

Risk Factors Associated with Coronary Artery Calcification Should Be Examined before Kidney Transplantation

Sanja Simic-Ogrizovic,^{1,2} Natasa Bogavac-Stanojevic,³ Maja Vuckovic,⁴
Violeta Dopsaj,³ Voja Giga,⁵ Milica Kravljaca,¹ Milan Stosovic¹ and Visnja Lezaic^{1,2}

¹Nephrology Clinic, Clinical Centre of Serbia, Belgrade, Serbia

²School of Medicine, Belgrade University, Belgrade, Serbia

³Institute of Medical Biochemistry, Faculty of Pharmacy, Belgrade, Serbia

⁴Institute of Radiology, Clinical Centre of Serbia, Belgrade, Serbia

⁵Cardiology Clinic, Clinical Centre of Serbia, Belgrade, Serbia

The best treatment for end stage renal disease (ESRD) patients is kidney transplantation, but the renal transplant recipients still have a higher incidence of cardiovascular events compared with general population. Cardiovascular risk factors were imposed long before ESRD, as the majority of patients starting dialysis or kidney transplantation already have signs of advanced atherosclerosis. Artery calcification is an organized, regulated process similar to bone formation. Coronary artery calcification (CAC) is found frequently in advanced atherosclerotic lesions and could be a useful marker of them. We evaluated the prevalence of CAC in 49 stable renal transplant recipients and in 48 age- and gender-matched patients with chronic kidney disease (CKD) in stages 2-5 not requiring dialysis to assess risk factors associated with CAC. Computed tomography was used for CAC detection and quantification (CAC score). The prevalence of CAC was 43.8% in transplant recipients and 16.7% in CKD patients ($p < 0.001$). Transplant recipients with CAC were significantly older and had longer duration of CKD and/or dialysis than recipients without CAC. In contrast, the serum levels of fetuin A (an inhibitor of vascular calcification) and albumin were significantly lower in CKD patients with CAC than those without CAC. During the observation period (30 months), 30 patients, including 23 CKD patients, began dialysis, and 4 transplant recipients and 2 CKD patients died. Independent predictors of mortality were age, serum amyloid A and the CAC score. In conclusion, the examination and prevention of risk factors associated with atherosclerosis should be started at the beginning of renal failure.

Keywords: chronic kidney disease; coronary artery calcification; fetuin A; inflammation; renal transplant recipient
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The standard treatment for patients with end stage renal disease (ESRD) is kidney transplantation as it considerably prolongs life mainly by impeding the progression of cardiovascular disorders by improving renal function (Meier-Kriesche et al. 2004). On the other hand, although the transplanted kidney provides cardiac and renal benefits, kidney function still remains poorer than that of healthy subjects. Renal transplant recipients have a much higher incidence of cardiac death and non-fatal cardiovascular events compared with the general population (Liefeldt and Budde 2010).

During the last 15 years after the introduction of more sophisticated imaging techniques including multi-slice or electron beam computed tomography, coronary artery calcification (CAC) has not ceased to be a topic of interest for many researchers in the field of nephrology. Vascular calcification is more frequent (2-5 fold) and more severe in uremic patients than in age- and gender-matched individuals in

the general population (Braun et al. 1996). Goodman et al. (2000) indicated that a high prevalence of CAC in young patients is especially marked in those who had been on dialysis for more than 10 years. In addition, both intimal and medial artery calcification occur in uremic patients and represent significant predictors for CV morbidity and mortality in this population (Schwarz et al. 2000).

During the last decade, significant attention has been focused towards factors implicated in the pathophysiology of CAC in end stage renal disease. Some of the factors include poor calcium and phosphate homeostasis, intact parathyroid hormone (iPTH) concentration, oxidative stress, inflammation and levels of vascular and soft tissue calcification inhibitors, together with age, dialysis vintage, dyslipidemia, diabetes, some medication and genetic factors (Martola et al. 2005). Currently accepted opinions propose that in the uremic milieu, vascular calcifications may result from both an imbalance in divalent ions and a deficit of

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Correspondence: Sanja Simic-Ogrizovic, Nephrology Clinic, Clinical Centre of Serbia, Pasterova 2, Belgrade 11000, Serbia.
e-mail: ssogrizovic@gmail.com

inhibitory proteins, eventually leading to phenotypic change and calcium deposition (Ketteler et al. 2005). It could be expected that, after successful kidney transplantation and definite improvement of the uremic milieu, the imbalance between promoters and inhibitors of vascular calcification would be lessened (Mazzaferro et al. 2007). However, the prevalence of renal transplant patients with CAC still remains very high.

