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Vascular access calcification predicts mortality in hemodialysis patients

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Vascular calcification is a recognized risk factor for cardiovascular mortality in patients with end-stage renal disease. The aim of this study was to identify risk factors for vascular access calcification and to determine if patients with this disorder are at increased risk of death. Vascular access calcification was found in 49 of 212 hemodialysis patients as measured by plain X-ray (arteriovenous fistula or synthetic graft) in two dimensions. Male gender, diabetes mellitus, and length of time on dialysis were independent predictors for access calcification determined by logistic regression multivariate analysis. Serum parameters were not independently related to access calcification. Kaplan–Meier analysis showed an increased mortality risk, and Cox regression analysis confirmed that vascular access calcification was an independent mortality predictor. Our study suggests that detection of vascular access calcification is a cost-effective method to identify patients at increased mortality risk.

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KEYWORDS: AV fistula; calcification inhibitors; hemodialysis patients; mortality; vascular calcification

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Patients with end-stage renal disease exhibit a dramatically increased cardiovascular mortality in comparison with the normal population.[1] Vascular calcifications are important predictors of mortality in end-stage renal disease patients on hemodialysis.[2] Uremia-associated risk factors like chronic inflammation, hyperphosphatemia, and an increased calcium–phosphate product and deficiencies of calcification inhibitors contribute to progressive vascular calcification in end-stage renal disease patients.[3–5] Different methods have been used to detect the influence of vascular calcifications on mortality risk of dialysis patients.[2,6] Nevertheless, easy to perform single modality methods for the detection of calcification are limited.

Vascular access calcification (VAC)—calcification of the arteriovenous fistula or the synthetic graft—can be detected easily by plain X-ray. However, there is only limited information on the prevalence of VAC and risk factors for the development of such calcifications. Moreover, there are no data indicating whether the presence of VAC is associated with increased mortality risk. Thus, the main objectives of this study were first to analyze risk factors associated with VAC and second to investigate whether VAC predict mortality in a cohort of hemodialysis patients.

RESULTS

Clinical risk factors for vascular access calcification

Characteristics of the dialysis population are given in Table 1. Using plain radiographs calcifications of the vascular access could be detected in 49 of the 212 hemodialysis patients (23%; Figure 1). Male patients were 3.95-times more likely to develop VAC. Patients with VAC had been on hemodialysis treatment 2.3 years longer than patients lacking VAC. Diabetic patients had a 3.43-fold risk for VAC compared with nondiabetic patients. Age, hypertension, and body mass index were not related to the presence of VAC, whereas smokers had the tendency to develop more VAC (odds ratio (OR) 1.73). Age of the vascular access and history of vascular access thrombosis were no relevant risk factors for VAC (Table 1).

Table 1 | Characteristics of patients with vs without vascular access calcification

	All patients (*n*=212)	No VAC (*n*=163)	With VAC (*n*=49)	Odds ratio	95% confidence interval	*P* value
Age (years)	59 ± 11	60 ± 10	58 ± 12	0.99	0.96–1.02	NS
Male/female	114/98	76 (47%)/87 (53%)	38 (78%)/11(22%)	3.95	1.89–8.27	0.0001
Diabetes mellitus	26 (12%)	14 (8.6%)	12 (24%)	3.43	1.46–8.03	0.003
Hypertension	185 (87%)	142 (87%)	43 (88%)	1.06	0.40–2.79	NS
Smoking	63 (30%)	44 (27%)	19 (39%)	1.73	0.88–3.35	NS
Body mass index (kg/m^2)	23.4 ± 3.7	23.6 ± 3.7	22.7 ± 3.6	0.93	0.85–1.02	NS
Dialysis vintage (years)	6.71 ± 4.55	6.2 ± 4.0	8.5 ± 5.8	1.11	1.04–1.19	0.0015
Vascular access age (months)	89 ± 134 (median 67)	80 ± 120 (median 66)	112 ± 190 (median 72)	1.00	1.00–1.00	NS
History of vascular access thrombosis	74 (35%)	58 (36%)	16 (33%)	0.91	0.46–1.79	NS
Synthetic graft (PTFE)	11 (5.2%)	8 (4.9%)	3 (6.1%)	1.26	0.32–4.96	NS
Iliacal/femoral calcification	116 (55%)	76 (47%)	40 (83%)	5.72	2.52–12.98	<0.0001
Carotid calcification (*n*=208)	150 (71%)	108 (66%)	42 (86%)	1.94	0.87–4.32	NS
Cardiac valve calcification (*n*=202)	88 (42%)	64 (39%)	24 (49%)	1.67	0.85–3.23	NS
IMT (mm) (*n*=208)	0.83 ± 0.43	0.82 ± 0.37	0.87 ± 0.58	1.28	0.67–2.45	NS
PWV (m/s) (*n*=184)	9.7 ± 2.1	9.6 ± 2.0	10.3 ± 2.5	1.16	0.98–1.37	NS

