Progressive Vascular Calcification over 2 Years Is Associated with Arterial Stiffening and Increased Mortality in Patients with Stages 4 and 5 Chronic Kidney Disease

Mhairi K. Sigrist,*† Maarten W. Taal,*† Peter Bungay,‡ and Christopher W. McIntyre*†

*Departments of *Renal Medicine and ‡Imaging, Derby City General Hospital, Derby, and †School of Health, University of Nottingham, Nottingham, United Kingdom*

Background and objectives: Vascular calcification is increasingly recognized as an important component of cardiovascular disease in chronic kidney disease. The objective of this study was to investigate prospectively the determinants, cardiovascular functional consequences, and survival associated with vascular calcification over 24 mo.

Design, setting, participants, & measurements: A total of 134 patients (60 on hemodialysis, 28 on peritoneal dialysis, and 46 with stage 4 chronic kidney disease) were studied. Vascular calcification of the superficial femoral artery was assessed using multislice spiral computed tomography; pulse wave velocity; all medications and time-averaged biochemical parameters were recorded at baseline and 12 and 24 mo.

Results: A total of 101 patients remained at 24 mo. Progressive calcification was seen in 58 of 101 patients. Most (31 of 46) patients with an initial calcification score of zero did not develop calcification. The hemodialysis group demonstrated a greater degree of progression than patients who were on peritoneal dialysis or had stage 4 chronic kidney disease. Progressive calcification was associated with age, male gender, serum alkaline phosphatase, β blockers, and lipid-lowering agents. Increases in vascular calcification correlated with increased arterial stiffness. Vascular calcification was present in 20 of 21 patients who died. Cox proportional hazard analysis identified change in calcification score, calcium intake from phosphate binders, and low albumin as risk factors for death.

Conclusions: Patients with stages 4 and 5 chronic kidney disease and preexisting vascular calcification exhibit significantly increased calcification over 24 mo. Rapid progression of calcification is associated with arterial stiffness and mortality.

Clin J Am Soc Nephrol 2: 1241–1248, 2007. doi: 10.2215/CJN.02190507

Vascular calcification (VC) and arterial stiffness have been identified as independent predictors of all-cause and cardiovascular (CV) mortality in stage 5 chronic kidney disease (CKD) (1). More than 40% of dialysis patients will die from CV-related causes (2). The pathophysiology of vascular disease in CKD is increasingly recognized to be distinct from that related to atherosclerosis in the general population (3). Additional risk factors for vascular disease in CKD include inflammation, malnutrition, oxidative stress, and abnormal mineral metabolism (4).

The functional hemodynamic alterations that are associated with VC and the mechanisms by which arterial calcification leads to CV death are poorly understood. We and others (5–7) have found associations between VC and arterial stiffening as well as autonomic dysfunction in cross-sectional studies of patients with stages 4 to 5 CKD, but no longitudinal studies are available.

Cross-sectional studies, including our own, (8–11) have identified multiple associations with VC, including age, gender, diabetes, length of time on dialysis, markers of mineral metabolism, bone turnover, malnutrition, and inflammation, but in the absence of longitudinal data, the relevance of these factors to the progression of VC remains unclear. The small number of published studies on the progression of VC are limited by small subject numbers, qualitative assessments of calcification, and indirect associations with functional alterations of the vasculature.

The aim of this study was to evaluate prospectively the relative contributions of multiple factors that are implicated in the development and progression of VC in CKD. We aimed to compare the progression of VC in patients with stage 4 CKD with dialysis patients. Furthermore, we sought to study prospectively the association between VC and mortality. It is hoped that these data will allow identification of targets for potential modification to reduce VC and improve CV risk among patients with CKD.

Concise Methods

Patients

A total of 134 patients (60 on hemodialysis [HD], 28 on peritoneal dialysis [PD], and 46 with stage 4 CKD) were recruited from Derby City General Hospital. Patients were eligible unless they had previously undergone renal transplantation or limb amputation. Patients with

Received May 24, 2007. Accepted July 17, 2007.

Published online ahead of print. Publication date available at www.cjasn.org.

Correspondence: Dr. Christopher W. McIntyre, Department of Renal Medicine, Derby City General Hospital, Derby, DE22 3NE, UK. Phone: +44-01332-625675; Fax: +44-01332-625975; E-mail: Chris.McIntyre@nottingham.ac.uk

ISSN: 1555-9041/206–1241

CKD stage 4 were staged on four-variable Modification of Diet in Renal Disease (MDRD) estimated GFR (eGFR) with values between 15 and 29 ml/min per 1.73 m^2. The Local Regional Ethics Committee granted project approval, and written consent was obtained from participants.

Study Design

Multislice computed tomography (CT) and measurement of hemodynamic variables were performed at baseline and 12 and 24 mo by a single observer. Baseline data have been published previously with a full description of the method (7). Briefly, multislice CT was used to quantify calcification in a standardized section of the superficial femoral artery. Each slice was scored individually, and a calcification score (CaSc) was generated. Calcification was considered to be present when an area ≥ 1 mm^2 displayed a density >130 Hounsfield units (12). Validation studies confirmed that the scoring technique is highly reproducible. Interobserver reproducibility between the investigator and a consultant radiologist was assessed in a 1 in 20 sample. The intraclass correlation was 1 (confidence interval 1 to 1), and the coefficient of variation (CoV) was 3.9%. Repeatedly scored scans showed an intraobserver intraclass correlation of 1 (confidence interval 1 to 1) and a CoV of 2.4%. Applanation tonometry was performed at the radial artery using a SphygmoCor (AtCor Medical Pty. Ltd., West Ryde, NSW, Australia). Augmentation was assessed as a derived central pressure wave. Electrocardiogram gated pulse wave velocity (PWV) was assessed at the radial and dorsalis pedis pulses before dialysis in the HD patients. Three BP recordings were taken using an automated AND UA-767 oscillometric device (A&D Medical, San Jose, CA).

