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Symmetric Dimethylarginine as Predictor of Graft loss and All-Cause Mortality in Renal Transplant Recipients

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Background. Elevated symmetric dimethylarginine (SDMA) has been shown to predict cardiovascular events and all cause mortality in diverse populations. The potential role of SDMA as a risk marker in renal transplant recipients (RTR) has not been investigated.

Methods. We analyzed SDMA in the placebo arm of the Assessment of Lescol in Renal Transplantation study, a randomized controlled trial of fluvastatin in RTR. Mean follow-up was 5.1 years. Patients were grouped into quartiles based on SDMA levels at study inclusion. Relationships between SDMA and traditional risk factors for graft function and all-cause mortality were analyzed in 925 RTR using univariate and multivariate survival analyses.

Results. In univariate analysis, SDMA was significantly associated with renal graft loss, all-cause death, and major cardiovascular events. After adjustment for established risk factors including estimated glomerular filtration rate, an elevated SDMA-level (4th quartile, >1.38 μmol/L) was associated with renal graft loss; hazard ratio (HR), 5.51; 95% confidence interval (CI), 1.95–15.57; P=0.001, compared to the 1st quartile. Similarly, SDMA in the 4th quartile was independently associated with all-cause mortality (HR, 4.56; 95% CI, 2.15–9.71; P<0.001), and there was a strong borderline significant trend for an association with cardiovascular mortality (HR, 2.86; 95% CI, 0.99–8.21; P=0.051).

Conclusion. In stable RTR, an elevated SDMA level is independently associated with increased risk of all-cause mortality and renal graft loss.

Keywords: Symmetric dimethylarginine, Renal transplantation, Survival, Graft loss.

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Increased risk of cardiovascular (CV) disease and premature death in renal transplant recipients (RTR) cannot fully be explained by traditional risk factors (*1*). In addition to established predictors of CV disease, numerous modifiable and nonmodifiable emerging risk factors have been proposed to contribute to the excessive incidence of CV events in RTR. Increased symmetric dimethylarginine (SDMA), which is an established risk factor for CV events and all-cause mortality in other populations, has not been investigated in transplant patients.

The introduction of new and more efficient immunosuppressive agents has significantly reduced the incidence of acute rejection episodes. However, less has been achieved for long-term allograft survival, and chronic allograft dysfunction remains a major clinical challenge (*2*). To improve risk stratification and to identify potential treatment targets for this

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patient group, further investigations on novel risk factors for allograft loss are of importance.

We and others have shown that markers of inflammation are associated with impaired long-term patient and graft outcome (*3–5*). Inflammation seems to correlate with endothelial dysfunction and early atherosclerotic changes in patients with chronic kidney disease (CKD) (*6*). It has been hypothesized that some of the excessive CV risk associated with CKD can be attributed to mechanisms involving oxidative stress (*7*), development of endothelial dysfunction, and reduced bioavailability of nitric oxide (NO). Nitric oxide relaxes vascular smooth muscle and suppresses processes involved in vascular disease, including smooth muscle cell proliferation, leukocyte adhesion, and platelet aggregation (*8*). Oxidative stress is also believed to be a key element in the progression of chronic renal disease (*9, 10*) and chronic allograft dysfunction (*11, 12*).

Methylarginines are formed when constituent methylated arginine residues are released from intracellular proteins. These arginine analogues may interfere with NO production. High levels of asymmetric dimethylarginine (ADMA) may reduce the levels of NO by inhibiting the enzyme NO synthase (*13*). Asymmetric dimethylarginine is an established risk factor for CV events and mortality in different populations, and we have previously shown an association with both renal and CV long-term outcomes in RTR (*14*). The structural isomer, SDMA, does not directly inhibit NO synthase but might indirectly reduce its activity by limiting arginine supply (*15, 16*). Furthermore, SDMA seems to participate in the inflammatory pathways in CKD by activation of nuclear factor-κB and increased expression of interleukin (IL)-6 and tumor necrosis factor-α (*17*). Plasma concentrations of ADMA and SDMA are elevated in patients with CKD (*18*). The role of endogenous inhibitors of NO in transplantation is not fully elucidated. We therefore investigated the association between SDMA and long-term outcomes in a well-characterized cohort of stable RTR.