We evaluated the prevalence of CAC in our stable renal transplant recipients as well as age- and gender-matched patients with chronic kidney disease (CKD) in stages 2-5 not requiring dialysis, and assessed possible demographic and laboratory risk factors associated with CAC in these two different groups. We thus identified significant predictors of patient mortality after a 30-month follow-up period.

Patients and Methods

Patients

This prospective (30 month duration) observational study involved 49 stable renal transplant (Tx) recipients, at least 6 months after transplantation and 48 CKD patients in stages 2-5 not requiring dialysis. CKD patients were selected from the population regularly monitored at the Nephrology Clinic in order to obtain a group of similar age and gender as the Tx patients. Diabetics and patients with acute inflammatory diseases were excluded.

The characteristics of all study participants are shown in Table 1. Thirty-six patients had received a kidney from a related living donor and thirteen from a deceased donor. These patients had previous transplantation duration of 9.56 ± 5.27 years. Any history of hypertension, ischemic vascular disease (myocardial infarction, angina pectoris, cerebral stroke), heart failure and smoking habits was noted at the study entry interview.

The original kidney diseases in our patients were: chronic glomerulonephritis ($n = 29$ in Tx and $n = 16$ in CKD patients), chronic pyelonephritis and congenital urinary tract anomalies ($n = 5$ in Tx and $n = 4$ in CKD patients), nephrosclerosis ($n = 3$ in Tx and $n = 14$ in CKD patients), polycystic kidney disease ($n = 2$ in Tx and $n = 6$ in CKD patients), other ($n = 3$ in Tx and $n = 8$ in CKD patients) and unknown ($n = 7$ in Tx and $n = 0$ in CKD patients). The immunosuppressive protocol for Tx patients consisted of calcineurin inhibitors (cyclosporine or tacrolimus), mycophenolate acids or azathioprine and prednisolone. CKD patients with some kind of glomerulonephritis as the original disease received corticosteroids or other immunosuppressive therapy according to protocols.

Clinical and immunological observations, as well as laboratory parameters and coronary calcium score using computed tomography were determined on the same day.

All patients gave informed consent prior to their enrolment in the study, which was planned according to the ethical guidelines following the Declaration of Helsinki. The institutional review committee approved our study protocol thereby following local biomedical research regulations.

Laboratory methods

A fasting blood sample was drawn from each patient once in order to measure the following parameters: complete blood count and serum concentrations of uric acid, creatinine (s-creatinine), albumin,

pre-albumin, transferrin, calcium, phosphate, iPTH, cholesterol, triglycerides, high sensitive CRP (hs-CRP), serum amyloid A (SAA), fetuin A, total homocysteine (tHcy) and interleukin-6 (IL-6). Hematological profiles were determined using an LH 750 hematology analyser (Beckman Coulter Inc., California, USA). Creatinine, uric acid and albumin were analyzed employing routine methods (Olympus System Reagents using an AU 2700 Olympus analyser, Hamburg, Germany). iPTH was determined by ELISA-PTH, (CIS bio international, GIF-sur-Yvette Cedex, France). hs-CRP, SAA, pre-albumin and transferrin were measured using immunonephelometric assays (Dade-Behring, BN II, Marburg, Germany). Serum IL-6 levels were determined with a highly sensitive colorimetric sandwich ELISA kit (Human IL-6 Quantikine HS ELISA kit; R&D Systems, GmbH, Germany). Serum fetuin A was determined by ELISA (Epitope Diagnostics, Inc., San Diego, California, USA).

Serum tHcy concentration was measured by high-performance liquid chromatography (HPLC) after reduction of the disulfide-bonds by dithiothreitol (normal range: 10-15 $\mu\text{mol/l}$).

Glomerular filtration rate (GFR) was estimated using the abbreviated Modification of Diet in Renal Disease (MDRD) formula study equation (Levey et al. 2006).

Arterial hypertension was diagnosed when the systolic blood pressure was ≥ 140 mmHg and/or diastolic pressure was ≥ 90 mmHg, or if antihypertensive treatment was prescribed.

Body mass index (BMI) was calculated according to the formula: $\text{weight (kg) / height}^2 (\text{m}^2)$.

