

CHAPTER 7

CIRCULATING MARKERS OF ENDOTHELIAL DYSFUNCTION INTERACT WITH PROTEINURIA IN PREDICTING MORTALITY IN RENAL TRANSPLANT RECIPIENTS

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Abstract

Background

Proteinuria is associated with endothelial dysfunction (ED) and increased mortality. We investigated whether urinary protein excretion (UPE) is correlated with markers of ED and whether these markers affect the association of proteinuria with mortality in renal transplant recipients (RTR).

Methods

Six hundred four RTR with a functioning graft for more than 1 year were included. RTR were divided according to UPE: less than 0.3, 0.3 to 1.0, and more than 1.0 g/24hr. Soluble intercellular adhesion molecule type 1 (sICAM-1) and soluble vascular cellular adhesion molecule type 1 (sVCAM-1) were measured using ELISA.

Results

UPE (0.2 [0.0–0.5] g/24 hr), sICAM-1 (603 [514–721] ng/mL), and sVCAM-1 (952 [769–1196] ng/mL) were measured at 6.0 (2.6–11.4) years posttransplant. During follow-up for 5.3 (4.7–5.7) years, 94 (16%) RTR died. UPE was correlated with sVCAM-1 (standardized $\beta=0.13$, $P=0.001$) but not with sICAM-1 (standardized $\beta=0.04$, $P=0.3$). RTR with UPE more than 1.0 g/24 hr and high sICAM-1 (hazard ratio=4.7, 95% confidence interval 2.3–9.7, $P<0.0001$) or sVCAM-1 (hazard ratio=4.2, 95% confidence interval 2.0–8.6, $P=0.0001$) concentrations were at increased risk for death, whereas RTR with UPE more than 1.0 g/24 hr and low concentrations of sICAM-1 and sVCAM-1 were not.

Conclusions

In RTR, UPE is correlated with sVCAM-1 but not with sICAM-1. Furthermore, RTR with proteinuria and high concentrations of sICAM-1 or sVCAM-1 have an increased risk for death, compared with RTR without proteinuria, whereas this is not the case in RTR with proteinuria but low concentrations of sICAM-1 and sVCAM-1. These results suggest that ED plays a role in the association of proteinuria with mortality after renal transplantation.

INTRODUCTION

Proteinuria is an established predictor of mortality, in particular cardiovascular mortality, not only in patients with diabetes and hypertension, but also in the normal population without overt renal disease.¹⁻⁶ The association of proteinuria with endothelial dysfunction (ED) is considered to be a mechanism underlying the elevated mortality in proteinuria.⁷⁻⁹

Proteinuria is not limited to native kidney disorders, but also develops in up to 30% of all long-term renal transplant recipients (RTR) where it can be due to renal diseases such as *de novo* glomerulonephritis, allograft glomerulopathy, and chronic rejection.¹⁰⁻¹³ It has been shown that proteinuria is a predictor for mortality in RTR as well.^{14,15} Cardiovascular mortality is the main cause of death in RTR,^{16,17} and ED might be an important early phenotype. However, whether ED is involved in the prognostic impact of proteinuria in RTR is unknown, as, first, it is unknown whether transplant proteinuria is associated with markers of ED, and, second, no data are available on the possible dependency of the prognostic impact of proteinuria in RTR on presence of markers of ED.

In the current study we investigated whether urinary protein excretion (UPE) is associated with markers of ED after renal transplantation and whether markers of ED affect the association of proteinuria with increased risk for mortality in RTR.

MATERIALS AND METHODS

Research design and subject

In this prospective cohort study, all RTR who visited our out-patient clinic between August 2001 and July 2003 and had a functioning graft for at least 1 year were eligible to participate at their next visit to the out-patient clinic. Recipients were asked to participate at a later visit to the out-patient clinic if they were ill or had signs of an infection. A total of 606 RTR signed written informed consent, from an eligible 847 (72% consent rate). The group that did not sign informed consent was comparable with the group that signed informed consent with respect to age, sex, body mass index (BMI), serum creatinine, creatinine clearance, and proteinuria. At inclusion, proteinuria was not determined in two RTR, leaving 604 RTR for analyses. Further details of this study have been published previously.¹⁸ The Institutional Review Board approved the study protocol (METc 01/039) which was in adherence to the Declaration of Helsinki.