RESULTS

Baseline Characteristics

Participants in the Assessment of Lescol in Renal Transplantation (ALERT) study (*19*) were kidney allograft recipients with a stable graft function. Mean time from transplantation to randomization was 5.1 years. Baseline patient data including patient demographics, risk factors, and comorbidity have previously been presented (*20*).

Table 1 shows baseline characteristics for patients according to quartiles of SDMA. The groups were comparable in

TABLE 1. Demographic and baseline data according to quartiles of SDMA

	SDMA quartiles μmol/L				
Variables	**Q1 (n=248) 0.46–0.88 μmol/L**	**Q2 (n=216) 0.88–1.08 μmol/L**	**Q3 (n=232) 1.08–1.38 μmol/L**	**Q4 (n=229) 1.38–4.41 μmol/L**	***P***
Age at baseline, yr	51.7(10.4)	51.1 (11.2)	49.0 (10.7)	47.7 (11.2)	<0.001
Male sex	138 (55.6)	138 (63.9)	171 (73.7)	161 (70.3)	<0.000
Current smoker	36 (14.5)	29 (13.4)	48 (20.1)	53 (23.2)	0.003
Body mass index, kg/m^2	25.9 (4.2)	26.2 (4.3)	25.5 (4.7)	25.1 (4.5)	0.015
Diabetes mellitus	60 (24.2)	32 (14.8)	39 (16.8)	46 (20.1)	0.316
Hypertension	170 (68.5)	150 (69.4)	181 (78.0)	173 (75.5)	0.023
Systolic blood pressure, mm Hg	143.0 (18.8)	143.0 (18.0)	145.2 (18.0)	147.0 (21.2)	0.012
Diastolic blood pressure, mm Hg	85.7 (9.3)	85.6 (8.8)	86.5 (9.5)	85.9 (10.8)	0.548
Coronary heart disease	19 (7.7)	16 (7.4)	22 (9.4)	30 (13.1)	0.033
ADMA, μmol/L	0.72 (0.10)	0.76 (0.10)	0.80 (0.12)	0.86 (0.16)	<0.001
Serum creatinine, μmol/L	104.1 (17.8)	124.3 (24.2)	146.6 (29.0)	196.2 (60.9)	<0.001
eGFR, mL/min	63.2 (12.5)	53.3 (12.0)	45.9 (10.4)	34.6 (11.6)	<0.001
Proteinuria, g/24 hr	0.22 (0.37)	0.27 (0.57)	0.42 (0.79)	0.59 (0.99)	<0.001
HDL cholesterol, mmol/L	1.42 (0.45)	1.39 (0.46)	1.35 (0.44)	1.20 (0.39)	<0.001
LDL cholesterol, mmol/L	4.17 (0.98)	4.24 (1.10)	4.15 (0.96)	4.12 (1.02)	0.441
Triglycerides, mmol/L	2.19 (2.13)	2.14 (1.13)	2.22 (1.39)	2.37 (1.28)	<0.001
hsCRP, mg/L	2.80 (4.27)	3.64 (5.75)	3.46 (6.46)	4.23 (8.14)	0.646
IL-6, pg/mL	2.60 (1.52)	2.88 (1.89)	2.83 (1.80)	3.24 (2.02)	0.006
Time since last transplantation, yr	4.9 (3.3)	5.1 (3.3)	4.9 (3.4)	5.6 (3.6)	0.186
Time on dialysis, yr	2.0 (3.6)	2.1 (3.6)	2.5 (3.6)	2.8 (4.0)	<0.001
Cold ischemia time, hr	20.0 (7.8)	18.5 (7.8)	20.2 (7.1)	19.9 (7.9)	0.535
Panel reactive antibodies	43 (18.5)	39 (19.5)	30 (14.5)	30 (16.1)	0.305
Delayed graft function	31 (12.6)	31 (14.6)	47 (20.4)	54 (24.3)	<0.000
Treatment of cytomegalovirus	23 (9.4)	30 (14.4)	34 (15.5)	30 (13.6)	0.149

Total n=925.