Coronary artery calcification (CAC) score

CAC score was evaluated using multi-detector row spiral computed tomography (MSCT) (General Electric Medical System, USA) operating with the following parameters: 64 slices with 912 detectors, 0.625 mm distance between slices, 0.35s rotation time and a tube current of 800 mA at 120 kV. Data were obtained during the diastolic phase of the heart cycle. CAC score calculations were based on formulae using measurements of total volume and area of calcified lesions, as well as mean and maximum density. Individual CAC scores were calculated for the left main coronary artery, descending branch of the left coronary artery, circumflex branch of the left coronary artery and right coronary artery. Scores then were summed to calculate the total coronary CAC score. The final score is expressed in modified Agatston units (Agatston et al. 1990).

To identify factors associated with CAC, renal Tx and CKD patients were separated into two sub-groups according to the presence or absence of significant CAC using a cut-off score of 100. This calcium score was chosen according to the latest guidelines from the American College of Cardiology and the American Society of Nuclear Cardiology endorsed by the American Heart Association (ACC/ASNC) (Greenland et al. 2007).

The database included demographic (age and gender), clinical (CKD, dialysis and Tx duration, BMI, hypertension), CAC score as well as hematological, biochemical and immunological variables.

Statistics

Differences in continuous variables between the groups were analysed by the Student's *t*-test for normally distributed variables. Values for S-creatinine, proteinuria, triglycerides, hs-CRP, IL-6 and SAA were log-transformed to achieve normality. Due to the fact that the CAC score distribution and disease duration were skewed after log-transformation, these variables were compared by the Mann-

Whitney test. Adjusted mean levels were estimated by analysis of covariance (ANCOVA). Group differences for categorical variables were examined by the Chi-square test. Uni-variate associations were evaluated by Spearman's correlation analysis.

Clinical accuracy of the examined parameters was assessed using receiving operative characteristic (ROC) curve analysis. The area under the ROC curve (AUC) is considered to be a useful quantitative measure of accuracy. The ROC curve allows variable thresholds to be determined on a target sensitivity or specificity. We set the cut-off value at the point when discrimination between the cases with positive and negative diagnosis was optimal.

In order to analyse the death risk, uni-variate survival analysis with the Cox proportional hazard model was obtained. The primary dependent variable was the time to death measured in months. Variables that were potential predictors of death in uni-variate analy-

sis were tested in the multi-variate Cox proportional hazard model.

All calculations were performed using MS Excel, EduStat 2.01 (2005, Alpha Omnia, Belgrade, Serbia) and MedCalc for Windows version Version 9.6.3 (Mariakerke, Belgium). Minimal statistical significance was set at $p < 0.05$.

Results

Comparisons between renal Tx and CKD patients are presented in Table 1. Renal Tx patients had a significantly higher CAC score, CKD and/or dialysis duration and significantly higher inflammatory markers (hs-CRP, SAA and IL-6) along with lower serum fetuin A levels but better kidney function, higher tHcy concentrations and significantly lower serum phosphate levels and $\text{Ca} \times \text{P}$ compared with

Table 1. Demographic characteristics and laboratory data of renal Tx and CKD patients.

	Renal Tx patients	CKD patients	<i>p</i>
<i>N</i>	49	48	
Age (years)	41.98 ± 10.58	38.21 ± 15.56	ns
Gender (M/F)	31/18	25/23	ns
Smoking history (%)	20	26.5	ns
Hypertension (%)	58	51	ns
IVD (%)	14.2	10.4	ns
CKD and/or D duration (months) †	143 (97-206)	24 (12-33)	< 0.001 ^a
Transplantation duration (months)	114.28 ± 63.28	/	/
CAC score †	51 (0-461.25)	0 (0-7.5)	0.002 ^a
BMI (kg/ m ²)	24.94 ± 3.56	24.40 ± 3.42	ns
S. creatinine (µmol/L)*	172.44 (154.64-192.29)	249.84 (208.11-299.94)	< 0.001
GFR (ml/min/1.73 m ²)	39.60 ± 15.04	30.86 ± 22.15	0.026
Haemoglobin (g/L)	124.92 ± 22.14	128.34 ± 22.31	ns
Uric acid (µmol/L)	412.02 ± 91.3	470.46 ± 112.85	0.006
Albumin (g/L)	41.17 ± 6.09	42.20 ± 3.57	ns
Pre-albumin (g/L)	0.32 ± 0.11	0.32 ± 0.10	ns
Transferrin (g/L)	2.16 ± 0.53	2.16 ± 0.50	ns
Calcium (mmol/L)	2.42 ± 0.18	2.34 ± 0.20	0.56
Phosphate (mmol/L)	1.16 ± 0.28	1.48 ± 0.41	< 0.001
Ca × P (mmol ² /L ²)	2.7 ± 0.56	3.44 ± 0.86	< 0.001
intact PTH (pg/mL) *	103.49 (77.53-138.14)	86.35 (67.05-111.21)	0.360
Cholesterol (mmol/L)	5.8 ± 1.11	5.88 ± 1.24	ns
Triglycerides (mmol/L) *	1.89 (1.66-2.17)	1.81 (1.55-2.12)	ns
hs-CRP (mg/L) *	1.38 (0.897-2.131)	0.73 (0.477-1.118)	0.037
SAA (mg/L) *	8.54 (6.389-11.427)	3.38 (2.585-4.311)	< 0.001
IL-6 (pg/mL) *	4.16 (3.43-5.039)	2.40 (1.95-2.957)	< 0.001
Homocysteine (umol/l)	24.27 ± 11.0	19.91 ± 6.71	0.016
Fetuin A (g/L)	0.397 ± 0.133	0.463 ± 0.111	0.014