Outcome events

All participating subjects visit the out-patient clinic at least once a year. Information on mortality is recorded by our renal transplant center through close contact with general practitioners and referring nephrologists. Mortality of all RTR were recorded until August 2007. There was no loss to follow-up.

Renal transplant characteristics

Relevant transplant characteristics were taken from the Groningen Renal Transplant Database. This database holds information on all renal transplantations performed at our center since 1968, including dialysis history. Standard immunosuppressive treatment and current medication were described previously.¹⁸ BMI, waist circumference, and blood pressure were measured as described previously.¹⁸ Smoking status and cardiovascular history were recorded with a self-report questionnaire. Cardiovascular disease history was considered positive if there was a previous myocardial infarction (MI), transient ischemic attack (TIA) or cerebrovascular accident (CVA).

Laboratory and clinical assessments

Plasma soluble intercellular adhesion molecule type 1 (sICAM-1) and plasma soluble vascular cellular adhesion molecule type 1 (sVCAM-1) concentrations were measured as markers of ED¹⁹ by enzyme-linked immunosorbent assay (ELISA) kits (Diaclone Research, Besanc, on, France). High sensitivity C-reactive protein (CRP) concentrations were determined using in-house enzyme-linked immunosorbent assays. Total cholesterol was determined using the CHOD PAP method (MEGA AU 510; Merck Diagnostica, Darmstadt, Germany). Low density lipoprotein (LDL) was calculated using the Friedewald formula. High density lipoprotein cholesterol (HDLc) was determined using the CHOD PAP method on a Technikon RA-1000 (Bayer Diagnostics b.v., Mijdrecht, The Netherlands). Plasma glucose was determined by the glucose-oxidase method (YSI 2300 Stat plus; Yellow Springs, OH, USA). Plasma creatinine concentrations were determined using a modified version of the Jaffé method (MEGA AU 510, Merck Diagnostica, Darmstadt, Germany). Total protein concentration was analyzed using the Biuret reaction (MEGA AU 510, Merck Diagnostica, Darmstadt, Germany).

Statistical analyses

Analyses were performed with SPSS version 14.0 (SPSS Inc., Chicago, IL) and Sigma Plot version 10 (Systat software Inc., Germany). Parametric parameters are given as means \pm standard deviation (SD), whereas non-parametric parameters are given as median[interquartile range]. Hazard ratio's (HR) are reported with [95% confidence interval (CI)]. A two-sided *P*-value less than $P < 0.05$ indicated statistical significance. For interaction terms, two-sided *P*-values < 0.10 were considered to indicated statistical significance.

Baseline characteristics are shown according to subgroups of UPE: (1) < 0.3 g/24hr, (2) $0.3-1.0$ g/24hr, and (3) > 1.0 g/24hr. Relationships between UPE and baseline characteristics were investigated by Pearson chi-square analyses for percentages and by univariate linear regression analyses for UPE versus baseline characteristics with a continuous distribution (log-transformation was applied for variables with a skewed

distribution). Potential correlations of UPE with markers of ED were explored using scatter-plots. Statistical significance was tested using linear regression analyses.

To analyze proteinuria as potential predictor of mortality, we first performed Kaplan-Meier analyses with a Log-Rank test. Predictive performance of individual parameters for mortality was assessed by determining area under the curve (AUC) of receiver operating characteristic (ROC) curves. Statistical differences between AUCs were compared nonparametrically by the method of DeLong *et al*²⁰. Cox-proportional hazard regression was used to estimate the effect of UPE and concentrations of adhesion molecules on mortality. In the multivariate analysis the associations of UPE and concentrations of adhesion molecules with mortality were adjusted for time between transplantation and inclusion date and creatinine clearance (Model 2), subsequently for recipient age and sex (Model 3), and for risk factors for atherosclerosis: systolic blood pressure, smoking, myocardial infarction, cerebrovascular attack, concentration of triglycerides, HDL, and LDL, concentrations of glucose and insulin, percentage HBA1c, and diabetes mellitus (Model 4). In addition, the association of UPE with mortality and the associations of concentrations of adhesion molecules with mortality were adjusted for each other (Model 5).

