Original Articles

Time course of asymmetric dimethylarginine and symmetric dimethylarginine levels after successful renal transplantation

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ABSTRACT

Background. Although renal transplantation (Tx) improves the outcome of patients with renal disease, cardiovascular (CV) risk remains high. Recently, it was demonstrated that asymmetric dimethylarginine (ADMA) levels predict CV events and graft survival in renal transplant recipients (RTRs). Little is known about the impact of renal Tx on the plasma levels of ADMA and symmetric dimethylarginine (SDMA). The present study aimed to define the time course of ADMA and SDMA after Tx.

Methods. We prospectively followed 167 incident RTRs with visits at the time of Tx and 3 and 12 months thereafter. At all visits, demographics and relevant biochemistry were recorded and blood was sampled for analysis of ADMA and SDMA (high-performance liquid chromatography). Eighty-four patients had an additional sampling in the immediate postoperative period. In a case-controlled substudy (n = 31), we compared ADMA and SDMA levels between RTRs and chronic kidney disease (CKD) patients, matched for glomerular filtration rate, gender, age, CV history and diabetes.

Results. Overall, plasma ADMA and SDMA levels decreased after Tx. The decline of SDMA was more pronounced and paralleled the recovery of renal function. Interestingly, the decline of ADMA was preceded by an increase in the immediate postoperative period. In the case-controlled substudy, SDMA levels were similar, whereas ADMA levels were significantly higher in RTRs compared with the CKD counterparts (P = 0.003).

Conclusion. ADMA levels follow a biphasic pattern after successful renal Tx with a transient rise in the immediate postoperative period followed by a decline. Levels remain elevated compared with CKD patients, matched for age, gender, diabetes, CV history and renal function.

Keywords: ADMA, DDAH, renal, SDMA, transplantation

INTRODUCTION

Asymmetric dimethylarginine (ADMA) is increasingly recognized as a uraemic toxin. It is an endogenous inhibitor of nitric oxide synthase and thereby mediates endothelial dysfunction, hypertension and vascular remodelling. Elevated plasma ADMA concentrations have been associated with the presence of numerous traditional and non-traditional risk factors, such as hypertension, diabetes, left ventricular hypertrophy, low-density lipoprotein and increased intima media thickness [1–6]. Levels of ADMA and its isomer symmetric dimethylarginine (SDMA) are both elevated in patients with chronic kidney disease (CKD). For SDMA, renal excretion is the major pathway of elimination. Elevated plasma ADMA concentrations have been associated with the presence of numerous traditional and non-traditional risk factors, such as hypertension, diabetes, left ventricular hypertrophy, low-density lipoprotein and increased intima media thickness [1–6]. Levels of ADMA and its isomer symmetric dimethylarginine (SDMA) are both elevated in patients with chronic kidney disease (CKD). For SDMA, renal excretion is the major pathway of elimination, whereas for ADMA both renal and extra-renal degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), constitutes the major route of disposal. Therefore, SDMA levels are more closely related to glomerular filtration rate (GFR) than ADMA levels [7–10].

Although the incidence of cardiovascular (CV) events and death in renal transplant recipients (RTRs) is lower when
compared with dialysis patients remaining on the transplant waiting list, it remains high compared with the general population. A recent study of prevalent transplant recipients, enrolled in the Assessment of LEscol in Renal Transplantation (ALERT) trial, found that plasma levels of ADMA were associated with increased incidence of CV events, all-cause mortality and deterioration of graft function mirroring previous reports in the non-transplanted CKD population [11–16].

After transplantation (Tx), it can be assumed that, parallel to the improvement of renal function and the increase of renal mass, both renal and enzymatic clearance of ADMA and SDMA might improve. Data with regard to SDMA uniformly show a decline after renal Tx. Studies evaluating ADMA levels after renal Tx, conversely, have yielded inconsistent data. In animal models, a decrease in DDAH activity with concomitant increase in ADMA levels was found in association with acute rejection and ischaemia/reperfusion injury [17, 18]. Yilmaz et al., on the other hand, demonstrated a reduction in ADMA levels in the immediate postoperative period in a cohort of 27 recipients of a living donor kidney transplant. Other investigators reported high levels of ADMA to persist in RTRs [7, 19–22]. Recently, obesity was suggested to be a risk factor of persistently elevated ADMA levels after renal Tx [23].