Continuous variables are shown as mean (SD); categorical variables as n (% of total).

ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high sensitivity CRP; IL-6, interleukin-6. *P* values for linear trends (ANOVA/χ^2 test) are presented in the rightmost column, SD, standard deviation.

relation to time since transplantation, cold ischemia time, presence of panel reactive antibodies, and treatment of cytomegalovirus. There were, however, more patients with delayed graft function in the higher SDMA quartiles, and they experienced generally longer time on dialysis before transplantation. Symmetric dimethylarginine was positively associated with proteinuria and inversely correlated with estimated glomerular filtration rate (eGFR). Also, patients with elevated SDMA at baseline were slightly younger, more likely to be men and smokers, and showed a higher burden of coronary heart disease, hypertension, and dyslipidemia. Also, IL-6 values were significantly higher in the 4th SDMA quartile, whereas high sensitivity C-reactive protein (hsCRP) showed no such association with SDMA. *P* values for linear trends (analysis of variance and chi-square test) are presented in the rightmost column of Table 1.

Correlation Analysis

Correlation analyses were performed between SDMA and measures of kidney function as well as ADMA and selected parameters of inflammation (calculations not shown). The correlation coefficient was 0.77 for creatinine and 0.72 for eGFR. Symmetric dimethylarginine was significantly correlated with ADMA (r=0.40) and IL-6 (r=0.09), but not hsCRP (r=0.02, nonsignificant).

Survival Analysis

Results from univariate and multivariate Cox regression analyses are presented in Table 2. Fifty-two participants had missing values for one or more of the covariates and were excluded from the multivariate model. Hazard ratios (HRs) with corresponding 95% confidence intervals (95% CI) are shown for all study outcomes. The multivariate model is adjusted for baseline characteristics and potentially important risk factors including age, sex, smoking habit, established coronary heart disease, systolic blood pressure, low-density lipoprotein cholesterol, diabetes mellitus, hsCRP, ADMA, and eGFR split into quartiles.

Symmetric dimethylarginine showed a positive association with all study outcomes in univariate analysis, whereas after multivariate adjustment, there was a significant increased risk of death (HR, 4.56; 95% CI, 2.15–9.71; *P*<0.001) and graft loss (HR, 5.51; 95% CI, 1.95–15.57; *P*=0.001) in the highest SDMA quartile. When the mortality variable was further subdivided by cause of death, there was a strong association between SDMA and non-CV death (HR, 7.54; 95% CI, 2.54–22.40; *P*<0.001). For CV death, the increased risk associated with SDMA in quartile 4 was borderline significant (HR, 2.86; 95% CI, 0.99–8.21; *P*=0.051). A higher frequency of major adverse CV events (MACE) was seen in the 4th SDMA quartile, although this trend was not statistically

TABLE 2. Cox Regression Analysis for study outcomes using SDMA quartile 1 (Q1) as reference

	SDMA quartiles			
	Q1 0.46–0.88 μmol/L	Q2 0.88–1.08 μmol/L	Q3 1.08–1.38 μmol/L	Q4 1.38–4.41 μmol/L
Outcome	n=248	n=216	n=232	n=229
MACE				
Number of events	26 (10.5%)	24 (11.1%)	30 (12.9%)	40 (17.5%)
Univariate HR (95% CI)	1	1.03 (0.59–1.80)	1.24 (0.73–2.09)	1.88 (1.15–3.09)
Multivariate HR (95% CI)	1	0.99 (0.54–1.83)	1.10 (0.56–2.14)	1.64 (0.75–3.58)
All-cause mortality				
Nr of events	21 (8.5%)	20 (9.3%)	23 (9.9%)	61 (26.6%)
Univariate HR (95%CI)	1	1.04 (0.56–1.91)	1.15 (0.64–2.08)	3.53(2.15–5.79)
Multivariate HR (95%CI)	1	1.40 (0.72–2.71)	1.70 (0.83–3.47)	4.56 (2.15–9.71)
Cardiovascular death				
Number of events	12 (4.8%)	11 (5.1%)	13 (5.6%)	29 (12.7%)
Univariate HR (95% CI)	1	1.06 (0.48–2.37)	1.12 (0.51–2.46)	2.91 (1.48–5.70)
Multivariate HR (95%CI)	1	1.34 (0.56–3.22)	1.47 (0.56–3.87)	2.86 (0.99–8.21)
Noncardiovascular death				
Number of events	9 (3.6%)	9 (4.2%)	10 (4.3%)	32 (14.0%)
Univariate HR (95% CI)	1	0.99 (0.38–2.57)	1.19 (0.48–2.93)	4.36 (2.08–9.14)
Multivariate HR (95%CI)	1	1.41 (0.51–3.92)	1.97 (0.68–5.74)	7.54 (2.54–22.40)
RGL				
Number of events	6 (2.4%)	14 (6.5%)	24 (10.3%)	80 (34.9%)
Univariate HR (95%CI)	1	2.65 (1.02–6.88)	4.44 (1.82–10.87)	19.70 (8.59–45.16)
Multivariate HR (95%CI)	1	1.62 (0.59–4.48)	1.86 (0.68–5.11)	5.51 (1.95–15.57)