CKD, chronic kidney disease; D, dialysis; Tx, transplantation; CAC, coronary artery calcification; GFR, glomerular filtration rate; hs-CRP, high sensitive C-reactive protein; SAA, serum amyloid A; IL-6, interleukin 6.

Continuous variables are presented as mean ± standard deviation and compared by Student *t* test, whereas categorical variables are presented as relative frequencies and compared using the Chi-square test.

†Values for CKD and/or D duration and CAC score are presented as median and 25th and 75th percentile.

*Values for S-creatinine, intact PTH, triglycerides, hs-CRP, IL-6 and SAA are presented as geometrical mean and 95% confidence intervals (CI). Logarithmic transformation of the values was performed before the analysis.

^a variables are compared using the Mann-Whitney test.

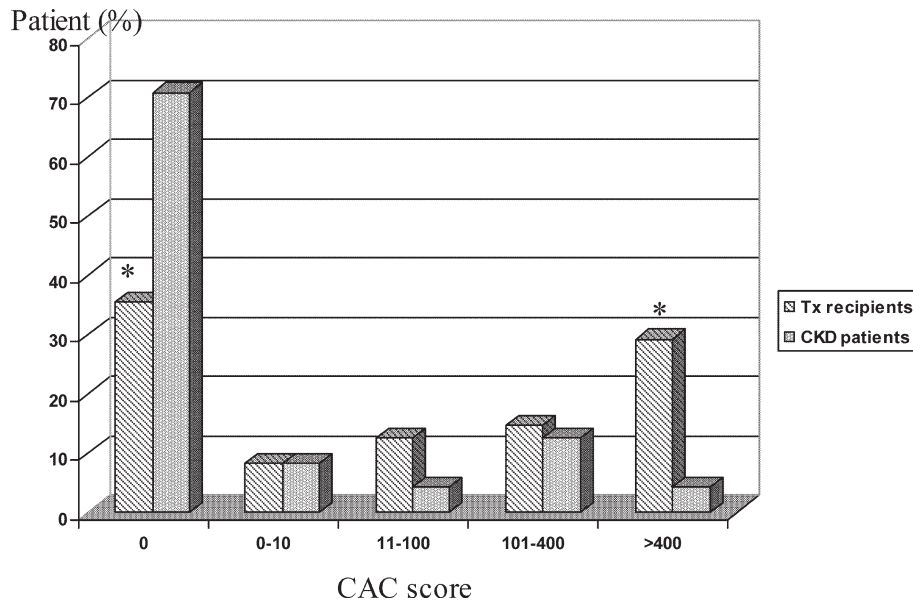


Fig. 1. CAC score in transplant recipients and CKD patients.

Tx, renal transplant recipients

CKD, chronic kidney disease patients

CKD patients.

The prevalence of patients with a CAC score > 100 was 31% for all the examined patients; it was 43.8% in the Tx group and 16.7% in the CKD patients ($p < 0.001$).

More than half the patients (54%) had a CAC score of zero (35.4% in the Tx recipients compared with 70.8% in CKD patients; $p < 0.001$), while 7% had CAC scores 1-10 (8.3% compared with 8.3%; $p = 0.976$), 8% values of 11-100 (12.5% compared with 4.2%; $p = 0.148$), 14% CAC scores of 101-400 (14.6% compared with 12.5%, $p = 0.796$) and 17% of the patients had CAC scores > 400 (29.2% compared with 4.2%; $p = 0.001$) (Fig. 1).