Biochemistry

Monthly blood samples were collected for HD patients and at regular clinic visits for PD patients and patients with stage 4 CKD. Serum phosphate (PO_4), Ca, albumin corrected calcium, albumin, intact parathyroid hormone (PTH), lipid profile, and alkaline phosphatase (ALP) were analyzed using standard autoanalyzer techniques (Roche Diagnostics [Basel, Switzerland] Modular IIP). Serum PTH was measured using the immunometric, Immulite 2000 assay (Siemens Medical, New York, NY) (normal range 7 to 53 pg/ml). A time-averaged value is given for baseline (results averaged over the 6 mo before the study) and 12- and 24-mo results (values averaged over each 12-mo period between study visits). Commercially available ELISA kits were used to assess osteoprotegerin (OPG), fetuin-A, and high-sensitivity C-reactive protein (hsCRP) from samples that were obtained at baseline and 12 mo. The OPG ELISA (Immunodiagnostic Systems, Boldon, UK) had a detection limit of 0.14 pmol/L and a CoV of 10 to 4%. The fetuin-A ELISA (Biovendor, Modrice Czech Republic) had a detection limit of 0.35 ng/ml, and the hsCRP (DRG Instruments, Marburg/Iahn, Germany) detection limit was 0.1 mg/L and CoV was 7 to 2%.

Statistical Analyses

Results are reported as means ± SD for normally distributed data and median (inter quartile range [IQR]) otherwise. Comparisons of paired data were performed using paired sample *t* test for parametric data and Wilcoxon matched paired test for nonparametric data. Comparisons of more than two sets of data were performed using one-way ANOVA (with Bonferroni *post hoc* analysis for multiple comparisons) or Kruskal-Wallis tests depending on distribution. χ^2 tests were used to compare categorical data. Correlation coefficients were generated using R Spearman (*r*) tests for nonparametric data (*i.e.*, bimodal distribution of CaSc). Mann-Whitney *U* tests were used to compare skewed variables between two groups.

Multiple attempts were made to normalize the distribution of CaSc; however, the bimodal distribution of CaSc, the high number of zero scores, and tails at both extremes prevented successful transformation of the data for linear regression analysis. Instead, ordinal regression analysis, with CaSc divided into quartiles, was chosen as the most meaningful method of analysis. The following baseline independent categorical variables were entered into regression analysis as determinants of CaSc: Modality group (HD, PD, or stage 4 CKD); ethnicity; gender; diabetes (yes/no); previous CV morbidity (yes/no); history of parathyroidectomy (yes/no); and the use of phosphate binders, 1α calcidiol, corticosteroids, lipid-lowering agents, diuretics, angiotensin-converting enzyme inhibitors, Ca channel antagonists, warfarin, erythropoietin, or β blockers. The following baseline independent continuous variables were entered into regression analysis as determinants of CaSc: Age, weight, body mass index (BMI), smoking history (pack-years), alcohol intake, eGFR, elemental Ca intake from phosphate binders, time-averaged PO_4, time-averaged albumin corrected calcium, time-averaged albumin, time-averaged PTH, time-averaged ALP, time-averaged lipids, baseline OPG, baseline hsCRP, and baseline fetuin-A. Cox proportional hazard analysis was performed using time to death as the dependent variable and all of the previously mentioned variables as well as change in CaSc (ΔCaSc; divided by 10 for increased units). A Kaplan-Meier plot was used to compare the survival of patients with and without VC. Statistics were generated using SPSS 12.0.1 (SPSS, Chicago, IL).

Results

Study Population

Baseline characteristics of study patients are shown in Table 1. Only CaSc and eGFR as well as use of phosphate binders, angiotensin-converting enzyme inhibitors, and β blockers were significantly different between the groups at baseline. At 24 mo, 101 of 134 patients remained to be studied, including four patients who had received a transplant (12 patients were lost to follow-up, and 21 died). Twenty-five of 46 patients with stage 4 CKD initiated dialysis during the study period (11 PD and 14 HD). Table 2 shows time-averaged biochemical variables at 12 and 24 mo. The population had good biochemical control, particularly serum PO_4 and Ca.

VC

During 24 mo, overall CaSc increased from a median of 12 (IQR 0 to 334) at baseline to 53 (IQR 0 to 480; $P < 0.0001$). CaSc increased in 58 of 101 patients ($P < 0.0001$). Figure 1 shows the increase in CaSc by modality group (categorized according to modality at 24 mo) at 12 and 24 mo. The HD group demonstrated a greater degree of progression than the PD or stage 4 CKD group at both 12 ($P = 0.002$) and 24 mo ($P = 0.01$). The rate of progression of CaSc was constant during the 24 mo of follow-up (median progression was 65 CaSc units per year between baseline and 12 mo in comparison with 54 CaSc units per year between 12 and 24 mo).