IMT, intima–media thickness; NS, not significant; PWV, pulse wave velocity; PTFE, polytetrafluoroethylene; VAC, vascular access calcification.
Given are numbers, percent, or mean ± s.d., range, relative risk (odds ratio) and 95% confidence intervals for the presence of VAC (univariate logistic regression).

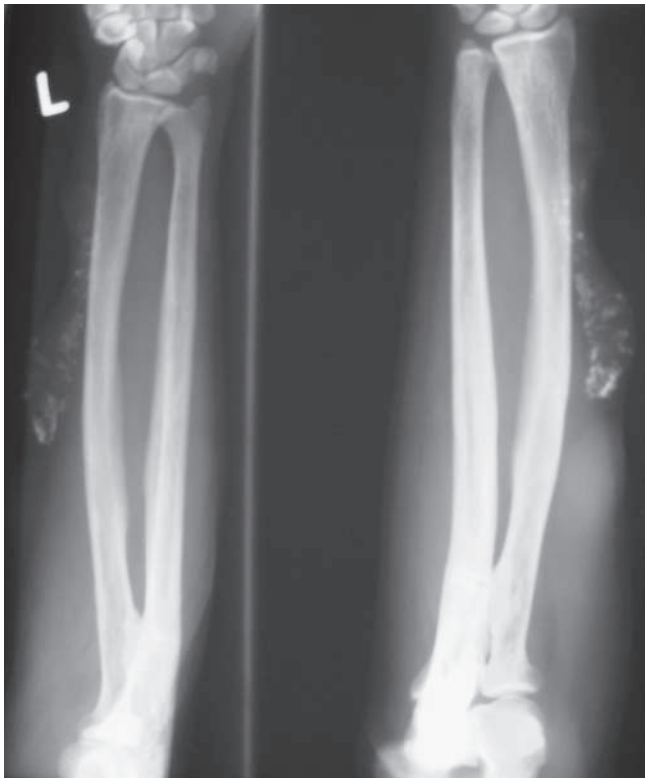

Figure 1 | Two-dimensional X-ray with calcification of the vascular access.

Biochemical risk factors for VAC

Patients with VAC exhibited a tendency toward higher phosphate levels in comparison to patients without VAC (Table 2). Serum cholesterol levels were 11% lower in patients with VAC as compared with patients without VAC, whereas triglyceride levels were only slightly lower in the group with VAC. Intact parathyroid hormone and serum high-sensitivity C-reactive protein were not significantly altered in patients with VAC in comparison to patients without VAC (Table 2). Levels of the calcification inhibition markers like serum fetuin-A and undercarboxylated matrix Gla protein were not significantly different in patients with vs patients without VAC.

In the multivariate analysis (logistic regression), only male gender (OR 5.08, 95% confidence interval (CI) 2.18–11.86, $P=0.00016$), diabetes mellitus (OR 4.57, CI 1.75–11.95, $P=0.0019$), and dialysis vintage (OR 1.15, CI 1.06–1.25, $P=0.0012$) remained significant predictors for VAC.

Relation of VAC to other calcification sites and cardiovascular parameters

Patients with calcifications of carotid arteries or heart valves had a tendency toward VAC (OR 1.94 and 1.67, $P=0.10$ and $P=0.14$, respectively), whereas the presence of iliacal/femoral calcifications exhibited a significant correlation with VAC (OR 5.72, $P<0.0001$). Carotid intima-media thickness (IMT) and carotid-femoral pulse wave velocity (PWV) were only slightly higher in patients with VAC compared with patients without VAC (Table 1).