Finally, we investigated whether there was an interaction between proteinuria and concentrations of sICAM-1 and sVCAM-1 in predicting mortality. The interactions were tested by entering proteinuria, concentrations of sICAM-1 or sVCAM-1, and their product term in Cox-regression analyses as continuous variables. To visualize this, HRs were reported according to subgroups of proteinuria and dichotomized concentrations of sICAM-1 and sVCAM-1.

RESULTS

Baseline characteristics according to subgroups of UPE (<0.3 g/24hr, 0.3-1.0 g/24hr, and >1.0 g/24hr) are shown in table 1. A total of 604 RTR (55% male, aged 51.4±12.1 years, 86% cadaveric transplants) were analyzed. Median [interquartile range] sICAM-1 concentration was 603 [515-720] ng/mL and median sVCAM-1 concentration was 952 [769-1196] ng/mL. Median UPE was 0.2 [0.0-0.5] g/24hr. Median time between transplantation and inclusion date was 6.0 [2.6-11.4] years. The prevalence of male RTR was significantly higher in the proteinuric subgroup with UPE 0.3-1.0g/24hr than in the non-proteinuric subgroup with UPE <0.3 g/24h and in the proteinuric subgroup with UPE >1.0 g/24h. With increasing amount of UPE, RTR were more often smoker, had higher systolic and diastolic blood pressure, had lower concentrations of HDL-c, higher concentrations of triglycerides, higher concentrations of sVCAM-1, higher concentrations

of CRP, higher concentrations of serum creatinine, lower creatinine clearance and more often a history of acute rejection.

Table 1. Baseline characteristics according to subgroups of UPE.