All these studies are hampered by small sample size or short follow-up. To overcome these limitations and definitely establish the post-transplant time course of ADMA, we performed a prospective observational study in 167 incident RTRs with visits up to 1 year after engraftment.

MATERIALS AND METHODS

Patient population

The present study consisted of a prospective observational study and a case-controlled substudy. All recipients of a single kidney, transplanted at the University Hospitals Leuven, who consented to participate in our protocol biopsy programme, were eligible for inclusion in the prospective observational part. For the present analysis, patients consenting to be enrolled in an ongoing observational trial examining the evolution of arterial stiffness, calcifications and cardiac risk factors after Tx (NCT00547040) and a graft survival exceeding 1 year were recruited. For the case-controlled (1 : 1) substudy, 31 patients were eligible for inclusion. For the present analysis, patients consenting to be enrolled in the prospective observational study and a case-controlled substudy, 31 patients were recruited. For the case-controlled (1 : 1) substudy, 31 patients were recruited. For the case-controlled (1 : 1) substudy, 31 patients were recruited. For the case-controlled (1 : 1) substudy, 31 patients were recruited.

Demographic data

Demographic and clinical data were prospectively recorded and retrieved from an electronic database. CV history was defined as the occurrence of positive stress test or angiographic significant stenosis (>60%), myocardial infarction, percutaneous coronary artery intervention, cardiac surgery, peripheral artery disease, cerebrovascular disease or significant (>70%) stenosis on carotid ultrasound. Hyperlipidaemia was defined as low-density lipoprotein (LDL) cholesterol >100 mg/dL, total cholesterol >200 mg/dL or usage of lipid-lowering drugs. New Onset Diabetes after Tx (NODAT) was defined as the need for glucose-lowering medication for an uninterrupted period of at least 3 months. Different combinations of immunosuppressive drugs were used during the study. Patients were stratified according to the following maintenance immunosuppressive regimens: (i) Tacrolimus (Tac), mycophenolic acid (MPA) and corticosteroids (CS), (ii) Cyclosporin (CsA), MPA and CS, (iii) Sotrastaurin, MPA, CS or JAK3, MPA, CS. The latter patient \( n = 1 \) was part of a Phase 2 trial A3921030. Data on delayed graft function (DGF) and clinically and subclinically biopsy-proven acute rejections (number, time interval since Tx) were prospectively collected. Acute rejections were treated with high-dose intravenous CS during 5 days followed by oral, daily tapered, therapy. DGF was defined as the need for dialysis therapy during the first 7 days after Tx. CV events were defined as major adverse cardiac events, cerebrovascular accidents, peripheral arterial disease and sudden death. Tobacco use was categorized as ‘none’, ‘past’ or ‘current’ smoking.

Procedures, analytical techniques and calculations

In RTRs, baseline (BL) blood samples were collected immediately before Tx (random, non-fasting). Samples taken at Months 3 (M3) and 12 (M12) were taken in a fasting condition. In CKD patients, samples were also taken in a fasting state. After processing, the samples were stored at −80°C until batch analysis. Serum haematocrit (Hct), C-reactive protein (CRP), urea nitrogen, creatinine, uric acid, immunosuppressive drug trough levels were measured with standard laboratory techniques. Serum albumin was measured with the bromocresol green method. The eGFR was measured with the Modification of Diet In Renal Disease equation (MDRD). Change in parameter levels are expressed as a percentage of BL. We divided the patients into two groups: decrease (<−5%) and stable or increase (change >−5%) in ADMA levels between and BL three months, BL and 1 year and three months and BL.

ADMA and SDMA measurements

Plasma ADMA and SDMA levels were analysed by means of a high-performance liquid chromatography method (see Supplementary data, Materials and Methods). The sample preparation for the measurement of ADMA and SDMA was adopted and modified from a method described earlier [24]. The limit of detection (LOD) was determined according to Armbruster et al. [25]. The lower limit of quantification (LLOQ) was defined as the lowest concentration of the analyte that could be quantified with a precision <15%. The LOD was 0.05 μM for all compounds, and the LLOQ for ADMA and SDMA was 0.1 μM. Method imprecision was assessed according to the NCCLS EP5–T guideline. Changes in plasma levels of
ADMA and SDMA over time were expressed as percentage change versus BL values.