For the Tx patients Spearman analysis revealed significant correlations only between CAC score and age ($r = 0.480$, $p = 0.001$), hypertension ($r = 0.303$, $p = 0.036$) and uric acid ($r = 0.321$, $p = 0.026$). In CKD patients the CAC score was positively associated with age ($r = 0.693$, $p < 0.001$), hypertension ($r = 0.349$, $p = 0.025$), CKD duration ($r = 0.442$, $p = 0.002$) and albumin ($r = 0.303$, $p = 0.037$) but negatively with serum fetuin A concentration ($r = -0.419$, $p = 0.004$).

Table 2 shows the comparison between patients with a CAC score ≤ 100 (without CAC) and with a CAC score > 100 (with CAC). Tx patients with CAC were significantly older and had longer duration of CKD and/or dialysis than Tx patients without CAC. In addition, Tx patients with CAC showed significantly increased serum uric acid, compared to those without CAC. The serum IL-6 level tended to be higher and the serum fetuin A level tended to be lower in renal Tx recipients with CAC, compared to those without CAC, although the differences were not statistically significant.

CKD patients with CAC were older, had longer CKD

duration and higher tHcy concentrations but lower serum levels of albumin, pre-albumin and fetuin A (Table 2).

To explore whether the association between albumin, pre-albumin, tHcy and fetuin A and CAC score were confounded by other factors, the concentrations of these parameters in CKD patients with and without CAC were compared after adjusting for age and duration of disease. Serum albumin concentrations ($F = 4.551$, $p = 0.039$) and fetuin A ($F = 15.897$, $p = 0.001$) were significantly lower in CKD patients with CAC than the corresponding values in CKD patients without CAC. However, no significant differences were found for adjusted means of the other parameters between CKD patients with and without CAC. In Tx patients adjusted uric acid for age and CKD duration were not significantly different between Tx patients with and without CAC.

The AUC was calculated to assess the accuracy of the examined parameters in screening a CAC score > 100 in CKD patients. The significant ROC curves with specificities and sensitivities for optimal cut-off values are presented in Table 3. Only fetuin A and albumin had acceptable discriminative ability for detecting CAC presence (Swets 1988). The ROC curves for hsCRP, IL-6 and pre-albumin indicated moderate accuracy for discrimination between patients with and without CAC. No other parameters indicated significant discriminative ability for detecting CAC. Although the sensitivity of fetuin A, albumin, pre-albumin and hsCRP were equal (100%), their specificities ranged from 72.2% for fetuin A to 61.1% for pre-albumin. In Tx recipients no parameter had significant discriminative ability for detecting CAC.

During the observational period (30 months), 30 patients began dialysis (7 Tx recipients and 23 CKD

Table 2. Demographic characteristics and laboratory data in renal Tx and CKD patients without CAC and with CAC (CAC score > 100).