Influence of VAC on mortality

In the second part of our study, we performed a survival analysis to determine the relevance of VAC for an increased mortality risk. Mean cumulative survival of patients with VAC was 946 days, whereas patients without VAC had a mean cumulative survival of 1089 days. Kaplan–Meier analysis showed that the presence of VAC significantly increased the risk for death (hazard ratio 2.14, 95% CI 1.11–4.12, $P=0.023$; Figure 2). When accounting for age, diabetes, dialysis vintage, *Kt*/*V*, and vascular disease, the presence of VAC remained a significant predictor for mortality as the Cox regression analysis confirmed that VAC were an important mortality

Table 2 | Serum and dialysis parameters and medication of patients with vs without vascular access calcification

	All patients (*n*=212)	No VAC (*n*=163)	With VAC (*n*=49)	Odds ratio	95% confidence interval	*P* value
Serum parameters						
Protein (g/l)	67.2 ± 5.0	66.9 ± 4.8	68.4 ± 5.6	1.06	0.99–113	NS
Calcium (mmol/l)	2.30 ± 0.18	2.30 ± 0.18	2.28 ± 0.18	0.46	0.082–2.61	NS
Phosphate (mmol/l)	1.62 ± 0.42	1.59 ± 0.41	1.71 ± 0.45	2.01	0.94–4.31	NS
Ca × PO_4 product ($mmol^2/l^2$)	3.74 ± 1.06	3.68 ± 1.04	3.93 ± 1.13	1.24	0.92–1.67	NS
iPTH (pg/ml)	370 ± 458 (median 192)	367 ± 455 (median 194)	381 ± 473 (median 170)	1.00	1.00–1-00	NS
Cholesterol (mmol/l)	5.14 ± 1.18	5.28 ± 1.13	4.67 ± 1.22	0.57	0.40–0.81	0.001
Triglycerides (mmol/l)	2.27 ± 1.23	2.35 ± 1.31	2.01 ± 0.88	0.76	0.55–1.05	NS
High-sensitive C-reactive protein (mg/l)	9.5 ± 17.4 (median 3.34)	8.62 ± 16.2	12.4 ± 20.9	1.01	0.99–1.03	NS
Serum fetuin-A (g/l)	0.55 ± 0.14	0.56 ± 0.14	0.53 ± 0.15	0.24	0.02–2.54	NS
ucMGP (nmol/l)	187 ± 101	190 ± 105	176 ± 88	1.00	1.00–1.00	NS
Dialysis parameters						
Dialysis hours per week	12.3 ± 1.4	12.4 ± 1.3	12.1 ± 1.4	0.87	0.69–1.11	NS
Calcium dialysate (mmol/l)	1.60 ± 0.21	1.61 ± 0.20	1.57 ± 0.22	0.46	0.10–2.06	NS
Kt/V	1.28 ± 0.19	1.30 ± 0.16	1.22 ± 0.26	0.11	0.02–0.69	0.017

iPTH, immunoreactive parathyroid hormone; NS, not significant; ucMGP, undercarboxylated matrix Gla protein; VAC, vascular access calcification.
VAC, number, percent, or mean ± s.d., range, relative risk (odds ratio), and 95% confidence intervals for the presence of vascular access calcifications (univariate logistic regression.

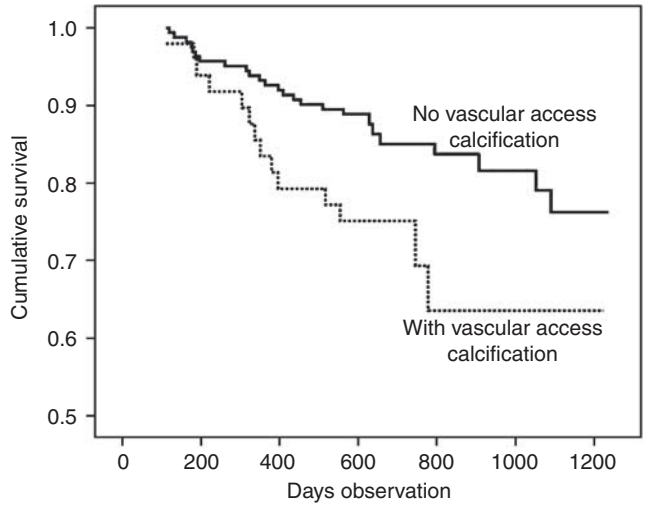

Figure 2 | Kaplan–Meier analysis of patients with vs no vascular access calcification (hazard ratio 2.14; 95% confidence interval 1.11–4.12; *P* = 0.023).