**Statistics**

Parametric and non-parametric parameters are expressed as mean ± SD and median (minimum–maximum) when appropriate. Differences between time points were analysed using paired t-test, Wilcoxon signed rank, Friedman and McNemar’s testing, for continuous and dichotomous variables, respectively. Post hoc analyses of differences between periods were performed using Dunn’s multiple comparison test. Differences between groups were assessed by Wilcoxon rank-sum, Mann–Whitney U, unpaired T-tests or chi-square when appropriate. Non-parametric variables, including ADMA and SDMA, were log-transformed for the regression analyses. Multivariate linear regression was performed including all univariately associated variables (P < 0.2). This subset was then subjected to a final backward elimination. Collinearity diagnostics was performed by examining the tolerance and variance inflation factor (VIF). Values of VIF > 2.5 were regarded as indicating multicollinearity. All statistical calculations were carried out using SAS version 9.2 (SAS, Inc., Cary, NC, USA).

### RESULTS

**Patient characteristics**

Tables 1 and 2 summarize the main BL characteristics of the RTR group. The majority (95%) was transplanted with a kidney from a deceased donor. In this cohort only one patient was transplanted pre-emptively. The main cause of renal failure is glomerular disease. The majority of patients were on an immunosuppressive regimen consisting of a calcineurin inhibitor (CNI), MPA and CS. Methylprednisolone was tapered to 4 mg at 3 months; in 23 patients treatment with CS was halted after 3 months. The mean eGFR according to the MDRD formula amounted to 49.45 ± 16 and 52.24 ± 15 mL/min/1.73 m² at M3 and M12 respectively. BL plasma ADMA and SDMA levels were 0.63 µM and 2.05 µM. In nine patients a CV event occurred.

**Natural history of ADMA and SDMA in RTRs**

Table 2 demonstrates the natural history of plasma ADMA and SDMA levels. Overall, both ADMA and SDMA showed a
levels after 3 months. Twenty-seven per cent (37%) patients showed no decrease in ADMA decrease over time. Differences between both metabolites are, however, remarkable. SDMA levels showed a dramatic and uniform decrease. The decrease of ADMA levels after Tx, conversely, was more subtle. The most important decrease of SDMA occurred within the first 3 months after Tx [−59%, (−85 to 1.6%), P < 0.01]. Within the same time period ADMA levels overall decreased by 6% (range −41 to 52%, P < 0.05). In the group from BL to 3 months, 44% (n = 73) remained stable or increased. Sixty-one (37%) patients showed no decrease in ADMA levels after 3 months. Twenty-seven per cent (n = 19) of the latter patient group had an increase in the first 3 months as well.

In 84 patients, ADMA and SDMA levels were also investigated in the immediate postoperative period, i.e. at Days 7–14 (8 [5–15] days) after Tx. Plasma ADMA and SDMA levels were 0.68 µM and 1 µM, respectively. Remarkably, plasma ADMA levels at this time point were significantly higher than levels observed at the time of Tx [10% (−34 to 93%), P < 0.01] (Figure 1). A similar increase was observed in a subgroup of patients free of acute rejection and delayed graft function (n = 67; P < 0.01) (data not shown). SDMA levels, to the contrary, showed a steady decline parallel to the recovery of renal function. [−55% (−84 to 1%), P < 0.0001]. Between Months 3 and 12, the percentage change of plasma SDMA levels was significantly correlated with the percentage change of eGFR (r = −0.45; P < 0.001). For change of ADMA levels, no correlation was found with percentage change of eGFR.

**Case-controlled substudy: RTRs versus CKD patients**

Table 3 shows that RTRs at 1 year after Tx (n = 31) and CKD patients (n = 31) were well matched for age, gender, eGFR and diabetic status. Because the CKD patient study included only patients without CV history, all patients were free from CV history. SDMA levels were similar, whereas ADMA levels were significantly higher in RTRs. Total cholesterol and LDL cholesterol levels were also higher in RTRs and more RTRs were active smokers (P = 0.07). More CKD patients were on renin–angiotensin–aldosterone system (RAAS) blockade therapy. In univariate analysis, plasma ADMA levels were directly associated with transplant status (P = 0.005, R² = 0.13), plasma SDMA levels (P = 0.0032; R² = 0.14) and CRP levels (P = 0.06, R² = 0.06). An inverse association was found with the use of RAAS blockade (P = 0.02; R² = 0.09) and serum albumin levels (P = 0.003; R² = 0.14). Multivariate linear regression in the overall group showed that renal transplant status is significantly associated with plasma ADMA levels (P = 0.02), independent of plasma SDMA (P = 0.001), 24-h proteinuria (P = 0.0037) and lower serum albumin levels (P = 0.002).