	Renal Tx patients			CKD patients		
	Without CAC (n = 28)	With CAC (n = 21)	P	Without CAC (n = 40)	With CAC (n = 8)	P
Age (years)	36.2 ± 8.96	46.67 ± 9.370	< 0.002	31.8 ± 9.72	62.6 ± 6.52	< 0.001
CKD and/or D duration (months) †	132 (62-154)	161 (113-228)	< 0.001 ^a	12 (6-24)	42 (42-105)	< 0.001 ^a
BMI (kg/m ²)	24.72 ± 3.68	24.87 ± 3.33	ns	24.0 ± 3.05	25.9 ± 4.48	ns
S. creatinine (µmol/L)*	164.93 (141.38-192.40)	181.22 (154.18-213.01)	ns	248.47 (202.16-305.39)	255.09 (157.93-412.03)	ns
GFR (ml/min/1.73 m ²)	41.54 ± 15.07	37.13 ± 14.42	ns	31.92 ± 23.07	26.87 ± 18.78	ns
Haemoglobin (g/L)	121.67 ± 20.56	126.37 ± 23.18	ns	124.53 ± 22.91	127.45 ± 21.72	ns
Uric acid (µmol/L)	385.48 ± 90.79	438.00 ± 87.54	0.048	485.84 ± 109.5	452.0 ± 111.43	ns
Albumin (g/L)	42.17 ± 4.59	42.28 ± 21.81	ns	45.8 ± 2.86	39.95 ± 6.08	0.005
Pre-albumin (g/L)	0.32 ± 0.13	0.33 ± 0.11	ns	0.35 ± 0.01	0.23 ± 0.12	0.002
Transferrin (g/L)	2.19 ± 0.64	2.13 ± 0.475	ns	2.16 ± 0.55	2.19 ± 0.27	ns
Calcium (mmol/L)	2.42 ± 0.13	2.42 ± 0.23	ns	2.34 ± 0.217	2.36 ± 0.17	ns
Phosphate (mmol/L)	1.14 ± 0.27	1.19 ± 0.31	ns	1.51 ± 0.433	1.39 ± 0.37	ns
Ca × P (mmol ² /L ²)	2.71 ± 0.62	2.84 ± 0.53	ns	3.50 ± 0.61	3.24 ± 0.69	ns
intact PTH (pg/mL) *	97.82 (62.73-152.54)	112.53 (74.62-169.72)	ns	77.41 (59.04-101.5)	130.09 (64.40-262.77)	ns
Cholesterol (mmol/L)	5.49 ± 1.17	6.07 ± 1.04	ns	5.78 ± 1.26	6.26 ± 1.14	ns
Triglycerides (mmol/L)*	1.68 (1.41-2.00)	1.97 (1.66-2.34)	ns	1.92 (1.61-2.29)	1.45 (1.01-2.10)	ns
CRP (mg/L)*	1.56 (0.70-3.51)	1.30 (0.75-2.26)	ns	0.61 (0.37-0.99)	1.74 (0.94-3.21)	ns
SAA (mg/L)*	8.54 (5.87-12.43)	8.55 (45.40-13.52)	ns	3.33 (2.43-4.55)	3.38 (2.26-5.05)	ns
IL-6 (pg/mL)*	3.65 (2.31-5.67)	4.63 (3.47-6.16)	ns	2.16 (1.41-2.74)	3.51 (2.28-5.38)	ns
Homocysteine (umol/l)	22.03 ± 6.48	25.94 ± 12.57	ns	18.5 ± 5.76	24.15 ± 7.96	0.037
Fetuin A (g/L)	0.411 ± 0.148	0.385 ± 0.120	ns	0.494 ± 0.101	0.354 ± 0.068	< 0.001

CKD, chronic kidney disease; D, dialysis; Tx, transplantation; CAC, coronary artery calcification; GFR, glomerular filtration rate; hs-CRP, high sensitive C-reactive protein; SAA, serum amyloid A; IL-6, interleukin 6.

Continuous variables are presented as mean ± standard deviation and compared by Student *t* test, whereas categorical variables are presented as relative frequencies and compared using the Chi-square test.

† Values for CKD and/or D duration are presented as median and 25th and 75th percentile.

* Values for S-creatinin, intact PTH, triglycerides, hs-CRP, IL-6 and SAA are presented as geometrical mean and 95% confidence intervals (CI). Logarithmic transformation of the values was performed before the analysis.

^a variables are compared using the Mann-Whitney test

Table 3. The results of ROC analysis for discriminating CKD patients without and with CAC.

	AUC	Confidence interval	Std. error	Cut-off	Specificity	Sensitivity
Fetuin A (g/L)	0.878	0.777-0.978	0.051	4.37	1.00	0.722
Albumin (g/L)	0.826	0.710-0.942	0.059	42.00	1.00	0.632
hs-CRP (mg/L)	0.763	0.631-0.895	0.067	0.57	1.00	0.684
Pre-albumin (g/L)	0.750	0.608-0.892	0.072	0.32	1.00	0.611
IL-6 (pg/mL)	0.744	0.572-0.917	0.088	4.10	0.600	0.889

AUC statistically different from 0.5

hs-CRP, high sensitive C-reactive protein; IL-6, interleukin 6; AUC, area under the ROC curve.

Table 4. Predictors of mortality using the multi-variate Cox proportional hazard model.

Variable	β	Hazard ratio (CI)	<i>p</i>
Age	.229	1.257 (1.006-1.482)	0.007
CAC score	.001	1.001 (1.000-1.002)	0.020
SAA	.074	1.077 (1.020-1.137)	0.008

CAC, coronary artery calcification; SAA, serum amyloid A.

patients), one Tx recipient suffered a myocardial infarction, and six patients died (2 CKD patients due to cardiovascular events and 4 renal Tx patients - 2 due to cardiovascular events, 1 from a malignancy and 1 from a severe pulmonary infection).