Table 3 | Effect of vascular access calcification on total mortality determined by univariate and multivariate Cox regression analysis

Vascular access calcification	HR	95% CI	*P*
Univariate analysis	2.14	1.11–4.12	0.023
Multivariate analysis	2.15	1.05–4.39	0.036

HR, hazard ratio; 95% CI, 95% confidence interval.
The variables age, diabetes mellitus, dialysis vintage, *Kt/V*, and presence of vascular disease at the start of the study were included in the multivariate analysis.

risk factor (Table 3). The hazard ratio of VAC for cardiovascular mortality was 1.79 (95% CI 0.81–3.97, $P = 0.14$).

Comparison of VAC with other risk factors for mortality

As VAC represents an important risk factor for mortality, we compared the crude effects of other risk factors in a univariate Cox regression analysis (Table 4). In our study, only age, high-sensitivity C-reactive protein, IMT, and iliacal/femoral calcification were significant predictors of mortality.

DISCUSSION

In this study, we investigated the prevalence of VACs in hemodialysis patients and analyzed risk factors associated with VAC. In addition, we assessed whether VAC were related to mortality risk.

In our study, about one-fourth (23%) of the patients tested positively for calcifications of the vascular access. To our knowledge, there is only one other study reporting on the prevalence of VAC.[7] Toussaint *et al.* used computed tomography fistulograms to assess calcification in the aorta, subclavian, carotid artery, and arteriovenous fistula in 28 patients. They found VAC in four of their patients (14%), which is within the same range of our study. However, to our knowledge, VAC has not been investigated systematically in relation to risk factors so far.

The first major finding of our study is that independent risk factors for VAC were male gender, diabetes mellitus, and dialysis vintage. Other clinical and biochemical parameters like age, calcium, calcium-phosphate product, parathyroid hormone, or fetuin-A[3–5,8] were not related to VAC in our study, whereas smokers and higher phosphate serum levels showed a tendency to be related with VAC. What could be the reason that we only found few risk factors for VAC? Arteriovenous fistulas differ from arteries as these are mainly arterialized veins. In the absence of pathological conditions, such as chronic venous insufficiency, veins usually do not calcify, but in a different setting, that is, when exposed to

Table 4 | Crude effect of vascular access calcification and clinical, biochemical, and cardiovascular parameters and other calcification sites on total mortality determined by univariate Cox regression analysis

Parameter	HR	95% CI	P
Age (years)	1.04	1.01–1.08	0.007
Dialysis vintage (years)	0.99	0.93–1.06	0.824
Hypertension	1.01	0.42–2.41	0.980
Diabetes mellitus	1.48	0.62–3.53	0.380
BMI (kg/m^2)	0.94	0.86–1.03	0.208
Serum calcium (mmol/l)	0.83	0.16–4.40	0.829
Serum phosphate (mmol/l)	0.66	0.31–1.41	0.281
Protein (g/l)	1.00	0.94–1.07	0.927
Hemoglobin (g/dl)	0.89	0.73–1.09	0.267
High sensitive C-reactive protein (mg/l)	1.03	1.02–1.04	<0.001
Pulse wave velocity (m/s) (n=184)	1.19	0.98–1.44	0.084
Carotid IMT (mm) (n=208)	1.83	1.21–2.78	0.004
Iliacal calcification	2.03	1.03–4.00	0.042
Cardiac valve calcification (n=202)	1.80	0.88–3.65	0.106
Carotid calcification (n=208)	1.99	0.87–4.57	0.103

BMI, body mass index; HR, hazard ratio; IMT, intima–media thickness; 95% CI, 95% confidence interval.

different hemodynamics with high blood flow, vascular remodelling occurs.[9] According to a recent study, cephalic veins can develop calcifications before creation of the vascular access.[10] Thus, whether VAC had developed solely after the surgery remains speculative.

Different types of cardiovascular calcification (i.e., atherosclerotic, medial artery, and cardiac valve calcification) are likely the consequence of distinct yet overlapping pathological mechanisms.[11] What are potential mechanisms leading to VAC? First, VAC could be a marker of systemic vascular calcification. In our study, patients with VAC showed increased iliacal/femoral calcification and a trend toward more frequent carotid or cardiac valve calcifications. The high correlation of VAC with iliacal/femoral calcification suggests that VAC and iliacal/femoral calcification share common pathogenic mechanisms. Second, VAC could be a marker of insufficient dialysis as VAC was related to decreased Kt/V and increased dialysis vintage. Third, hemodynamic factors in the vascular access such as turbulences or high flux, which were not assessed in our study, could have contributed to local calcification. Taken together, several factors seem be involved in the development of VAC. Interestingly, a small study investigated the ultrastructural basis of VAC and found brushite in addition to hydroxyl apatite as calcium depositions[12] whereas others found only hydroxyl apatite.[13] Thus, one may speculate that the mechanism(s) of calcification may be different in VAC when compared with other vascular sites; however, further investigations are clearly warranted.