Number of events (in percentage of total) registered in each SDMA quartile during a mean of 5.1 years of follow-up. Univariate and multivariate hazard ratios with 95% confidence intervals (HR, 95% CI) for study outcomes for each SDMA quartile compared with the first quartile. In the multivariate model adjustments were made for: age, sex, diabetes mellitus, smoking status, systolic blood pressure, LDL cholesterol, coronary artery disease, ADMA, hsCRP and eGFR in quartiles.

MACE, major adverse cardiovascular events; RGL, renal graft loss; cardiovascular death, cardiac, vascular, and cerebrovascular deaths; HR, hazard ratio; 9%% CI, 95% confidence interval; hsCRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein.

significant in multivariate analysis (HR, 1.64; 95% CI, 0.75–3.58; *P*=0.212). We were not able to reveal any significant competing risks between nonfatal MACE and all-cause mortality (data not shown).

Figure 1 presents adjusted HRs with corresponding *P* values for the three most important causes of death: CV events, infection, and cancer. Compared with the first quartile, there was a more than ninefold increase in the risk of dying from cancer and a more than sevenfold increased risk of death by infection in the fourth SDMA quartile. The occurrence of death from cancer or infection according to SDMA quartiles are shown in Table S1 (**SDC,** http://links.lww.com/TP/A994).

Figures 2 to 3 show adjusted Cox hazard functions for all-cause death and renal graft loss according to SDMA quartiles, illustrating the relationship between SDMA level and the risk of adverse events as a function of time.

In an initial analysis, IL-6 replaced CRP without causing noticeable changes in HR for any of the study outcomes (data not shown). Furthermore, we constructed an extended model for the prediction of graft loss, adding to the multivariate analysis the following plausible risk factors for adverse renal outcome: time since last transplantation, total time on renal replacement therapy, baseline level of proteinuria, delayed graft function, and treatment of rejection (before randomization). Importantly, this extensive multivariate adjustment rendered the HR for SDMA quartile 4 essentially unchanged (HR, 4.02; CI, 1.37–11.80; *P*=0.011).

DISCUSSION

This study is the first to report that increased serum levels of SDMA, adjusted for traditional and nontraditional risk factors, are associated with reduced long-term graft and patient survival in kidney transplant recipients. The association between SDMA and clinical outcomes does not appear fully linear, the risk increasing substantially from the third to the fourth quartile. The increased HR for all-cause mortality in the fourth quartile was mainly driven by non-CV causes, but there was still borderline significance for an association between SDMA and CV death.