Predictors of mortality using the uni-variate Cox proportional hazard model in all examined patients were: age ($p = 0.003$), CKD/dialysis duration ($p = 0.004$), CAC score ($p = 0.001$), tHcy ($p = 0.014$), SAA ($p = 0.001$), IL-6 ($p = 0.005$) and fetuin A ($p < 0.001$). All significant predictors of mortality were subjected to a forward stepwise selection process. In multi-variate analysis age, SAA and CAC score were found to be independent predictors of patient death (Table 4).

Discussion

In the present study the prevalence of CAC in Tx patients was 43.8% and was significantly higher than in CKD patients (16.7%). Rosas et al. (2005) found CAC in 65% of Tx recipients but 66% of the examined patients were diabetics. In other studies with significantly fewer diabetics a similar prevalence of CAC was found in Tx patients (Bargnoux et al. 2009; Seyahi et al. 2011). In the large Dallas Heart Study among 2,660 participants aged 43.9 years, Kramer et al. (2005) observed a 25% prevalence of a CAC score of >10 and 15% of CAC scores > 100 in 28 non-diabetic stage 3-5 CKD patients, but in 13 diabetics stage 3-5 CKD patients the prevalence of a CAC score >10 was 85%. In a multi-ethnic study of atherosclerosis Kestenbaum et al. (2009) found a prevalence of CAC at 66% in much older patients (68.7 years) with CKD stage 3.

Moe et al. (2004) and Mazzaferro et al. (2007) compared mean CAC score, potential promoters and inhibitors for CAC and outcome in Tx recipients with values for dialysis patients. The Tx patients had CAC scores of lower severity, no net change in CAC with time (Moe et al. 2004), less inflammation (Mazzaferro et al. 2007), higher levels of calcification inhibitors (Mazzaferro et al. 2007) and a better survival rate than dialysis patients (Moe et al. 2004). However, we evaluated Tx recipients with age and gender matched stage 2-5 CKD patients not requiring dialysis. Although the Tx patients had better kidney function and lower serum uric acid levels, they had significant higher CAC scores and positive acute phase proteins (hs-CRP, SAA and IL-6) along with lower serum fetuin A levels than CKD patients. After successful kidney transplantation

inflammation markers significantly decreased because of faster cytokine clearance and an improved uremic milieu but some degree of inflammation still persisted or was elevated despite immunosuppressive therapy. Vascular inflammation is an important factor in the development of atherosclerosis as illustrated by the increased number of post-transplant cardiovascular events with elevated levels of hs-CRP in transplant recipients (Bakri et al. 2004). In our study Tx patients had greater CAC scores as a surrogate of atherosclerosis than CKD patients and this could be the reason for greater inflammation in these patients despite better kidney function.

In addition, our renal Tx recipients had significantly lower serum phosphate levels and $\text{Ca} \times \text{P}$ than CKD patients with no difference in iPTH levels. It is well known that after successful kidney transplantation significant changes occur in divalent ion concentrations. In particular, serum phosphate may reach normal or even low levels (Mazzaferro et al. 2009).

As in most other studies examining dialysis (Kraśniak et al. 2007) or Tx patients (Rosas et al. 2005; Mazzaferro et al. 2007; Seyahi et al. 2011) CAC significantly correlated with age in both examined groups. In addition, patients from both subgroups with CAC had undergone significantly longer CKD and/or dialysis duration than patients without CAC. An association between CAC and dialysis vintage has already been demonstrated (Rosas et al. 2005). Contrary to Seyahi et al. (2011), we found no association between CAC and transplantation duration.

The differences between renal Tx patients with and without CAC with respect to serum IL-6 and fetuin A levels were not statistically significant. In CKD patients, along with age and CKD duration, both negative acute phase reactants (serum albumin and fetuin A) significantly correlated with CAC. Positive acute phase protein levels tended to be higher in CKD patients with CAC compared to those without CAC. Moreover, after adjusting for age and CKD duration, serum albumin and fetuin A concentrations were significantly lower in the CKD patients with CAC. It is well known that hypo-albuminemia is a marker of inflammation rather than malnutrition. By searching Medline and PubMed databases, Hamirani et al. (2008) found variable association between CAC and inflammation in the general population. Nevertheless, in Tx patients (Mazzaferro et al. 2007) and dialysis patients (Kraśniak et al. 2007; Coen et al. 2006), this relationship was closer in cross-sectional studies and in CAC progression investigations (Mazzaferro et al. 2009; Coen et al. 2010).