Thirty-one patients with a CaSc of zero at baseline remained free of calcification throughout the study (eight with stage 4 CKD, 11 on PD, and 11 on HD). During the first 12-mo period, calcification was initiated in eight (17%) of 46 patients (median CaSc 4) followed by another six (15%) of 38 patients during the second 12-mo period (median CaSc 4). Regression of CaSc was observed in five patients at 12 mo (median decrease 28 ± 20%) and eight patients at 24 mo (median decrease 52 ± 40%). No

Table 1. Baseline patient data[a]

Parameter	Stage 4 CKD (n = 46)	PD (n = 28)	HD (n = 60)	P
Age (yr; mean ± SD)	60 ± 14	61 ± 14	60 ± 15	NS
Male gender (n [%])	26 (56)	17 (60)	42 (70)	NS
Diabetes (n [%])	10 (21)	8 (29)	16 (27)	NS
Smokers (n [%])	6 (13)	4 (14)	6 (10)	NS
Dialysis vintage (mo;)	N/A	34 ± 23	36 ± 25	NS
BMI (kg/m^2; mean ± SD)	25 ± 9	25 ± 11	23 ± 9	NS
eGFR (ml/min; mean ± SD)	19 ± 6	8 ± 3	2 ± 5	0.001
Dialysis adequacy (Kt/V; mean ± SD)[b]	–	2.5 ± 0.5	1.2 ± 0.2	–
Previous CV comorbidities (n [%])[c]	11 (23)	12 (42)	12 (20)	NS
Baseline CaSc (median [IQR])	2 (0 to 197)	21 (0 to 343)	121 (0 to 610)[e]	0.008
No. of patients with VC (n [%])	22 (47)	20 (71)	44 (73)	0.02
Vitamin D (n [%])	17 (37)	17 (61)	33 (55)	NS
Lipid-lowering therapy (n [%])	15 (33)	13 (46)	18 (30)	NS
Non–calcium-based phosphate binders (n [%])	1 (2)	16 (57)	26 (43)	< 0.001
Calcium-based phosphate binders (n [%])[d]	17 (37)	13 (46)	37 (61)	0.04
Dose of calcium based binder (g/d; mean ± SD)	1.9 ± 1.0	1.9 ± 0.8	2.3 ± 1.3à	0.003
Calcium channel blockers (n [%])	23 (50)	11 (39)	13 (21)	NS
Use of ACE inhibitors (n [%])	17 (37)	11 (89)	11 (18)	0.02
Use of β blockers (n [%])	20 (43)	3 (10)	15 (38)[e,f]	0.009

[a]ACE, angiotensin-converting enzyme; BMI, body mass index; CaSc, calcification score; CKD, chronic kidney disease; CV, cardiovascular; eGFR, estimated GFR; HD, hemodialysis; IQR, interquartile range; N/A, not applicable; PD, peritoneal dialysis; VC, vascular calcification.

[b]Kt/V in PD is weekly and in HD is per single session.

[c]Defined as any previous description of ischemic heart disease, heart failure, cerebrovascular disease, or peripheral vascular disease recorded in the patients medical chart.

[d]Some patients are prescribed both calcium-containing and non–calcium-containing phosphate binders.

Bonferroni *post hoc* analysis: [e]Significant difference between stage 4 CKD and HD ($P > 0.05$); [f]Significant differences between PD and HD ($P > 0.05$).

clear determinants of VC regression were identified. Of the four patients who received a transplant, two had progression and one had no change from zero. The fourth patient, a 36-yr-old man who had diabetes and received a kidney and pancreas transplant, saw his CaSc fall from 269 to zero.

Determinants of VC

Table 2 shows results of biochemical measurements that were performed on the study patients. Progressive calcification (ΔCaSc baseline to 24 mo) correlated with baseline CaSc and age and inversely with albumin and the use of lipid-lowering agents (Table 3). No associations were found between ΔCaSc and time-averaged Ca, PO_4, PTH, hsCRP, Ca from phosphate binders, the use of sevelamer, or any other prescribed medications. Ordinal regression analysis showed that the progression of VC was associated with gender, age, time-averaged ALP, and the use of lipid-lowering agents and β blockers (model r^2 = 0.64; Table 4).

Analysis of the modality groups separately showed similar overall associations with VC, with a few exceptions. It is interesting that in patients with stage 4 CKD, the reduction in eGFR correlated with ΔCaSc ($r = -0.478$, $P < 0.05$). When patients who were free of VC were compared with those with VC, patients who were free of calcification had significantly lower weight (69 ± 12 *versus* 79 ± 12 kg; $P = 0.001$), lower BMI (26 ± 4 *versus* 27 ± 4 kg/m^2; $P = 0.04$), and higher HDL cholesterol (1.5 ± 0.4 *versus* 1.3 ± 0.4; $P = 0.014$). In contrast to previous reports, we found no association between VC and fetuin-A in the whole population or within modality groups. Low fetuin-A levels were observed in all three groups (0.22 ± 0.1, 0.24 ± 0.2, and 0.25 ± 0.1 g/L for PD, HD, and stage 4 CKD, respectively; normal range 0.4 to 1 g/L) (13). In addition, we observed no association between fetuin-A and hsCRP (Figure 2).