| | UPE | | | P-value |
|---|------------------|------------------|------------------|---------|
| | <0.3g/24hr | 0.3-1.0g/24hr | >1.0g/24hr | |
| n (%) | 320 (53) | 228 (38) | 56 (9) | |
| Recipient demographics | | | | |
| Age (years) | 51.6 ± 12.4 | 51.8 ± 11.5 | 48.8 ± 13.0 | 0.2 |
| Male sex, n (%) | 153 (48) | 147 (65) | 30 (54) | 0.0006 |
| Body composition | | | | |
| BMI (kg/m ²) | 26.0 ± 4.4 | 26.0 ± 4.1 | 26.1 ± 4.7 | 1.0 |
| Waist circumference (cm) | 96.1 ± 13.9 | 98.4 ± 13.4 | 97.7 ± 14.2 | 0.2 |
| Smoking, n (%) | 59 (18) | 56 (25) | 18 (32) | 0.04 |
| Blood pressure | | | | |
| Systolic pressure (mmHg) | 150 ± 22.3 | 155 ± 22.9 | 160 ± 22.6 | 0.001 |
| Diastolic pressure (mmHg) | 88.7 ± 9.8 | 90.8 ± 9.8 | 92.8 ± 9.7 | 0.003 |
| Use of ACE-inhibitor or All-antagonist, n (%) | 97 (30) | 78 (34) | 26 (46) | 0.06 |
| Use of β-blocker, n (%) | 184 (58) | 148 (65) | 40 (71) | 0.06 |
| History of cardiovascular disease | | | | |
| MI, n (%) | 24 (8) | 22 (10) | 2 (4) | 0.3 |
| TIA/CVA, n (%) | 19 (6) | 10 (4) | 4 (7) | 0.6 |
| Lipids | | | | |
| Total cholesterol (mmol/L) | 5.6 [5.0-6.1] | 5.6 [4.8-6.3] | 5.7 [4.8-6.6] | 0.9 |
| LDL (mmol/L) | 3.5 [2.9-4.0] | 3.5 [2.9-4.1] | 3.6 [2.9-4.3] | 0.8 |
| HDL-c (mmol/L) | 1.1 [0.9-1.3] | 1.0 [0.9-1.3] | 1.0 [0.8-1.3] | 0.009 |
| Triglycerides (mmol/L) | 1.8 [1.3-2.5] | 2.0 [1.5-2.8] | 2.1 [1.4-3.1] | 0.03 |
| Use of statin at inclusion, n (%) | 160 (50) | 114 (50) | 25 (45) | 0.7 |
| Glucose homeostasis | | | | |
| Glucose (mmol/L) | 4.5 [4.1-5.0] | 4.6 [4.2-5.1] | 4.5 [4.0-5.1] | 0.3 |
| Insulin (μmol/L) | 10.7 [7.8-16.1] | 11.6 [8.1-15.8] | 11.9 [8.0-18.5] | 0.6 |
| Diabetes after transplantation, n (%) | 58 (18) | 38 (17) | 10 (18) | 0.9 |
| Use of antidiabetic drugs (%) | 45 (14) | 28 (12) | 6 (11) | 0.7 |
| Endothelial function parameters | | | | |
| sICAM-1 (ng/mL) | 603 [510-712] | 597 [526-721] | 637 [504-787] | 0.3 |
| sVCAM-1 (ng/mL) | 922 [765-1161] | 946 [755-1235] | 1124 [913-1330] | 0.001 |
| CRP (mg/L) | 1.8 [0.8-4.6] | 2.3 [1.0-4.8] | 2.9 [0.7-7.1] | 0.02 |
| Time after transplantation (years) | 10.3 [7.1-15.9] | 10.7 [7.0-15.8] | 10.4 [7.6-15.0] | 0.6 |
| Donor demographics | | | | |
| Age (years) | 35.4 ± 15.1 | 39.3 ± 15.2 | 36.8 ± 17.0 | 0.01 |
| Male sex, n (%) | 173 (55) | 126 (55) | 28 (50) | 0.8 |
| Renal allograft function | | | | |
| Serum creatinine concentration (μmol/L) | 126 [103-150] | 141 [120-175] | 175 [130-251] | <0.0001 |
| Creatinine clearance (mL/min) | 65.7 ± 21.4 | 59.7 ± 21.3 | 49.9 ± 27.4 | <0.0001 |
| UPE (g/24hr) | 0.0 [0.0-0.4] | 0.4 [0.3-0.6] | 1.7 [1.3-2.7] | <0.0001 |
| Prior dialysis duration (mo) | 29.0 [13.0-50.0] | 26.0 [14.0-47.0] | 24.5 [10.3-39.8] | 0.2 |
| Transplantation type, n (%) | | | | |
| Cadaveric donor | 270 (84) | 204 (89) | 47 (84) | 0.2 |
| Living donor | 50 (16) | 24 (11) | 9 (16) | |
| Ischemia times | | | | |
| Cold ischemia times (hr) | 22.0 [14.0-27.0] | 22.0 [16.0-27.0] | 20.0 [14.0-26.5] | 0.4 |
| Warm ischemia times (min) | 35.0 [30.0-45.0] | 35.0 [30.0-45.0] | 37.0 [30.3-45.8] | 1.0 |
| HLA mismatches, n | | | | |
| HLA-AB | 1.3 ± 1.0 | 1.3 ± 1.0 | 1.3 ± 1.2 | 0.9 |
| HLA-DR | 0.4 ± 0.6 | 0.4 ± 0.6 | 0.4 ± 0.6 | 0.9 |

Table 1 continued

| | UPE | | | P-value |
|----------------------------------|-----------------|-----------------|------------------|---------|
| | <0.3g/24hr | 0.3-1.0g/24hr | >1.0g/24hr | |
| Acute rejection, n (%) | 129 (40) | 110 (48) | 32 (57) | 0.03 |
| Acute rejection treatment, n (%) | | | | |
| High doses corticosteroids | 89 (28) | 78 (34) | 20 (36) | 0.09 |
| Antilymphocyte antibodies | 40 (13) | 32 (14) | 12 (21) | |
| Immunosuppression | | | | |
| Prednisolone dose, (mg/day) | 10.0 [7.5-10.0] | 10.0 [8.8-10.0] | 10.0 [10.0-10.0] | 0.1 |
| Calcineurine inhibitor, n (%) | 258 (81) | 178 (78) | 37 (66) | 0.05 |

Values are presented as mean ± SD, median [interquartile range] or percentages. Relationships between UPE and baseline characteristics were investigated by Pearson χ^2 analyses for percentages and by univariate linear regression analyses for UPE versus baseline characteristics with a continuous distribution (log-transformation was applied for variables with a skewed distribution).