An association between SDMA and mortality has previously been reported in several nontransplanted populations: in patients referred for angiography (*21, 22*), in patients after an ischemic stroke (*23*) and in stable coronary heart disease patients(*24*). Recently, SDMA was found to independently predict mortality in a large (n=3523) multiethnic cohort representative of the general population (*25*). One study indicates that in an older population, plasma levels of SDMA seem predictive of CV events (*26*). Significant relationships between SDMA and development of major CV events have been found in patients undergoing elective diagnostic cardiac catheterization (*21, 22*), patients with stable coronary heart disease (*24*)

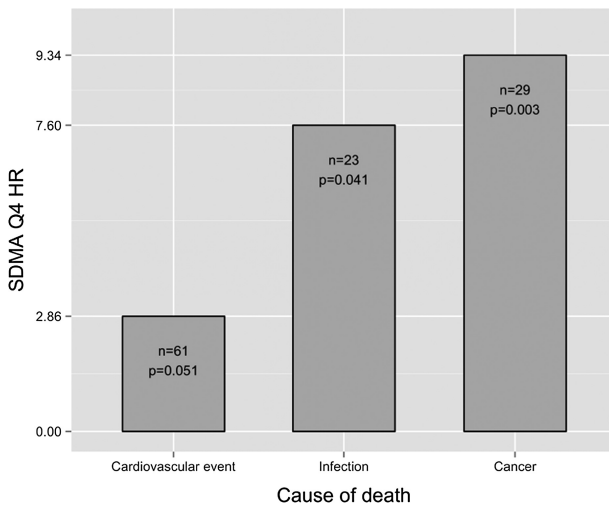

FIGURE 1. Hazard ratios with corresponding *P* values in the fourth SDMA quartile (SDMA Q4) stratified by cause of death. Multivariate Cox regression analysis adjusted for age, sex, diabetes mellitus, smoking status, systolic blood pressure, LDL cholesterol, coronary artery disease, hsCRP, ADMA and eGFR in quartiles. SDMA, symmetric dimethylarginine; hsCRP, high sensitivity C-reactive protein; ADMA, asymmetric dimethylarginine; LDL, low-density lipoprotein.

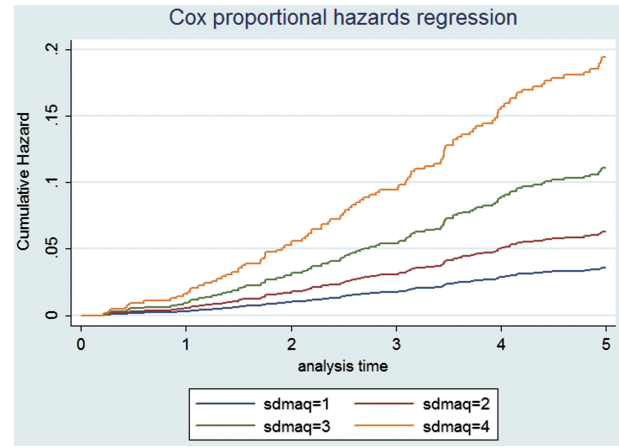

FIGURE 2. Adjusted cumulative hazard curves for all-cause mortality by quartiles of SDMA (SDMAq=1–4). SDMA, symmetric dimethylarginine.

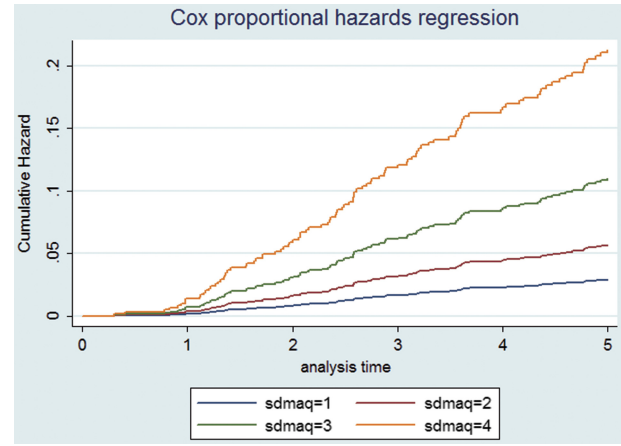

FIGURE 3. Adjusted cumulative hazard curves for renal graft loss by quartiles of SDMA (SDMAq=1–4). SDMA, symmetric dimethylarginine.

and patients with non-ST-elevation myocardial infarction (*27*). In patients with CKD (*28, 29*) and in primary care patients with and without peripheral arterial disease (*30*), increased CV risk was not related to SDMA.