The second major finding of our study is that the presence of VAC is an important risk factor for mortality. This result is well in agreement with previous findings showing vascular calcification as being a risk factor for death in dialysis patients.[2,6,14] However, the studies of London *et al.* and Adragao *et al.* needed measurement of calcification at different sites and/or with different scoring methods. In contrast, the method by X-ray of the vascular access has the advantage of being less time and cost consuming. Our measurement of calcification can easily be applied in dialysis patients by using a standard clinical procedure (i.e., X-ray). Especially, those patients who do not have easy access to a cardiac spiral computed tomography or where technical issues prevent its application (e.g., those with atrial fibrillation or massive obesity) can be assessed concerning their cardiovascular profile and risk. Moreover, this rather simple method provides a cost-effective measure of calcification and thus could become a standard screening method for vascular calcification in hemodialysis patients with a vascular access including arteriovenous fistulas or synthetic grafts. In clinical practice, ultrasound can probably be used to detect VAC with a similar sensitivity as an X-ray examination. In this context, it is important to note that a recent study comparing a simple calcification score with the more sensitive assessment of coronary calcifications by spiral computed tomography found a very good correlation between these two assessments.[15]

There are several limitations of our study. The cross-sectional character of our study, assessing most serum parameters at only one single time point, decreases the potential predictive power of individual serum parameters. This is, why, at least in the case of rapidly fluctuating parameters such as phosphate and calcium, we used time-averaged values for the analyses. Another limitation of our study is that we did not perform flow measurements of the vascular access as at the start of the study there was no Doppler ultrasound available. Finally, the radiographic method to detect calcifications was a simple X-ray technique, thus, we might have missed early stages of calcification, which are already present in the form of microcalcifications. These might be detected by a computed tomography scan but are too small to be visualized by plain X-ray films. Again, widespread applicability, ease of performing the method and cost were our main motivations to omit such more sophisticated approaches.

In conclusion, we report for the first time a systematic analysis of predictors and consequences of VAC in hemodialysis patients. The detection of VAC represents a cost-effective and easy to perform method to identify patients at increased mortality risk.

MATERIALS AND METHODS

Patients

We prospectively analyzed 212 hemodialysis patients (i.e., all patients with a vascular access like arteriovenous fistula or synthetic graft) from the Zvezdara University Medical Centre, Belgrade, Serbia (Table 1). All chronic hemodialysis patients were eligible to enter the study if they agreed to participate and had a two-dimensional X-ray of the vascular access. The vascular access was mainly located on the distal part of the upper extremity (76%) whereas 24% were proximally located. Synthetic grafts (polytetrafluoroethylene) were used in 11 patients (5%). Patients were enrolled between December

2003 and October 2005 and observed for 4–40 months until May 2007 (mean follow-up 692 days). During that observation period, 39 deaths occurred (cardiovascular, 28; malignancy, 6; sepsis, 4; ileus, 1). Gender was equally distributed (114 male gender, 98 female gender). Etiologies for end-stage renal disease were hypertensive nephrosclerosis, 115 (54%); glomerulonephritis, 26 (12%); autosomal-dominant polycystic kidney disease, 21 (10%); pyelonephritis, tubulointerstitial disease and obstructive nephropathy, 25 (12%); diabetic nephropathy, 12 (6%); systemic lupus erythematosus, 5 (2%); and Balkan endemic nephropathy, 8 (4%). The following phosphate binders and vitamin D medications were given: calcium carbonate, 159 (75%); aluminum hydroxide, 21 (10%); calcium carbonate + aluminium hydroxide, 24 (11%); no phosphate binder, 8 (4%); 1,25-OH-vitamin D3, 146 (69%); 1-α-OH-vitamin D3, 14 (6.6%); warfarin, 5 (2.4%); calcium antagonists, 89 (42%). The presence of vascular disease was defined as a preexisting diagnosis of coronary artery disease, cerebrovascular disease, or peripheral artery disease. The study protocol was approved by the Ethics Committee of the Zvezdara University Medical Centre, Belgrade, Serbia, and each patient gave informed consent.