BMI, body mass index; ACE, angiotensin-converting enzyme; LDL, low-density lipoprotein; HDL, high-density lipoprotein; MI, myocardial infarction; TIA, transient ischemic attack; CVA, cerebrovascular accident; HLA, human leukocyte antigen; sICAM, soluble intercellular adhesion molecule type 1; sVCAM, soluble vascular cellular adhesion molecule type 1; CRP, C-reactive protein.

Correlations of UPE with sICAM-1 and sVCAM-1 are shown in figures 1A and 1B respectively. UPE was not significantly correlated with sICAM-1 (standardized $\beta=0.04$, $P=0.3$), while UPE was significantly correlated with sVCAM-1 (standardized $\beta=0.13$, $P=0.001$).

A total of 94 (16%) RTR died during median follow up for 5.3 [4.7-5.7] years. UPE in RTR who stayed alive during follow up was lower compared to UPE in RTR that died (0.2 [0.0-0.5] g/24hr versus 0.3 [0.2-0.8] g/24hr, $P<0.01$). In the non-proteinuric subgroup of RTR with UPE <0.3 g/24hr 39 (12%) RTR died, whereas 39 (17%) and 16 (29%) RTR died in the proteinuric subgroups of RTR with UPE 0.3-1.0 g/24hr and >1.0 g/24hr respectively (Log-Rank test: $P=0.002$).

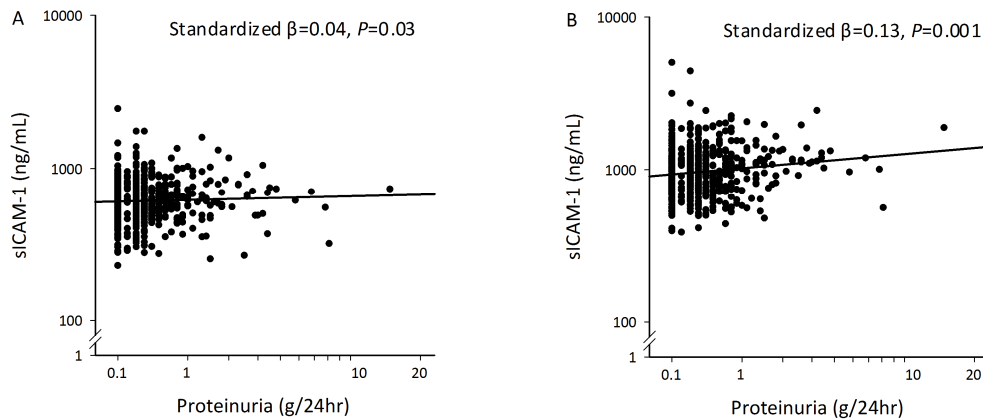


Figure 1. Scatter-plots of proteinuria versus (A) sICAM-1 and (B) sVCAM-1 concentrations. Significance was tested using linear regression analyses.

ROC analysis of the prediction of mortality revealed a mean (SE) AUC of 0.58 (0.03) for sICAM-1, 0.64 (0.03) for sVCAM-1, 0.57 (0.04) for serum creatinine concentration, 0.60 (0.03) for UPE, and 0.67 (0.03) for creatinine clearance. The AUCs of serum creatinine and sICAM-1 were significantly lower (both $P < 0.05$) than the AUC of creatinine clearance. No statistically significant differences were found for comparisons of predictive performance of other pairs of parameters ($P > 0.2$ for the other comparisons).

Table 2 shows the univariate and multivariate Cox regression analyses for mortality in RTR.