CV Consequences of VC

Hemodynamic variables that were measured at baseline and 12 and 24 mo are shown in Table 2, with significant changes highlighted. ΔCaSc correlated with pulse pressure ($r = 0.216$,

Table 2. Biochemical and hemodynamic variables in all patients at baseline and 12 and 24 mo[a]

Variable	Baseline (n = 134)	12 Mo (n = 117)	24 Mo (n = 101)
Total cholesterol (mmol/L)	4.6 ± 1.0	4.2 ± 0.9[b]	4.0 ± 1.0[c]
HDL cholesterol (mmol/L)	1.30 ± 0.30	1.32 ± 0.30	1.37 ± 0.40[c,d]
LDL cholesterol (mmol/L)	2.29 ± 0.90	1.84 ± 0.70[b]	1.78 ± 0.80[c,d]
PO_4 (mmol/L)	1.59 ± 0.36	1.62 ± 0.35	1.55 ± 0.30[d]
CCa (mmol/L)	2.45 ± 0.13	2.45 ± 0.14	2.45 ± 0.12
Ca × PO_4 ($mmol^2/L^2$)	3.90 ± 0.90	3.98 ± 0.88	3.78 ± 0.84
PTH (pg/ml)	257 ± 233	379 ± 367[b]	404 ± 383[c]
ALP (IU/L)	102 ± 55	123 ± 93[b]	128 ± 75[c]
Albumin (g/L)	32 ± 5	33 ± 4	34 ± 4[c,d]
hsCRP (mg/L)	9.6 ± 18	10.9 ± 21	–
Osteoprotegerin (pmol/L)	27 ± 9	30 ± 11[b]	–
Fetuin-A (ng/ml)	0.24 ± 0.14	0.27 ± 0.09[b]	–
Systolic BP (mmHg)	149 ± 28	144 ± 24[b]	138 ± 23[c]
Diastolic BP (mmHg)	80 ± 15	77 ± 12[b]	76 ± 13[c]
Pulse pressure (mmHg)	68 ± 23	67 ± 23	62 ± 20[c]
Heart rate (bpm)	70 ± 13	72 ± 16	74 ± 16[c]
Augmentation (mmHg)	15 ± 10	16 ± 14	14 ± 11
Augmentation index (p1/p2)	139 ± 22	137 ± 34	116 ± 48[c,d]
PWV (m/s)	10.3 ± 3.4	11.2 ± 4.7	9.7 ± 3.5[d]

[a]Biochemical results are time averaged. Data are means ± SD. ALP, alkaline phosphatase; CCa, albumin corrected calcium; hsCRP, high-sensitivity C-reactive protein; PTH, parathyroid hormone.
[b]Significant differences between baseline and 12 mo ($P > 0.05$).
[c]Significant difference between baseline and 24 mo ($P > 0.05$).
[d]Significant differences between 12 and 24 mo ($P > 0.05$).

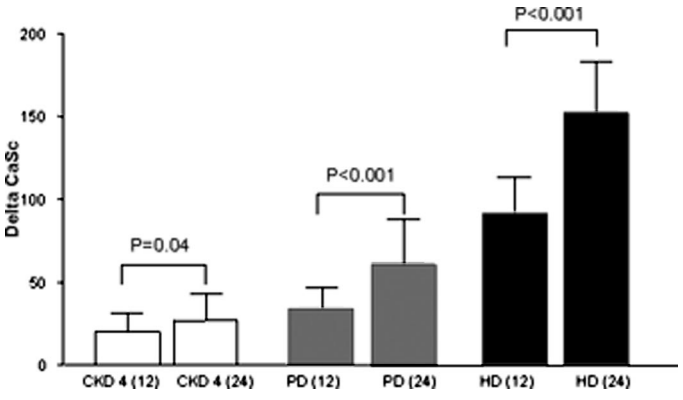

Figure 1. Change in calcification score over 24 mo. Calcification scores (CaSc) are shown for the groups (patients with stage 4 chronic kidney disease [CKD; n = 16], peritoneal dialysis [PD; n = 26] patients, and hemodialysis [HD; n = 55] patients) at 12 and 24 mo. CaSc significantly increased from baseline at both 12 (stage 4 CKD $P = 0.005$, PD $P < 0.001$, and HD $P < 0.001$) and 24 mo (stage 4 CKD $P = 0.001$, PD $P < 0.001$, and HD $P < 0.001$). The HD group demonstrated a significantly higher increase in CaSc than the PD or stage CKD 4 group at both 12 ($P = 0.002$) and 24 mo ($P = 0.01$).

$P < 0.05$) and PWV ($r = 0.329$, $P < 0.05$) and inversely with diastolic BP ($r = -0.294$, $P < 0.05$) at 24 mo. It is interesting that ΔCaSc correlated significantly with increased arterial stiffening (ΔPWV) at both 12 ($r = 0.52$, $P < 0.001$) and 24 mo ($r = 0.33$, $P = 0.003$; Figure 3).

Table 3. Univariate correlations between Δ CaSc during 24 mo

Variable	Correlation Coefficient	P
Baseline CaSc	0.648	<0.001
Age (yr)	0.410	<0.001
Albumin (g/L)	−0.263	0.004
Lipid-lowering agents (yes)	−0.259	0.005

Mortality

Of the original 134 patients, 21 died. Twenty of 21 patients who died had VC. The single mortality without VC was due to metastatic uroepithelial malignancy (Figure 4). Forty percent of patients died of CV-related causes. Mortality in the modality groups was 52% in HD, 33% in PD, and 14% stage 4 CKD. Cox proportional hazard analysis identified three factors that were associated with reduced survival: ΔCaSc, calcium intake from phosphate binders, and low albumin at baseline (Table 5).

