, and not secreted. Urinary excretion of cystatin C has been shown to predict the requirement for renal replacement therapy in patients with established AKI \sim 1 day earlier than creatinine [21]. In the intensive care setting, a 50% increase in serum cystatin C predicted AKI at 1 and 2 days before the rise in serum creatinine [22]. Our results, which show that cystatin C is significantly increased already 6 h after a reduction of GFR by 50%, are in line with previous data [3]. After contrast administration in children, a significant rise of cystatin C was detected already after 8 h [23]. However, in a previously reported study by Ahlström *et al.*, serum cystatin C did not outperform serum creatinine in the early diagnosis of AKI in men [24]. In mice, a significant elevation of cystatin C after bilateral nephrectomy could be seen already 2 h post-surgery [25]. Also, 12 h after unilateral nephrectomy in mice, cystatin C was significantly elevated [25].

Creatinine

Creatinine is derived from the metabolism of creatinine in skeletal muscle and from dietary meat intake; it is released

into the circulation at a relatively constant rate and has a stable plasma concentration. Although freely filtered across the glomerulus, ~10–40% of urinary creatinine is derived from tubular secretion by the organic cation secretory pathways in the proximal tubule [26]. Several limitations are known for the use of serum creatinine values like the variation in creatinine production caused by dietary intake or different muscle mass, and the variations in creatinine secretion, all hampering the detection of small losses of renal function using this marker. Hence, it came as a surprise that we could already detect a significant increase in creatinine by 1 h after unilateral nephrectomy, which might be related to the surgical procedure itself resulting in significant muscle damage. To our knowledge, only one previous study investigated the influence of unilateral nephrectomy on serum creatinine within 24 h [2]. Interestingly, these authors could not find a significant increase of creatinine even 24 h after nephrectomy. The difference might be based on the different sample size as these authors investigated 10 patients in contrast to 24 patients in our study. Also, the side distribution of renal function is not provided in their study; hence, it is possible that the drop in GFR after unilateral nephrectomy was <50%. Lastly, differences in the surgery itself might have resulted in differences in muscle damage. Interestingly urea did not significantly increase during our observation period, confirming its limited suitability as a marker of glomerular filtration, as it is influenced by many other factors such as hydration status and liver function. This is in line with follow-up data in kidney donors where BUN did not significantly increase comparing pre- and 20-year post-donation levels [27], which in that case might however be related to the hyperfiltration of the remaining kidney.

Limitations of the study

Our single-centre study is hypothesis generating in nature, suggesting that the temporal resolution of SDMA in detecting major changes in GFR is not inferior to the clinically used markers like cystatin C and might even offer some theoretical advantages like the lack of inflammation and steroid treatment on SDMA levels. Yet, we did not investigate patients in different clinical settings; therefore, we do not know whether SDMA would exhibit the same characteristics in patients with co-morbidities.

Above all, as pointed out by Manolio, there are problems inherent to new biomarkers [28]. Although initial reports about novel markers provide exciting clues into the pathophysiology of diseases and enable us to improve diagnostic capabilities, translating these into clinical application requires replication in multiple settings. For SDMA, we lack sensitivity and specificity data in humans and animals before this compound could be advocated as a robust parameter of GFR. Last but not least, at this time, the very early detection of a decrease in GFR or renal injury cannot be used for interventions that will improve the clinical outcome of patients.

Acknowledgements. J.T.K. is supported by a grant of the Else-Kröner-Fresenius Foundation (P63/06/EKMS 06/03). H.V. is supported by the StrucMed program of the Medical School Hannover, Germany.

Conflict of interest statement. None declared.