Table 2. Univariate and multivariate Cox regression analyses for mortality in RTR.

| | ² log sICAM-1 | | ² log sVCAM-1 | | ² log UPE | |
|---------|--------------------------|---------|--------------------------|---------|----------------------|---------|
| | HR [95% CI] | P-value | HR [95% CI] | P-value | HR [95% CI] | P-value |
| Model 1 | 2.2 [1.4-3.4] | 0.001 | 2.6 [1.8-3.7] | <0.0001 | 1.7 [1.3-2.3] | 0.0003 |
| Model 2 | 1.8 [1.2-2.8] | 0.008 | 2.5 [1.7-3.6] | <0.0001 | 2.0 [1.5-2.6] | <0.0001 |
| Model 3 | 1.9 [1.2-3.0] | 0.005 | 2.0 [1.4-3.0] | 0.0005 | 1.5 [1.1-2.1] | 0.009 |
| Model 4 | 1.7 [1.0-2.7] | 0.04 | 1.9 [1.3-2.9] | 0.003 | 1.6 [1.1-2.2] | 0.02 |
| Model 5 | 1.7 [1.1-2.8] | 0.02 | 1.9 [1.3-3.0] | 0.003 | 1.6 [1.1-2.3] | 0.01 |

Model 1: crude model

Model 2: adjustment for recipient age and sex

Model 3: Model 2 + additional adjustment for time between transplantation and inclusion date and creatinine clearance

Model 4: Model 3 + additional adjustment for systolic blood pressure, smoking, MI/CVA/TIA, concentration triglycerides, HDL, and LDL, fasting concentration glucose and insulin, percentage HBA1c, and diabetes mellitus

Model 5: Model 4 + additional adjustment for

- UPE in case of sICAM-1
- UPE in case of sVCAM-1
- sICAM-1 and sVCAM-1 in case of UPE

sICAM-1, sVCAM-1, and urinary protein excretion were entered in the regression analyses as ²log transformed variables in all analyses.

sICAM-1, soluble intercellular adhesion molecule type 1; sVCAM-1, soluble vascular cellular adhesion molecule type 1; UPE, urinary protein excretion; HR, hazard ratio; CI, confidence interval.

Two-fold increases in sICAM-1, sVCAM-1 and UPE were associated with hazard ratios (HRs) of 2.2 (95% CI 1.4-3.4, $P=0.001$), 2.6 (95% CI 1.8-3.7, $P<0.0001$), and 1.7 (95% CI 1.3-2.3, $P=0.0003$) respectively for the prediction of all-cause mortality (table 2, Model 1). Adjustment for recipient age and sex and further adjustment for time between transplantation and inclusion date and creatinine clearance (table 2, Model 2 and 3) did not materially change these results. Finally, the associations were adjusted for atherosclerotic risk factors: systolic blood pressure, smoking, myocardial infarction, cerebrovascular attack, concentration of triglycerides, HDL, and LDL, concentrations of glucose and insulin, percentage HBA1c, and diabetes mellitus. After this adjustment, two-fold increases in sICAM-1, sVCAM-1 and UPE were associated with HRs of 1.7 (95% CI 1.0-

2.7, $P=0.04$), 1.9 (95% CI 1.3-2.9, $P=0.003$), and 1.6 (95% CI 1.1-2.2, $P=0.01$) respectively for prediction of all-cause mortality (table 2, Model 4). Further adjustment of the association of sICAM-1 and sVCAM-1 with mortality for UPE and of the association of UPE with mortality for sICAM-1 and sVCAM-1 did not materially change these results (table 2, Model 5).

To investigate whether there was an interaction between proteinuria and concentrations of sICAM-1 and sVCAM-1 in predicting mortality, the interactions were tested by entering UPE, concentrations of sICAM-1 or sVCAM-1, and their product term in Cox-regression analyses as continuous variables. We found a significant interaction between UPE and concentration of sICAM-1 ($P=0.04$) for prediction of mortality and a borderline significant interaction between UPE and concentration of sVCAM-1 ($P=0.1$).