**Determinants of ADMA at BL and 12 months**

Table 4 summarizes the results of the univariate and multivariate regression analyses with plasma ADMA levels as dependent variable.

At BL, higher BL SDMA (P = 0.02), CRP (P = 0.04) and Hct levels (P = 0.02), the presence of CV disease (P = 0.02) and haemodialysis (P = 0.03) were associated with higher ADMA levels. There was an inverse association with residual diuresis (P = 0.0031) and high-density lipoprotein (HDL) cholesterol (P = 0.0016). The association of postoperative CV events and ADMA levels reached borderline significance (P = 0.08). In multivariate analysis, presence of CV history, BL SDMA levels, Table 3. Demographics of CKD patients and RTR without CV history

<table>
<thead>
<tr>
<th>Variable</th>
<th>CKD (n = 31)</th>
<th>RTR (n = 31)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49 ± 10.5</td>
<td>48.4 ± 11</td>
<td>0.67</td>
</tr>
<tr>
<td>Gender (M/F) (%)</td>
<td>42/58</td>
<td>42/58</td>
<td>1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.36 ± 4.9</td>
<td>24.9 ± 5.1</td>
<td>0.6</td>
</tr>
<tr>
<td>ADMA (µM)</td>
<td>0.47 (0.33–0.78)</td>
<td>0.5 (0.39–0.8)</td>
<td>0.003</td>
</tr>
<tr>
<td>SDMA (µM)</td>
<td>0.74 (0.41–1.4)</td>
<td>0.75 (0.38–1.43)</td>
<td>0.3675</td>
</tr>
<tr>
<td>CRP (g/L)</td>
<td>2 (1–33)</td>
<td>1 (0.6–19.8)</td>
<td>0.12</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>45 ± 3.5</td>
<td>44 ± 3.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Hct</td>
<td>0.41 ± 0.04</td>
<td>0.40 ± 0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Tot cholesterol (mg/dL)</td>
<td>195.8 ± 30</td>
<td>178.8 ± 28.6</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>112.2 ± 37.6</td>
<td>93.6 ± 21.6</td>
<td>0.05</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>58 (41–101)</td>
<td>52.5 (29–102)</td>
<td>0.2</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>50.4 ± 16</td>
<td>48.8 ± 16</td>
<td>0.7</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>0.14 (0.04–2.94)</td>
<td>0.14 (0.06–1.87)</td>
<td>0.6</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>46%</td>
<td>54%</td>
<td>0.5</td>
</tr>
<tr>
<td>ACE or ARB (%)</td>
<td>81%</td>
<td>40%</td>
<td>0.001</td>
</tr>
<tr>
<td>Active smoking (%)</td>
<td>8%</td>
<td>20%</td>
<td>0.07</td>
</tr>
</tbody>
</table>

GFR, glomerular filtration rate (Cockcroft); tot, total; ACE, ACE-inhibitor; ARB, angiotensin receptor blocker.
lower HDL levels and haemodialysis were independently associated with higher BL plasma ADMA levels ($r^2 = 0.23$).

At 12 months, a positive association was found with the presence of CV history ($P = 0.04$), proteinuria ($P = 0.02$), higher CRP ($P = 0.02$), SDMA levels ($P < 0.0001$) and BL ADMA levels ($P < 0.0001$). An inverse association was demonstrated with serum albumin ($P = 0.0007$) and BL HDL cholesterol ($P = 0.03$). There was a trend for a positive association with cumulative steroid dose ($P = 0.09$).

In multivariate analysis, higher ADMA levels were associated with higher BL ADMA ($P < 0.0001$), higher SDMA levels at 12 months ($P < 0.0001$), higher eGFR ($P = 0.026$) and lower albumin levels ($P = 0.0013$). This model explained 34% of the variation.