The relationship between high SDMA and increased risk of all-cause death seems robust in our cohort of RTR. We identified a trend, but no significant association between SDMA levels and the composite endpoint of major CV events. For CV death, there was an almost threefold higher risk associated with the highest SDMA-quartile, although just borderline significant. In conclusion, our findings extend and corroborate that SDMA may be a marker for CV events and all-cause death (*21*).

We also showed that a high SDMA level in clinically stable RTR is independently predictive of renal graft loss. The possibility of SDMA being a separate risk factor for adverse renal outcomes in RTR has not previously been studied. Busch et al. (*29*) looked at the prognostic role of both ADMA and SDMA in a heterogeneous population of 200 CKD-patients including 37 renal transplant patients. Their results indicate that an increased serum level of SDMA (but not ADMA) might be a predictor for the progression to end-stage renal disease.

Compared to the healthy general population, mean SDMA levels were elevated in our RTR cohort (*31, 32*). Our SDMA values were in concert with a review by Fleck et al. (*33*) demonstrating high levels of dimethylarginines in end-stage renal disease patients, SMDA levels decreasing after renal transplantation, though not reaching reference values for healthy subjects.

Possible mechanisms for the relationship between high SDMA and poor long-term outcomes are not well defined. Symmetric dimethylarginine is believed to be biochemically inert and eliminated solely by renal filtration (*34, 35*). Hence, most of the difference in SDMA concentrations between various populations could be explained by its strong covariance with kidney function; an association first shown in a study on hypertensive children (*36*). A meta-analysis by Kielstein et al. (*37*) reports a correlation coefficient of 0.77 and 0.78 between SDMA and various GFR estimates and insulin clearance, respectively. In our study, we found a similar level of correlation between SDMA and eGFR. Even so, after adjusting for eGFR, SDMA was still independently associated with graft loss and mortality.

Symmetric dimethylarginine may influence L-arginine availability (*35*). Associations between dimethylarginines and CV disease in different patient groups are believed mainly to result from negative effects of these substances on endothelial function (*38, 39*), although a recent publication by Siegerink et al. (*24*) questions this, suggesting that alternative mechanisms for both dimethylarginines should be considered.

Symmetric dimethylarginine may trigger vascular pathology by modulation of store-operated calcium channels in monocytes (*40*), thus exerting a proinflammatory effect. Symmetric dimethylarginine is also shown to be involved in inflammatory pathways in CKD patients by activating nuclear factor-κB, leading to enhanced expression of tumor necrosis factor-α and IL-6 (*17*). Hence, a raised SDMA level may contribute to increasing the inflammatory burden.

The mean level of SDMA was significantly higher in our cohort than in most populations. A high SDMA level seems to identify a subpopulation of RTR more likely do die of infections and cancer. There is no known mechanism of action providing a plausible link between high serum levels of SDMA and the development of infection or malignancy in RTR. A high proportion of cancers in RTR are lymphoproliferative and related to infections with Epstein-Barr virus, human herpesvirus 8, human papilloma virus or the hepatitis B and C viruses (*41*). Symmetric dimethylarginine residuals are shown to be important constituents of the Epstein-Barr virus–encoded nuclear antigen 2 which is responsible for growth transformations in B lymphocytes(*42, 43*), but whether virus-related malignancy is associated with increased plasma levels of SDMA is not known. Further studies are needed to elaborate on this as well as to investigate whether associations between SDMA and specific non-CV causes of death can be found in other populations.

The main analysis is based on the placebo arm of the study. This cohort was selected because we wished to avoid the risk of interactions with the active intervention (statin therapy), as it is possible that statin use could modify SDMA levels or the biologic actions of SDMA. Degree of endothelium-dependent vasodilatation achieved by simvastatin treatment was indeed shown to vary across levels of ADMA (*44*), pointing in the direction of a possible interaction between statins and dimethylarginines. Statin treatment might improve endothelial function both in RTR (*45*) and in the general population (*46*). Endothelial NO synthase is upregulated by statins (*47*). Possibly, some of the beneficial effects of statins are mediated through pathways involving dimethylarginines. In our study, SDMA was measured at baseline only, before the initiation of statin therapy. Hence, speculations on mechanisms involved in the suspected interaction between SDMA and statin treatment was beyond the scope of this article.

