Methods. Blood samples from 24 healthy living-related kidney donors (19 F/5 M), mean age 55.2 ± 8.4 years, were collected prior to donation of the kidney as well as prior to donation of the kidney as well as prior to 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 μmol/L (P < 0.001). Baseline cystatin C levels increased from 0.87 ± 0.16 to 1.07 ± 0.120 μmol/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 μmol/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 sensitiv-
ity, 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 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 99mTc-MAG3 as part of the routine workup for living kidney donation and is reported as percentage mean values of the renal clearance (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
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<tbody>
<tr>
<td>Mean age (years)</td>
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<tr>
<td>Male/female</td>
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<tr>
<td>Height (cm)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>Baseline creatinine (μmol/L)</td>
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<tr>
<td>Baseline haemoglobin (g/dL)</td>
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<td>BMI (kg/m²)</td>
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<tr>
<td>Cholesterol (mg/dL)</td>
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<tr>
<td>Triglycerides (mg/dL)</td>
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<tr>
<td>Renal function side distribution (R/L)</td>
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## 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).

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 ∼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 certain disease states, independently of renal function [18,19].

Whether the sustained elevation of SDMA will have any long-term deleterious effects on the kidney donor re-
mains 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 catalyzed 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 ~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 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 by all nucleated cells. 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.

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**Conflict of interest statement.** None declared.

**References**

Methylation cycle, arginine-creatine pathway and asymmetric dimethylarginine in paediatric renal transplant

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Abstract

Background. Hyperhomocysteinaemia represents an important cause of morbidity in recipients of renal transplants, but few investigations have been carried out to evaluate the status of the methylation cycle and its relation with levels of new cardiovascular biomarkers, such as asymmetric dimethylarginine (ADMA).

Methods. Twenty-six children and adolescents aged 7–18 years (17 male, 9 female) with stable renal transplants were recruited for the study. None had received treatment...