**Determinants of SDMA BL and at 12 months**

At BL, higher ADMA ($P = 0.02$) was associated with higher SDMA levels in univariate analyses. There was an inverse relation with age ($P = 0.0003$), haemodialysis ($P = 0.03$), diabetes ($P = 0.02$), BMI ($P = 0.0008$), HbA1c ($P = 0.0009$) and CRP ($P = 0.02$). In multivariate analysis, higher BL SDMA levels was independently associated with lower residual diuresis ($P = 0.0009$), peritoneal dialysis ($P = 0.0001$), lower age ($P = 0.002$), lower HbA1c ($P = 0.04$), lower CRP ($P = 0.001$) and higher BL ADMA ($P = 0.002$). These variables explained 28% of the variation of BL SDMA levels.

At 12 months, a positive association with SDMA levels was found with ADMA levels ($P < 0.0001$), lower Hct ($P = 0.001$) and lower eGFR ($P < 0.0001$). In multivariate analysis, higher BL SDMA ($P = 0.0003$), higher serum albumin ($P = 0.02$), lower Hct ($P = 0.001$) and lower eGFR ($P < 0.0001$) were independently associated with SDMA levels. These variables explained 61% of the variation of SDMA levels at Month 12.

**Determinants of ADMA change in the first 14 days**

In Spearman’s correlation analysis, we found a higher increase of plasma ADMA levels in the immediate postoperative period in patients with a higher glucose concentration ($r = 0.24; P = 0.04$), shorter time period since Tx ($r = -0.36; P = 0.008$) and a higher CS dose ($r = 0.21; P = 0.05$). Cold ischaemia time ($r = 0.24; P = 0.09$) and change in Hct level ($r = -0.20; P = 0.07$) reached borderline significance. Male patients ($P = 0.02$), patients with acute rejection ($P = 0.02$) and patients with acute tubular necrosis present on BL biopsy ($P = 0.06$) also exhibited a higher increase in ADMA levels. Multivariate linear regression could not be performed because of not normally distributed residuals in the linear regression analysis.

In the subgroup without acute rejection or DGF, proteinuria ($r = 0.29; P = 0.03$), cold ischaemia time ($r = 0.3; P = 0.05$) and less time elapsing since Tx ($r = -0.43; P = 0.0003$) were associated with a higher increase of ADMA levels.

**DISCUSSION**

This study reports on the time course of plasma ADMA and SDMA levels after successful renal Tx. SDMA levels showed a dramatic decline paralleling the recovery of renal function. ADMA levels, conversely, followed a biphasic time course with an early increase followed by a subtle decline. Moreover, ADMA levels, contrary to SDMA levels, are higher in RTRs when compared with CKD counterparts.

ADMA levels observed in the present cohort at the time of Tx were higher than levels previously reported in healthy controls, but at the lower end of the spectrum reported in dialysis patients [26–28]. The range of ADMA levels reported in dialysis patients is wide and shows an overlap with values observed in non-renal patients (e.g. hypertensives) [29]. This wide range is at least partly explained by the use of different biochemical techniques and lack of standardization [26, 30, 31]. CV history, HDL levels and dialysis modality were identified as independent determinants of plasma ADMA levels at the time of Tx. In agreement with Kielstein et al. [9], but contrary

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**Table 4. Uni- and multivariate analyses of ADMA BL and at 12 months**