Vascular access calcification and cardiovascular parameters

Calcification of the vascular access was assessed by plain two-dimensional X-ray of the arm with arteriovenous fistula or synthetic graft. X-ray images were analyzed by two experienced physicians blinded to the patient's condition. Only clearly visible extraosseous calcifications were counted as VAC. In addition, calcification of the iliacal/femoral arteries was determined on plain pelvic X-rays, which were analyzed by two experienced physicians.

Echocardiography was performed by one experienced investigator to detect calcifications of the aortic and mitral heart valves using an Aspen-Acuson device (Mountain View, CA, USA). To determine the intraobserver variability of echocardiographic detection of valvular calcification, one experienced investigator examined 30 randomly selected patients twice within 14 days. Intraobserver variability was 4%.

IMT was measured on both carotid arteries by performing a B-mode ultrasonography of the carotid arteries using an ALOCA SSD 2000 (Tokyo, Japan) system equipment with 7.5 MHz linear transducers. A trained investigator scanned both common carotid arteries, 4 cm from the bulbs, the carotid bulbs, and the first 2 cm of the internal and external carotid arteries. IMT was measured by one experienced investigator as the distance between adventitia and the lining of the arterial lumen/intima in a plaque-free area.[16,17] It was measured four times on both sides of the posterior wall (0.5, 1, 2, and 3 cm below the bifurcation) and the mean of these measurements was recorded. Calcified carotid plaques were defined as echogenic structures showing protrusion into the lumen with focal widening that was 50% greater than the IMT of adjacent sites. To determine the intraobserver variability of IMT measurements, one experienced investigator examined 30 randomly selected patients twice within 14 days. Intraobserver variability was 8%.

A Complior SP system (Artech Medical, Pantin, France) was used to assess the PWV by two trained investigators. PWV was measured utilizing two sensors (one carotid, one femoral) simultaneously to determine the velocity of the pulse in relation to the distance between the femoral artery and the suprasternal notch. Two measurements were performed and the mean value calculated. To determine the interobserver variability of PWV measurements, two experienced investigators independently analyzed 20 randomly selected patients. Interobserver variability was 7%.

The measurements of carotid calcification detection ($n = 208$), carotid IMT ($n = 208$), cardiac valve calcification detection ($n = 202$), and PWV ($n = 184$) were performed during the first months of the study; failure to perform the measurements in the remaining patients was mostly due to prior death of the patient.

Biochemistry

Blood was drawn from the arterial site after a long dialysis interval just before dialysis commenced. Biochemical analysis of serum risk factors (calcium, phosphate, lipids, protein, cholesterol, and triglycerides) was performed by standard laboratory procedure using an automated analyzer. Intact parathyroid hormone was assessed by a chemiluminescence assay (Diagnostic Product Corporation, Los Angeles, CA, USA). Serum analysis for high-sensitivity C-reactive protein was performed by particle-enhanced immunonephelometry using a standard 'CardioPhase hsCRP' for 'BNII' (Dade Behring Holding GmbH, Liederbach, Germany). The nephelometric method for association of serum fetuin-A serum was adopted from a serum ELISA method as previously described.[18] The ELISA measurement of undercarboxylated matrix Gla protein was conducted as previously described.[19] Calcium and phosphate measurements were calculated as mean values from four measurements within 4 months before the start of the study. All other parameters were single measurements at the beginning of the study.

Statistical analysis

Continuous variables were summarized by means and corresponding standard deviations. Comparisons of the values of continuous variables between two groups were made using an unpaired *t*-test. Categorical variables were summarized by relative frequencies. χ^2-Test was used for investigating associations between various categorical variables. Relative risk (odds ratio) and 95% CI for numerous variables were calculated by univariate logistic regression. Multivariate statistical analysis (logistic regression, enter method) was used to account for possible confounders (age, diabetes mellitus, dialysis vintage, hypertension, body mass index, serum levels of calcium, phosphate, iPTH, fetuin-A, undercarboxylated matrix Gla protein, triglyerides, and cholesterol). Cumulative survival was estimated using the Kaplan–Meier method. To identify prognostic factors of death, comparisons between survival curves were made by log-rank test. Cox regression was used (enter method) to determine the effect of AV-calicifications adjusted for variables (age, diabetes mellitus, dialysis vintage, *Kt/V*, and presence of vascular disease at the beginning of the study). Statistical analysis was performed with SPSS 14.0. A *P*-value of <0.05 was considered to be statistically significant.

DISCLOSURE

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