Discussion

We found that patients with stages 4 and 5 CKD and preexisting VC exhibit progression of calcification at a continuous rate over 24 mo. These data show that progressive calcification of

Table 4. Determinants of VC progression during 24 mo[a]

Variables	OR (95% CI)	*P*
Age (yr)	1.11 (1.05 to 1.16)	<0.001
Male gender	8.82 (1.82 to 42.65)	0.007
ALP (IU/L)	1.02 (1.00 to 1.03)	0.01
Lipid-lowering agents (yes)	0.27 (0.08 to 0.86)	0.03
β blockers (yes)	0.27 0.08 to 0.95)	0.04

[a]Ordinal regression analysis was used with Δ CaSc as the dependent variable (model $R^2 = 0.64$). CI, confidence interval; OR, odds ratio.

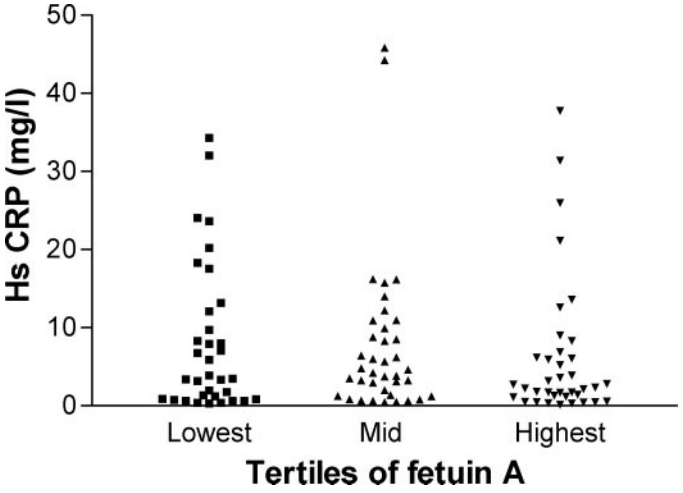

Figure 2. Scatter plot of the association between tertiles of fetuin-A and high-sensitivity C-reactive protein (hsCRP) in all study patients at baseline ($P = 0.42$).

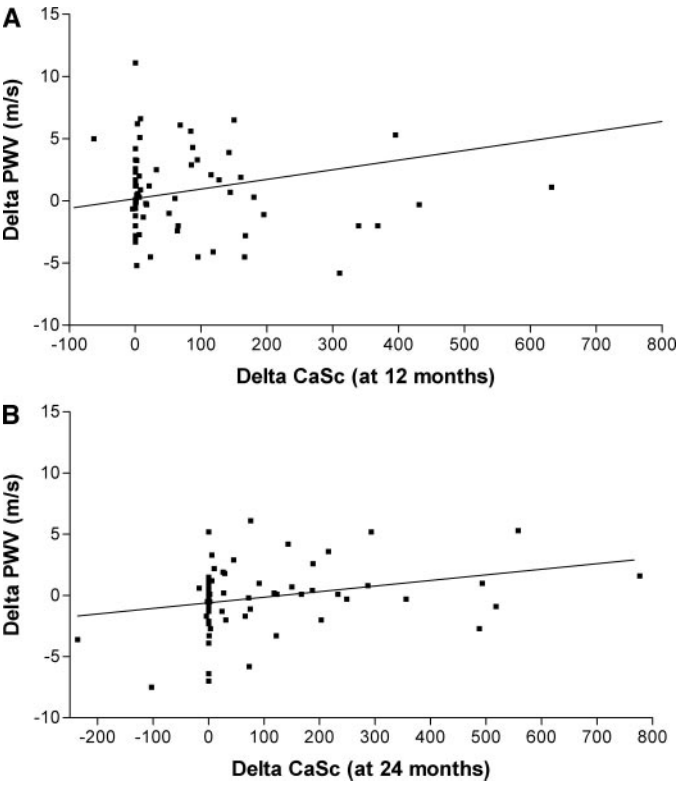

Figure 3. (A) Scatter plot of change in CaSc (ΔCaSc) plotted against change in pulse wave velocity (ΔPWV [m/s] at 12 mo ($r = 0.52$, $P < 0.001$). (B) Scatter plot of ΔCaSc plotted against ΔPWV (m/s) at 24 mo ($r = 0.33$, $P = 0.003$).

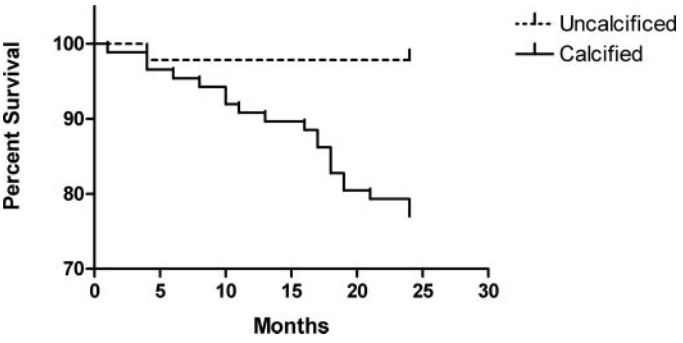

Figure 4. Kaplan-Meier plot of survival in patients with and without calcification of the superficial femoral artery at baseline.

the superficial femoral artery is associated with an incremental increase in arterial stiffness. In addition, the progression of VC, the use of calcium-containing phosphate binders, and reduced albumin were independent factors associated with death.