References

1. Centre for Disease Control. Hospitalization discharge diagnoses for kidney disease—United States, 1980–2005. *MMWR* 2008; 57: 309–312
2. Herget-Rosenthal S, Pietruck F, Volbracht L *et al.* Serum cystatin C—a superior marker of rapidly reduced glomerular filtration after uninephrectomy in kidney donors compared to creatinine. *Clin Nephrol* 2005; 64: 41–46
3. Haase-Fielitz A, Bellomo R, Devarajan P *et al.* Novel and conventional serum biomarkers predicting acute kidney injury in adult cardiac surgery—a prospective cohort study. *Crit Care Med* 2009; 37: 553–560
4. Goonasekera CD, Rees DD, Woolard P *et al.* Nitric oxide synthase inhibitors and hypertension in children and adolescents. *J Hypertens* 1997; 15: 901–909
5. Kielstein JT, Salpeter SR, Bode-Boeger SM *et al.* Symmetric dimethylarginine (SDMA) as endogenous marker of renal function—a meta-analysis. *Nephrol Dial Transplant* 2006; 21: 2446–2451
6. Kielstein JT, Martens-Lobenhoffer J, Vollmer S *et al.* L-Arginine, ADMA, SDMA, creatinine, MDRD formula—detour to renal function testing. *J Nephrol* 2008; 21: 963–965
7. Kielstein JT, Fliser D, Veldink H. Asymmetric dimethylarginine and symmetric dimethylarginine: axis of evil or useful alliance? *Semin Dial* 2009; 22: 346–350
8. Carello KA, Whitesall SE, Lloyd MC *et al.* Asymmetrical dimethylarginine plasma clearance persists after acute total nephrectomy in rats. *Am J Physiol Heart Circ Physiol* 2006; 290: H209–H216
9. Martens-Lobenhoffer J, Bode-Boeger SM. Fast and efficient determination of arginine, symmetric dimethylarginine, and asymmetric dimethylarginine in biological fluids by hydrophilic-interaction liquid chromatography–tandem mass spectrometry. *Clin Chem* 2006; 52: 488–493
10. Vallance P, Leiper J. Cardiovascular biology of the asymmetric dimethylarginine:dimethylarginine dimethylaminohydrolase pathway. *Arterioscler Thromb Vasc Biol* 2004; 24: 1023–1030
11. Kakimoto Y, Akazawa S. Isolation and identification of *N*-G, *N*-G- and *N*-G, *N*-G-dimethyl-arginine, *N*-epsilon-mono-, di-, and trimethyllysine, and glucosylgalactosyl- and galactosyl-delta-hydroxylysine from human urine. *J Biol Chem* 1970; 245: 5751–5758
12. Nijveldt RJ, van Leeuwen PA, van Guldener C *et al.* Net renal extraction of asymmetrical (ADMA) and symmetrical (SDMA) dimethylarginine in fasting humans. *Nephrol Dial Transplant* 2002; 17: 1999–2002
13. Bjarnadottir M, Grubb A, Olafsson I. Promoter-mediated, dexamethasone-induced increase in cystatin C production by HeLa cells. *Scand J Clin Lab Invest* 1995; 55: 617–623
14. Risch L, Herklotz R, Blumberg A *et al.* Effects of glucocorticoid immunosuppression on serum cystatin C concentrations in renal transplant patients. *Clin Chem* 2001; 47: 2055–2059
15. Uchida HA, Nakamura Y, Kaihara M *et al.* Steroid pulse therapy impaired endothelial function while increasing plasma high molecule adiponectin concentration in patients with IgA nephropathy. *Nephrol Dial Transplant* 2006; 21: 3475–3480
16. Zoccali C, Maas R, Cutrupi S *et al.* Asymmetric dimethyl-arginine (ADMA) response to inflammation in acute infections. *Nephrol Dial Transplant* 2007; 22: 801–806
17. Rule AD, Bergstralh EJ, Slezak JM *et al.* Glomerular filtration rate estimated by cystatin C among different clinical presentations. *Kidney Int* 2006; 69: 399–405
18. Billecke SS, D'Alecy LG, Platel R *et al.* Blood content of asymmetric dimethylarginine: new insights into its dysregulation in renal disease. *Nephrol Dial Transplant* 2009; 24: 489–496

19. Bode-Boger SM, Scalera F, Kielstein JT *et al.* Symmetrical dimethylarginine: a new combined parameter for renal function and extent of coronary artery disease. *J Am Soc Nephrol* 2006; 17: 1128–1134
20. Schepers E, Glorieux G, Dhondt A *et al.* Role of symmetric dimethylarginine in vascular damage by increasing ROS via store-operated calcium influx in monocytes. *Nephrol Dial Transplant* 2009; 24: 1429–1435
21. Herget-Rosenthal S, Poppen D, Husing J *et al.* Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. *Clin Chem* 2004; 50: 552–558
22. Herget-Rosenthal S, Marggraf G, Husing J *et al.* Early detection of acute renal failure by serum cystatin C. *Kidney Int* 2004; 66: 1115–1122
23. Hirsch R, Dent C, Pfiem H *et al.* NGAL is an early predictive biomarker of contrast-induced nephropathy in children. *Pediatr Nephrol* 2007; 22: 2089–2095
24. Ahlstrom A, Tallgren M, Peltonen S *et al.* Evolution and predictive power of serum cystatin C in acute renal failure. *Clin Nephrol* 2004; 62: 344–350
25. Song S, Meyer M, Turk TR *et al.* Serum cystatin C in mouse models: a reliable and precise marker for renal function and superior to serum creatinine. *Nephrol Dial Transplant* 2009; 24: 1157–1161
26. Shemesh O, Golbetz H, Kriss JP *et al.* Limitations of creatinine as a filtration marker in glomerulopathic patients. *Kidney Int* 1985; 28: 830–838
27. Najarian JS, Chavers BM, McHugh LE *et al.* 20 year follow-up of living kidney donors. *Lancet* 1992; 340: 207–210
28. Manolio T. Novel risk markers and clinical practice. *N Engl J Med* 2003; 349: 1587–1589

Received for publication: 3.2.10; Accepted in revised form: 15.6.10