Between examined subgroups in Tx and in CKD patients there were no differences in divalent ion concentrations or for $\text{Ca} \times \text{P}$ product and serum iPTH levels. It has been hypothesized that vascular calcifications in CKD are caused merely by passive deposition of $\text{Ca} \times \text{P}$ products in blood vessels owing to hyper-calcemia and hyper-phosphatemia in conditions of hyper-parathyroidism (Huybers & Bindels 2007). In dialysis patients, Kraśniak et al. (2007)

and Coen et al. (2010) found significant correlations between divalent ions and vascular calcification, but other studies did not confirm this (Guérin et al. 2000; Rosas et al. 2005).

During the last ten years, fetuin A, a negative acute phase protein and inhibitor of vascular calcification, has been the focus of vascular calcification by many researchers. Cottone et al. (2010) showed that serum fetuin A was decreased and inflammatory processes increased and linked to endothelial dysfunction even in early stages of CKD. Our results are partially in agreement with some published articles concerning fetuin A and the CAC score in CKD and Tx patients. The cut-off value of fetuin A to predict the presence of CAC is equal to 0.437 g/L in our study. In Mazzaferro et al. (2009) study, mean values in Tx and dialysis patients were from 0.45 g/L to 0.66 g/L. The optimal cut off values derived from ROC curves was 0.145 g/L for fetuin-A, but as a predictor of death in patients with ST-elevation acute myocardial infarction (Lim et al. 2007).

Only a few studies have evaluated the discriminative ability of biomarkers on the extent of CAC in CKD patients. When biological markers of vascular risk and their association with the extent of CAC were considered, fetuin A, malnutrition and inflammation were able to predict the appearance of a significant CAC score. These patients underwent MSCT for suspected coronary artery disease (Uz et al. 2009). In contrast to our study, the AUC for CAC identification was 0.574 for fetuin A. In a study concerning CKD patients, osteoprotegerin had an AUC of 0.79 and neither of calcium phosphate disorder markers had similar ability (Morena et al. 2009).

During the 30 month observation period six of our patients died, four from cardiovascular events. In multivariate analysis age, SAA and CAC score were found to be independent predictors of patient death. Although we only examined a small number of patients, inflammatory markers and severity of CAC together with age were significant predictors of death as already established for inflammatory markers (Simic-Ogrizovic et al. 2009) and CAC score (Coen et al. 2010; Shantouf et al. 2010) in dialysis patients.

Without doubt the present study confirmed the close relationship between age and renal failure and/or dialysis duration before and CAC after kidney transplantation. One could speculate that multiple established and non-established cardiovascular risk factors at different times during periods of diminished and end stage renal failure contribute to endothelial dysfunction, arterial stiffness and to atherosclerosis and vascular calcifications. At the time of renal transplantation the majority of ESRD patients already have an enormous burden of cardiovascular disease. Hernandez et al. (2005) found pre-existing vascular calcification to be an independent predictor of cardiovascular and all-cause mortality following transplantation. Relapse of pre-existing or appearance new risk factors in the post-transplantation period result, in part, from the atherogenic potential of immunosuppressive drugs (Ojo 2006). Bargnoux et al.

(2009) concluded that successful transplantation prevents *de novo* uremia-induced calcification in CAC-free patients, stops the progression of pre-existing mild calcification but CAC progression occurs in recipients with moderate to severe basal CAC.

Successful renal transplantation improves only some negative uremic effects and lowers, but does not halt CAC progression (Mazzaferro et al. 2009). During the post-transplantation period with deteriorating allograft function, recipients again suffer from uremia. Therefore, examination and prevention of risk factors must be started at the beginning of renal failure when the characteristics of favorable and non-favorable aspects of CAC are more obvious.

In conclusion, the prevalence of CAC in renal transplant patients is relatively high and clearly linked with age and chronic kidney and/or dialysis duration. In CKD patients the prevalence was lower and also correlated with age and CKD duration. Moreover, the negative acute phase markers, serum albumin and fetuin A levels, were associated with CAC. Along with age and serum amyloid A, CAC score was a predictor of patient death. Additional long-term longitudinal studies must incorporate patients at the beginning of chronic renal failure who will be monitored during disease progression, time on dialysis and throughout the kidney transplantation period.

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Conflict of Interest

All authors have no conflict of interest.

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