Rapid progression of VC was seen in 57% of patients. Other studies have found comparable rates of progression in PD and HD (8,14). This rate of progression has not previously been appreciated in patients with stage 4 CKD. Conversely, 4 to 8% of patients demonstrated regression. This shows that VC is a dynamic process, and its progression could be modified if appropriate strategies for therapeutic intervention were identified. Thirty percent of patients across all modalities remained free of VC after 24 mo. This finding is consistent with previous observational studies of VC (15). These patients are not, however, exempt from developing calcification. Seven percent of study patients developed VC during the first 12 mo, and in 5%, calcification started during the second 12-mo period. It is interesting that there were no significant differences between those who did and did not develop calcification other than weight, BMI, and HDL cholesterol, all features of the metabolic syndrome. Further studies of patients who are free of calcification could provide answers to the protective mechanisms involved.

The arterial calcification score generated by CT scanning is a composite of both medial and intimal calcification or calcified athero- and arteriosclerosis. These coexisting abnormalities have different pathologies. This is reflected in the multifactorial determinants of the progression of VC found in this and other studies. Consistent with other studies (8,10), we found that age and male gender were important determinants of VC. These patients are also the most likely to have intimal arterial disease (1). Other atherosclerotic risk factors were associated with VC in this study. The negative association seen in this study between the use of lipid-lowering agents and VC could reflect

Table 5. Factors that predicted patient survival during 24 mo[a]

Variable	HR (95% CI)	*P*
Albumin (g/L)	0.86 (0.76 to 0.97)	0.01
Δ CaSc (baseline to 12 mo)	1.03 (1.01 to 1.05)	0.03
Elemental calcium from binders (g/d)	1.41 (1.04 to 1.93)	0.03

[a]Cox proportional hazard analysis was used (−2 log likelihood = 71.2; $\chi^2 = 44.6$; $P < 0.0001$). HR, hazard ratio.

lower atherosclerotic burden as a result of a reduction in cholesterol. In addition, low serum albumin was associated with progressive VC, in keeping with previous studies of VC (11,16). Albumin, an abundant plasma protein, has been identified as a mediator of vascular damage in dialysis patients independent of calcification (17). Low serum albumin is directly predictive of CV death in dialysis patients (18). Hypoalbuminemia may reflect inflammation and/or malnutrition, both associated with atherosclerosis in CKD (19,20). Low albumin levels seen here could be due to either, because the hsCRP levels were moderately raised. We found no association between hsCRP and VC; however, the importance of inflammation cannot be discounted, because no other circulating markers of inflammation were measured. Fetuin-A, a negative acute-phase reactant in nonuremic individuals, has been identified as an inhibitor of calcification in murine models. Again, we found no relationship between fetuin-A and hsCRP, questioning whether it is truly a marker of inflammation in our population. In addition, all patients had low serum fetuin-A levels (13). We found no association between VC and circulating fetuin-A. Hermans *et al.* (21) investigated the potential relationship between fetuin-A and PWV and published similar negative findings.

In the general elderly population, progressive VC is solely associated with age and atherosclerotic risk factors (22). Calcification of the media seen predominately in patients with CKD is associated with additional factors, which may be unique to CKD. In other studies (15), elevated serum calcium and phosphate are commonly (but not consistently) associated with VC. Our population was characterized by excellent mineral control, which may explain why there was no association seen in this study. Braun *et al.* (23) showed that patients with the worst phosphate control had the most rapid progression of VC. The use of sevelamer, in comparison with calcium-based phosphate binders, is the only intervention that has been shown to attenuate the progression of VC; however, this could be related to the lipid-lowering properties of sevelamer (24). In this study, there was no association between progressive VC and the type or dosage of phosphate binder. It is important to note that calcification still advances in patients with well-controlled mineral levels and no calcium from phosphate binders (25). ALP was associated with the progression of VC. Circulating ALP in this setting could be directly involved in the calcification process or a marker of bone activity, although we found no association between PTH and VC. There is evidence that ALP has localized effects on the vessel, particularly in patients with diabetes, although this requires further elucidation (26). The use of β blockers was also associated with the progression of VC. β Blockers inhibit the β adrenoreceptors in the heart and peripheral vasculature and may promote VC through modulating sympathetic activity and trophic effects on peripheral vasculature. VC in HD patients is associated with reduced baroreflex sensitivity (6). In addition, β blockers can directly modulate skeletal function in murine models (27).

It is interesting that preexisting VC was a strong determinant of progression. Block *et al.* (15) also found this in 109 incident HD patients. In addition, HD seems to exacerbate calcification more than PD. PD patients had a similar rate of progression to patients who had not yet started on dialysis. The reasons for this are unclear, although the PD population had a higher residual kidney function and, less VC at baseline and used more Ca-containing phosphate binders. The ordinal regression analysis accounts for 64% of the variability, which suggests that additional factors are involved. Taken together, our data suggest that there is a complex interaction between atherogenic and CKD-specific risk factors along with additional unspecified risk factors, which drive the progression of VC in patients with CKD.