<table>
<thead>
<tr>
<th></th>
<th>ADMA BL</th>
<th></th>
<th></th>
<th>ADMA 12M</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PE</td>
<td>$R^2$</td>
<td>$P$</td>
<td>PE</td>
<td>$R^2$</td>
<td>$P$</td>
</tr>
<tr>
<td>Diuresis (mL)</td>
<td>-0.00007</td>
<td>0.06</td>
<td>0.003</td>
<td>0.079</td>
<td>0.026</td>
<td>0.038</td>
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<tr>
<td>CV history</td>
<td>0.09</td>
<td>0.04</td>
<td>0.016</td>
<td>0.07</td>
<td>0.04</td>
<td>ns</td>
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<tr>
<td>Smoker</td>
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<td>0.02</td>
<td>0.1</td>
<td>0.09</td>
<td>0.008</td>
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<tr>
<td>Haemodialysis</td>
<td>0.074</td>
<td>0.03</td>
<td>0.029</td>
<td>0.09</td>
<td>0.008</td>
<td>ns</td>
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<tr>
<td>SDMA (µM) BL</td>
<td>0.27</td>
<td>0.035</td>
<td>0.016</td>
<td>0.15</td>
<td>0.003</td>
<td>ns</td>
</tr>
<tr>
<td>CRP (g/L)</td>
<td>0.025</td>
<td>0.026</td>
<td>0.041</td>
<td>0.035</td>
<td>0.05</td>
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<tr>
<td>Hct (BL)</td>
<td>0.74</td>
<td>0.035</td>
<td>0.013</td>
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<td>HDL cholesterol</td>
<td>-0.003</td>
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<td>ACE or ARB</td>
<td>-0.06</td>
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<td>0.06</td>
<td>-0.002</td>
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<tr>
<td>ADMA (µM) BL</td>
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<td>0.34</td>
<td>0.11</td>
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<tr>
<td>CRP (g/L) 12 M</td>
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<td></td>
<td>0.07</td>
<td>0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SDMA (µM) 12 M</td>
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<td></td>
<td></td>
<td>0.25</td>
<td>0.14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
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<td></td>
<td></td>
<td></td>
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<td>0.001</td>
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<tr>
<td>Alb 12 m (g/L)</td>
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<td></td>
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<td>Proteinuria 12 m</td>
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<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>CS (g)</td>
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<td></td>
<td></td>
<td>0.00003232</td>
<td>0.017</td>
<td>0.095</td>
</tr>
</tbody>
</table>

Diuresis, residual diuresis; CV, cardiovascular; BL, baseline; 12M, 12 months; Hct, haematocrit; ACE, ACE-inhibition; ARB, angiotensin receptor blockade; CRP, C-reactive protein; eGFR, estimated GFR, MDRD formula; Alb, albumin; CS, cumulative dose corticoids.
to the Cardiovascular Risk Extended Evaluation in Dialysis Patients (CREED) investigators [32], we observed higher ADMA levels in patients on maintenance haemodialysis.

Data on levels of ADMA and SDMA in RTRs are scarce and moreover often inconsistent. Small sample size, case-mix including variable interval since Tx may explain this inconsistency. In the present study, ADMA levels were monitored in a large cohort of RTRs at Months 3 and 12 after Tx. In a subgroup of patients, ADMA and SDMA levels were also assessed during the second week after Tx. Plasma SDMA levels showed a steep decline after Tx which correlated highly with the improvement of renal function. Furthermore, SDMA levels at 1 year did not differ from CKD patients, matched for GFR, age, gender, diabetes and CV history. Our data confirm that renal excretion is the major determinant of SDMA levels [33].

The time course of ADMA levels after Tx conversely was biphasic with an early increase followed by a decline. Of note, the percentage change of plasma ADMA levels between Months 3 and 12 did not correlate with the improvement in renal transplant function within this time period. ADMA levels in RTR were also higher than levels observed in CKD counterparts. To our knowledge, there are four studies which compared the levels of ADMA and SDMA in different stages of CKD, including RTRs [7, 20, 21, 28]. In these four studies, no significant difference was found in ADMA levels in CKD and RTRs. This apparent controversy may be explained by the fact that we matched RTRs and CKD patients for various CV risk factors. In aggregate, these data suggest that transplant-related factors affect the synthesis and/or degradation by DDAH. Both the activated immune system, immunosuppressive agents and their metabolic side effects may account for the increased synthesis and decreased degradation in RTRs. Previous studies have indicated that CNI induce endothelial dysfunction and glucocorticoids (GC) therapy increases ADMA levels [34–36]. Furthermore, in the cross-sectional analysis, the use of angiotensin converting enzyme inhibitors or angiotensin receptor blocker blockade was associated with lower ADMA levels. We did not find any association of statin use with ADMA levels. This is consistent with previous data indicating that blockade of the RAAS lowers ADMA levels [37, 38]. Data on the effect of statin use on ADMA levels demonstrate conflicting results [39, 40].