Figure 2 shows the results of the Cox regression analysis for the interaction of UPE with (a) sICAM-1 and (b) sVCAM-1. sICAM-1 (≤ 603 and >603 ng/mL) and sVCAM-1 (≤ 951 and >951 ng/mL) concentrations were dichotomized for these analyses.

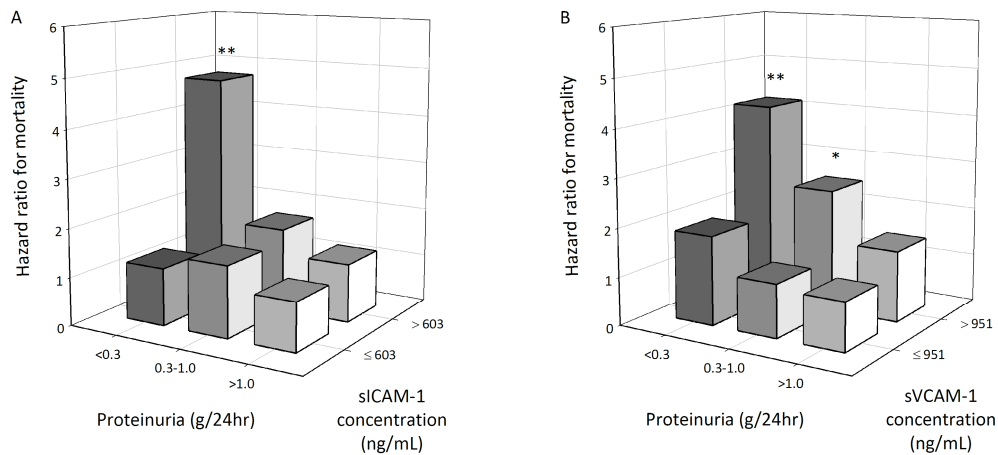


Figure 2. Interactions between UPE (<0.3 g/24hr, 0.3-1.0 g/24hr, and >1.0 g/24hr) and dichotomized concentrations of (A) sICAM-1 and (B) sVCAM-1 on the risk of mortality. Numbers in the bars represent the hazard ratio for mortality. * $P < 0.005$ and ** $P < 0.001$ compared to non-proteinuric RTR with UPE <0.3 g/24hr and low concentrations of sICAM-1 or sVCAM-1.

RTR with UPE >1.0 g/24hr and high concentration of sICAM-1 or sVCAM-1 were at highest risk for death (HR 4.7, 95% CI 2.3-9.7, $P < 0.0001$ and HR 4.2, 95% CI 2.0-8.6, $P = 0.0001$, respectively) compared to subjects without proteinuria (UPE <0.3 g/24hr) and low concentrations of sICAM-1 or sVCAM-1 (which were considered reference groups, with HRs of 1.0 by definition). RTR with UPE 0.3-1.0 g/24hr and high concentrations of sVCAM-1 also had an increased risk for death (HR=2.5, 95% CI 1.4-4.6, $P = 0.003$) compared

to the non-proteinuric reference group. RTR in the other subgroups did not have an increased risk for death. Importantly, RTR with UPE >1.0 g/24hr and low concentrations of markers of ED did not have a significantly increased risk for mortality. Additional adjustment for time between transplantation and inclusion date and creatinine clearance and further adjustment for recipient age and sex did not materially change these results.

DISCUSSION

This study shows that UPE and high concentrations of sICAM-1 and sVCAM-1 predict mortality late after renal transplantation, independent of risk factors for atherosclerosis. RTR with proteinuria and high concentrations of sICAM-1 or sVCAM-1 appear to be at highest risk for death.