Progressive calcification in this cohort is associated with an increase in arterial stiffness, high pulse pressure, and low diastolic BP. Cross-sectional studies (28) have shown that VC is associated with arterial stiffening. Blacher *et al.* (5,28) found that VC correlated with increased PWV, increased pulse pressure, and decreased diastolic BP. We have shown for the first time that these variables change in parallel over time. Incremental increases in PWV and pulse pressure have been observed in patients with increasing stages of CKD (29). Chronic hypertension rather than VC was previously used to explain this observation; however, Briet *et al.* (30) demonstrated altered vascular structure and arterial stiffening in patients with stages 3 and 4 CKD compared with a hypertensive population with normal renal function. It is clear, though, that PWV is not continuously on an increasing trajectory. PWV is affected by multiple factors, and the decreased PWV that is seen in some patients could reflect improved fluid management, improved BP control, or a change in vasoactive medication. This study demonstrates that VC is a highly active process that is directly related to vascular function, rather than an inert component of atherosclerosis or arteriosclerosis.

We observed that progressive calcification during 12 mo is independently predictive of death in patients with stages 4 and 5 CKD. This was despite the inclusion of more conventional mortality risk factors (*e.g.*, age, CV comorbidities) into the model. The association between VC at a single time point and mortality was observed previously in homogeneous HD groups (1,31). The mechanisms whereby VC contributes to CV death are not fully understood, but we propose that the arterial stiffness that is associated with VC leads to increased left ventricular afterload and decreased diastolic coronary perfusion. The amount of Ca delivered from calcium-based phosphate binders was also an independent predictor of death. Block *et al.* (31) recently demonstrated that death was three times more

likely in patients who used calcium-containing phosphate binders, rather than sevelamer, in a population of incident HD patients. The third determinant of reduced survival was a low serum albumin, which probably reflects malnutrition and has been associated with death in other studies conducted in patients with CKD (18).

Limitations to this study include the relatively small patient numbers in the three groups; this potentially limited the relevance of intergroup statistical analysis with particular reference to the drivers of VC. The observed associations between β blockers and lipid-lowering agents should be interpreted with caution, because these agents were not prescribed as part of a randomized study. In addition, a randomized, controlled trial would be necessary to confirm the observational findings from this study.

Conclusions

These data demonstrate rapidly progressive VC in comparable populations of HD and PD patients and patients with stage 4 CKD. Several potentially modifiable determinants of VC progression have been identified. Given its associations with CV mortality, preventing the progression of VC should become a clinical target in the treatment of patients with CKD. In view of our observations, in patients with stage 4 CKD, early intervention and aggressive management of the progression of VC needs to begin before the initiation of renal replacement therapy.

Acknowledgments

M.K.S. is funded to conduct this research through a 2-yr fellowship from Kidney Research UK/British Renal Society. Additional funding for this study was awarded through a Derby Hospitals NHS Foundation Trust research grant.

We acknowledge sister Carol Rhodes and staff on the renal unit in Derby for accommodating and supporting this project. In addition, we acknowledge Apostolos Fakis for expert statistical advice.

Disclosures

None.

References

1. London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H: Arterial media calcification in end-stage renal disease: Impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant* 18: 1731–1740, 2003
2. Foley RN, Parfrey PS, Sarnak MJ: Epidemiology of cardiovascular disease in chronic renal disease. *J Am Soc Nephrol* 9[Suppl]: S16–S23, 1998
3. Kalantar-Zadeh K, Block G, Humphreys MH, Kopple JD: Reverse epidemiology of cardiovascular risk factors in maintenance dialysis patients. *Kidney Int* 63: 793–808, 2003
4. Stenvinkel P, Pecoits-Filho R, Lindholm B: Coronary artery disease in end-stage renal disease: No longer a simple plumbing problem. *J Am Soc Nephrol* 14: 1927–1939, 2003
5. Blacher J, Guerin AP, Pannier B, Marchais SJ, London GM: Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. *Hypertension* 38: 938–942, 2001
6. Chesterton LJ, Sigrist MK, Bennett T, Taal MW, McIntyre CW: Reduced baroreflex sensitivity is associated with increased vascular calcification and arterial stiffness. *Nephrol Dial Transplant* 20: 1140–1147, 2005
7. Sigrist M, Bungay P, Taal MW, McIntyre CW: Vascular calcification and cardiovascular function in chronic kidney disease. *Nephrol Dial Transplant* 21: 707–714, 2006
8. Stompor TP, Pasowicz M, Sulowicz W, Dembinska-Kiec A, Janda K, Wojcik K, Tracz W, Zdzienicka A, Konieczynska M, Klimeczek P, Janusz-Grzybowska E: Trends and dynamics of changes in calcification score over the 1-year observation period in patients on peritoneal dialysis. *Am J Kidney Dis* 44: 517–528, 2004
9. Nitta K, Akiba T, Uchida K, Kawashima A, Yumura W, Kabaya T, Nihei H: The progression of vascular calcification and serum osteoprotegerin levels in patients on long-term hemodialysis. *Am J Kidney Dis* 42: 303–309, 2003
10. Barreto DV, Barreto FC, Carvalho AB, Cuppari L, Cendoroglo M, Draibe SA, Moyses RM, Neves KR, Jorgetti V, Blair A, Guiberteau R, Fernandes Canziani ME: Coronary calcification in hemodialysis patients: The contribution of traditional and uremia-related risk factors. *Kidney Int* 67: 1576–1582, 2005
11. Wang AY, Woo J, Wang M, Sea MM, Ip R, Li PK, Lui SF, Sanderson JE: Association of inflammation and malnutrition with cardiac valve calcification in continuous ambulatory peritoneal dialysis patients. *J Am Soc Nephrol* 12: 1927–1936, 2001
12. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R: Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 15: 827–832, 1990
13. Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Bohm R, Metzger T, Wanner C, Jahnen-Dechent W, Floege J: Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: A cross-sectional study. *Lancet* 361: 827–833, 2003
14. Braun J, Oldendorf M, Moshage W, Heidler R, Zeitler E, Luft FC: Electron beam computed tomography in the evaluation of cardiac calcification in chronic dialysis patients. *Am J Kidney Dis* 27: 394–401, 1996
15. Block GA, Spiegel DM, Ehrlich J, Mehta R, Lindbergh J, Dreisbach A, Raggi P: Effects of sevelamer and calcium on coronary artery calcification in patients new to hemodialysis. *Kidney Int* 68: 1815–1824, 2005
16. Chertow GM, Raggi P, Chasan-Taber S, Bommer J, Holzer H, Burke SK: Determinants of progressive vascular calcification in haemodialysis patients. *Nephrol Dial Transplant* 19: 1489–1496, 2004
17. Malatino LS, Benedetto FA, Mallamaci F, Tripepi G, Zoccali C, Parlongo S, Cutrupi S, Marino C, Panuccio V, Garozzo M, Candela V, Bellanuova I, Cataliotti A, Rapisarda F, Fatuzzo P, Bonanno G, Seminara G, Stancanelli B, Tassone F, Labate C: Smoking, blood pressure and serum albumin are major determinants of carotid atherosclerosis in dialysis patients. CREED Investigators. Cardiovascular Risk Extended Evaluation in Dialysis patients. *J Nephrol* 12: 256–260, 1999
18. Lowrie EG, Lew NL: Death risk in hemodialysis patients: The predictive value of commonly measured variables and