The transient increase of ADMA levels in the early post-transplantation period is remarkable, especially since Zocalli et al and Kielstein described that acute inflammation decreases ADMA levels [41, 42]. Comparable short-term studies are scant and moreover yielded discrepant data [43]. This discrepancy may be related to case-mix. Yilmaz et al. [19] found a decrease of ADMA and hsCRP levels from Day 1, along with an improvement of endothelial function. Contrary to our study, patients enrolled in the latter study were all young, free from CV disease and recipients of a living-related donor. Our patients were older and mainly recipients of a deceased donor. Esposito et al. [22], on the other hand, reported increasing ADMA levels up to 6 months after Tx. We found that acute rejection and glucose level were significant correlates of the increase of ADMA levels in the immediate postoperative period. However, alloimmune activation or intensified GC therapy are not the only explanation for the increase of ADMA in the postoperative period as the increase was also noted in a subgroup of patients free from acute rejection. Furthermore, the increase was time-dependent with a higher increase in patients with blood samples taken closer to Tx. Of note, this early increase is consistent with recent animal data [18]. Several hypotheses can be raised to explain the increase of ADMA levels in the immediate post-transplant period. First, after ischaemia/reperfusion injury of rat kidneys total DDAH function is impaired [17, 18]. This is consistent with our results as we found a higher increase in patients with acute tubular necrosis on biopsy. Second, dimethylarginines are released upon degradation of methylated proteins. Release of dimethylarginines can be triggered by surgery (tissue damage) or the catabolic effects of GC [18, 34, 44–46]. Third, hyperglycaemia, a common problem after renal Tx, impairs DDAH activity [47–49]. We also found in univariate analyses a correlation between the percentage change of plasma ADMA level and glycaemic control. In line with this hypothesis, maintenance of normoglycaemia with insulin improved plasma ADMA levels while preserving DDAH activity in intensive care patients [47, 50]. Fourth, we found a borderline significance of decrease in Hct and increasing ADMA levels. Indeed, intact erythrocytes play an important role in storage of ADMA, whereas upon erythrocyte lysis large amounts of free ADMA can be generated by proteolysis of methylated proteins [44, 45]. Finally, oxidative stress has been reported to increase during the first weeks after Tx. Oxidative stress is a well-known suppressor of DDAH activity [51–53]. Therefore, we suggest that an increase in protein breakdown and decrease in DDAH activity outweigh the benefits of improved renal function and renal mass in the immediate post-transplant period. Further research is necessary to elucidate the pathophysiological mechanisms as well as the clinical consequences of this early increase of plasma ADMA levels.

Several limitations of our study need to be acknowledged. First, it should be acknowledged that BL ADMA and SDMA levels were assessed at the time of renal Tx, which implies different time periods since the end of the last haemodialysis session. This is less important for ADMA since the dialysance of this molecule is considered to be negligible [54–56]. BL levels of SDMA, conversely, may be lower than ‘true’ predialysis values, and should be interpreted as such. Second, the decrease of ADMA levels was only mild and our study was underpowered to address the highly relevant question of whether ADMA levels relate to hard outcomes. Clinical data in non-dialysis CKD patients, however, indicate that even small increases of ADMA levels are associated with worse outcome [14, 57]. Third, we did not measure 1-arginine levels. ADMA levels are correlated with circulating 1-arginine levels in patients with CKD [12]. Consequently we do not have data on the 1-arginine/ADMA ratio which might be a better surrogate for NO production. Finally, the observational nature of our study makes it impossible to draw any firm conclusions on the pathophysiological mechanisms. On the other hand, our study is the largest to report on the time course of ADMA levels after successful renal Tx.

In conclusion, ADMA levels follow a biphasic pattern after successful renal Tx with a transient rise in the immediate post-operative period followed by a subtle decline. Of note, levels remain elevated compared with CKD patients, matched for age,
gender, diabetes, CV history and renal function. Additional studies are required to elucidate the consequences of the increase of plasma ADMA levels after Tx as well as the underlying pathophysiological mechanisms.

SUPPLEMENTARY DATA
Supplementary data are available online at http://ndt.oxfordjournals.org.

CONFLICT OF INTEREST STATEMENT
None declared.

ACKNOWLEDGEMENTS
The authors thank A. Herelixka, M. Dekens, G. Lemmens, E. Vanhalewijk, H. Wielandt, M. Dubois, A. Swinnen and C. Beerten for their excellent technical support. They thank Sigrid de Jong and Tom Teerlink from Metabolic Laboratory of VU University Medical Center, Amsterdam, for the development and support of sample preparation of ADMA and SDMA.

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Received for publication: 13.1.2014; Accepted in revised form: 19.5.2014