One-year graft survival after renal transplantation has steadily improved from approximately 40% in the 1970's to more than 90% nowadays.^{21,22} Long-term graft survival, however, has not paralleled this improvement. Approximately half of all cadaveric renal allografts are still lost within 10-12 years after transplantation.²³ One of the major causes of late allograft loss is death, mainly due to cardiovascular disease which is mostly a consequence of atherosclerosis.¹⁶ In this study we showed that UPE predicts all-cause mortality in RTR independent of risk factors for atherosclerosis and markers of ED. The observation that UPE is a risk factor for all-cause mortality is in accordance with other studies.^{14,15} Roodnat *et al*,¹⁴ showed that RTR with proteinuria defined as >0.2 g/L had an increased risk (HR=2.0) for death compared to non-proteinuric RTR. This was consistent with a report of Fernandez-Fresnedo *et al*,¹⁵ who found an increased risk for recipient death (HR=1.9) in proteinuric RTR (UPE >0.5g/day) compared to non-proteinuric RTR. Our study extends these findings, because we adjusted for risk factors for atherosclerosis and included data on endothelial function.

To our knowledge, our study shows for the first time that concentrations of sICAM-1 and sVCAM-1 are associated with increased mortality in RTR, even after adjustment for other risk factors for atherosclerosis. The main cause of mortality after renal transplantation is cardiovascular disease which is mostly a consequence of atherosclerosis.¹⁶ Both sICAM-1 and sVCAM-1 are considered markers of ED¹⁹ because they play a significant role in the development of atherosclerosis by facilitating the firm attachment of leukocytes and their migration into the arterial wall.²⁴ Studies in subjects of the general population and in patients with type 1 diabetes and end-stage renal disease indeed found that high serum concentrations of adhesion molecules predict future cardiovascular events and all-cause mortality.²⁵⁻²⁸

Several studies have shown that proteinuria predicts mortality, due to cardiovascular disease in particular, in patients with diabetes mellitus and in the general population.¹⁻⁶ Stroes *et al*⁷ have shown that proteinuria in the nephrotic range is accompanied by

impaired endothelium-dependent vasomotion. The literature concerning a potential correlation of proteinuria with sICAM-1 and sVCAM-1 is sparse. In patients with primary glomerulonephritis, Mackinnon *et al*⁹ found significant correlations between proteinuria and concentrations of sICAM-1 with $r=0.19$ and sVCAM-1 with $r=0.37$. Furthermore, it has been shown that in patients with macro-albuminuria concentrations of sVCAM-1 and sICAM-1 are increased compared to healthy controls, again with the strongest relation for sVCAM-1.⁸ Another study in patients with type 2 diabetes reported a similar correlation between proteinuria and sVCAM-1 as in our study, and also absence of a correlation between proteinuria and sICAM-1.²⁹ Proteinuria after renal transplantation is commonly believed to be a reflection of renal involvement.¹⁰⁻¹³ In this study, we found that sVCAM-1, albeit weakly, but similarly to patients with type 2 diabetes, is correlated with UPE. We also found that RTR with UPE >1.0 g/24hr and high concentrations of sICAM-1 or sVCAM-1 have an increased risk for death compared to RTR with UPE <1.0 g/24hr but low concentrations of sICAM-1 or sVCAM-1. These results suggest that generalized ED plays a role in linking proteinuria with increased risk for mortality. It furthermore suggests that the assessment of circulating adhesion molecules can help to identify RTR in which proteinuria is associated with vascular involvement or at least increased risk for mortality.

This study has some limitations. The RTR were included at different time points after transplantation; this could induce healthy survivor bias and therefore it would be interesting if future studies would investigate the influence of markers of ED on the development of proteinuria at a fixed time point (e.g., 1 year) after transplantation. Also in our study, markers of ED and rates of UPE were assessed from samples taken at one time point in each patient. It would be interesting to investigate in a future study whether sequential measurements of markers of ED could be used as an even earlier marker for proteinuria and can be used to predict development of proteinuria. An important strength of this study is that follow-up was complete for all patients.

In conclusion, this study shows that UPE and concentrations of sICAM-1 and sVCAM-1, as markers of ED, independently predict mortality late after transplantation. Furthermore, RTR with proteinuria and high concentrations of sICAM-1 or sVCAM-1 appear to be at highest risk for death. These results suggest that the association of proteinuria with increased mortality after renal transplantation is not merely a reflection of renal involvement but that ED also plays a role in this association. Future studies are needed to confirm the interaction between markers of ED and proteinuria in predicting mortality in RTR.

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