an evaluation of death rate differences between facilities. *Am J Kidney Dis* 15: 458–482, 1990
19. Tonbul HZ, Demir M, Altintepe L, Guney I, Ekrem Y, Turk S, Yeksan M, Yildiz A, Kaysen GA: Malnutrition-inflammation-atherosclerosis (MIA) syndrome components in hemodialysis and peritoneal dialysis patients Association between inflammation and malnutrition as risk factors of cardiovascular disease. *Ren Fail* 28: 287–294, 2006
20. Kaysen GA, Dubin JA, Muller HG, Mitch WE, Rosales LM, Levin NW: Relationships among inflammation nutrition and physiologic mechanisms establishing albumin levels in hemodialysis patients. *Kidney Int* 61: 2240–2249, 2002
21. Hermans MM, Brandenburg V, Ketteler M, Kooman JP, Sande FM, Gladziwa U, Rensma PL, Bartelet K, Konings CJ, Hoeks AP, Floege J, Leunissen KM: Study on the relationship of serum fetuin-A concentration with aortic stiffness in patients on dialysis. *Nephrol Dial Transplant* 21: 1293–1299, 2006
22. Messika-Zeitoun D, Bielak LF, Peyser PA, Sheedy PF, Turner ST, Nkomo VT, Breen JF, Maalouf J, Scott C, Tajik AJ, Enriquez-Sarano M: Aortic valve calcification: Determinants and progression in the population. *Arterioscler Thromb Vasc Biol* 27: 642–648, 2007
23. Braun J, Asmus HG, Holzer H, Brunkhorst R, Krause R, Schulz W, Neumayer HH, Raggi P, Bommer J: Long-term comparison of a calcium-free phosphate binder and calcium carbonate: Phosphorus metabolism and cardiovascular calcification. *Clin Nephrol* 62: 104–115, 2004
24. Chertow GM, Burke SK, Raggi P: Sevelamer attenuates the progression of coronary and aortic calcification in hemodialysis patients. *Kidney Int* 62: 245–252, 2002
25. Asmus HG, Braun J, Krause R, Brunkhorst R, Holzer H, Schulz W, Neumayer HH, Raggi P, Bommer J: Two year comparison of sevelamer and calcium carbonate effects on cardiovascular calcification and bone density. *Nephrol Dial Transplant* 20: 1653–1661, 2005
26. Fadini GP, Pauletto P, Avogaro A, Rattazzi M: The good and the bad in the link between insulin resistance and vascular calcification. *Atherosclerosis* 193: 241–244, 2007
27. Pierroz DD, Bouxsein ML, Rizzoli R, Ferrari SL: Combined treatment with a beta-blocker and intermittent PTH improves bone mass and microarchitecture in ovariectomized mice. *Bone* 39: 260–267, 2006
28. Haydar AA, Covic A, Colhoun H, Rubens M, Goldsmith DJ: Coronary artery calcification and aortic pulse wave velocity in chronic kidney disease patients. *Kidney Int* 65: 1790–1794, 2004
29. Wang MC, Tsai WC, Chen JY, Huang JJ: Stepwise increase in arterial stiffness corresponding with the stages of chronic kidney disease. *Am J Kidney Dis* 45: 494–501, 2005
30. Briet M, Bozec E, Laurent S, Fassot C, London GM, Jacquot C, Froissart M, Houillier P, Boutouyrie P: Arterial stiffness and enlargement in mild-to-moderate chronic kidney disease. *Kidney Int* 69: 350–357, 2006
31. Block GA, Raggi P, Bellasi A, Kooienga L, Spiegel DM: Mortality effect of coronary calcification and phosphate binder choice in incident hemodialysis patients. *Kidney Int* 71: 438–441, 2007