The prospective controlled design, the long time of follow-up, the large patient cohort, and the independent adjudication of all clinical endpoints are major strengths of our study. However, there are potential limitations which merit consideration. Although the statistics show significant associations between SDMA and mortality as well as the renal endpoint, the data do not prove a casual relationship. Furthermore, the study population, a cohort selected for entry into a clinical trial, is not necessarily fully representative of the general renal transplant population

In conclusion, this is the first study to report that increased plasma levels of SDMA in stable RTR are significantly associated with future graft loss and all-cause mortality.

MATERIALS AND METHODS

Study Design

A post hoc analysis was performed using the data from RTR included in the ALERT trial. Study design with baseline data has previously been described (*20*). In short, this randomized, double-blind, placebo-controlled study examined the effect of fluvastatin (40–80 mg daily) on cardiac and renal outcomes in 2102 RTR. Inclusion criteria were stable RTRs aged 30–75 years having received a renal transplant more than 6 months before study start and having serum cholesterol in the range of 4.0–9.0 mmol/L (155–348 mg/dL). Exclusion criteria were ongoing statin therapy, familial hypercholesterolemia, an acute rejection episode in the last 3 months before inclusion or predicted life expectancy of less than 1 year. The original trial had a follow-up mean time of 5.1 years.

The ALERT study adhered to the International Conference on Harmonization guidelines for Good Clinical Practice and was conducted in accordance with the Declaration of Helsinki Principles. Written informed consent was obtained from all patients included, and the trial was approved by the local ethics committee at each participating center.

Outcome Definitions

Cardiac study outcome was the original primary endpoint in the ALERT trial—a composite endpoint of MACE, defined as time to cardiac death, non-fatal myocardial infarction or performed coronary revascularization procedure. Renal outcome was time to first renal graft loss (death-censored). General survival outcome was time to death from all causes. An independent clinical endpoint committee blinded to study drug allocation validated all endpoints (*19, 20*).

Laboratory

Baseline laboratory values of the ALERT trial have been reported previously(*20*). Symmetric dimethylarginine level was measured at baseline to be assessed as a risk factor at inclusion, a mean 5.1 years after transplantation. Reversed-phased high-performance liquid chromatography was used to measure SDMA level in frozen serum (−80°C) obtained from 925 of the 1,052 participants in the placebo arm, the last 127 samples missing at random. Estimated GFR (mL/min per 1.73 m^2) was calculated by the formula from the Modification of Diet in Renal Disease study (*48*).

Statistical Analysis

In reviewing the literature before conducting our analyses, we found evidence of a possible effect of statins on endothelial function. Initial statistical analyses indicated a significant interaction between SDMA and randomization group. Consequently, for a clean approach, we used the placebo arm only in subsequent analyses.

Study participants were stratified into quartiles according to SDMA levels. For comparison of demographics and known risk factors across quartiles, *P* values for continuous variables were calculated using analysis of variance with linear trend, whereas for categorical variables, we used the chi-square linear test for proportions. The variables IL-6, hsCRP, triglycerides, ADMA, creatinine, proteinuria, time since last transplantation, and time on dialysis were logarithmically transformed to correct for skewness.

Survival analyses were performed by Cox proportional hazard models. We calculated HRs with 95% CI by comparing the upper three quartiles to the first. We did not calculate HRs for SDMA as a continuous variable because the association between SDMA and outcomes appeared nonlinear when using categorical approach. Crude and multivariate adjusted HRs are presented. The multivariate model was adjusted for plausible confounders based on clinical knowledge and published literature. Colinearity between eGFR and SDMA was not a problem because standard errors remained of acceptable size when including both parameters in the statistics. Because eGFR as a continuous variable did not fully meet the proportional hazards assumption, we split this variable into quartiles for inclusion in subsequent statistical analyses. Remaining covariates fulfilled the proportionality assumptions according to the Schoenfeld residuals test. Possible competing risks between MACE (as study endpoint) and all-cause mortality (as competing risk endpoint) were examined by sensitivity analysis (*49*), including the subdistribution hazards method described by Fine and Gray (*50*).

All analyses were performed using SPSS version 18.0 (IBM, New York) and STATA version 11 (StataCorp, College Station, TX).

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