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Associations between vascular calcification, arterial stiffness and bone mineral density in chronic kidney disease

Nigel D. Toussaint[1,2], Kenneth K. Lau[3], Boyd J. Strauss[2,4], Kevan R. Polkinghorne[1,2] and Peter G. Kerr[1,2]

[1]Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia, [2]Department of Medicine, Monash University, Clayton, Victoria, Australia, [3]Department of Radiology and [4]Clinical Nutrition and Metabolism Unit, Monash Medical Centre, Clayton, Victoria, Australia

Abstract

Background. Vascular calcification (VC) and arterial stiffness are major contributors to cardiovascular (CV) disease in chronic kidney disease (CKD). Both are independent predictors of CV mortality and are inversely correlated with bone mineral density (BMD). Few studies have addressed the extent of VC in the pre-dialysis CKD population, with associated measurements of BMD and arterial compliance.
Methods. We report cross-sectional data on 48 patients with CKD (GFR 17–55 ml/min) assessing the prevalence of VC and its associations. All patients had computed tomography (CT) scans through abdominal aorta and superficial femoral arteries (SFAs) to determine VC, pulse wave velocity (PWV) using SphygmoCor device (AtCor PWV Inc., Westmead, Australia) measuring arterial stiffness, and dual-energy X-ray absorptiometry (DEXA) scans to determine BMD, as well as serum markers of renal function and mineral metabolism.
Results. Patients, 71% male, 54% diabetic, had a median age 64.5 years. Mean estimated GFR was 35.1 ± 10 ml/min. Mean PWV was 10.0 ± 4.5 m/s and mean aortic VC score was 421.5 ± 244 Hounsfield units, with 90% of subjects having some aortic VC present. In univariate linear regression analysis, aortic VC correlated positively with age (r 0.50, $P < 0.001$), triglycerides (r 0.47, $P = 0.002$) and PWV (r 0.33, $P = 0.03$). There was also greater VC with declining renal function (r −0.28, $P = 0.05$). There was no significant association between VC and serum markers of mineral metabolism, however phosphate and Ca × P correlated positively with PWV (r 0.35, $P = 0.02$, r 0.36, $P = 0.02$, respectively). There was also a positive association between PWV and triglycerides ($P = 0.008$), and a trend towards greater PWV with increasing age ($P = 0.09$). In multivariate regression analysis only increasing age and triglyceride levels were significantly associated with aortic VC and PWV. Mean spine and femoral T-scores on DEXA were 0.48 and −1.31 respectively, with 13% of subjects having femoral T-score <−2.5 (osteoporotic range). SFA VC inversely correlated with femoral T-scores (r −0.43, $P = 0.004$); however, there was a positive (likely false) association between spine T-scores and aortic VC (r 0.37, $P = 0.01$), related to the limitation of vertebral DEXA in CKD.
Conclusion. There is a high prevalence of VC in pre-dialysis CKD patients, worse with increasing age, triglycerides and reducing renal function. Correlation exists between VC and PWV and determination of one or both may be useful for CKD patient CV risk assessment. Femoral BMD is inversely associated with SFA VC, but measurement of vertebral BMD by DEXA is unreliable in CKD patients with aortic VC.

Keywords: arterial stiffness; bone mineral density; cardiovascular disease; chronic kidney disease; mineral metabolism; vascular calcification

Cardiovascular (CV) disease is the leading cause of mortality in patients with chronic kidney disease (CKD) [1–3] and up to 45% of pre-dialysis CKD patients may die before receiving dialysis [4]. Although traditional CV risk factors are common in this population, much of the CV disease may relate to non-traditional CV risk factors such as vascular calcification (VC) and arterial stiffness [5–7]. Although the presence of increased VC in patients with CKD has been known for some time, the extent to which VC impacts on CV disease and mortality has only recently been appreciated. Studies have reported increased VC in end-stage kidney disease (ESKD) compared to the general population, with the predominant differences being earlier age of onset and greater distribution [8–10]. Studies involving VC measurement of CKD patients, not on dialysis, are limited but the prevalence of CV disease is also increased [11–15].

CKD patients also have stiffer vessels compared to the general population, contributing to reduced arterial compliance [16]. In CKD there is a similar magnitude of atherosclerotic plaque burden and intimal thickness but

Correspondence and offprint requests to: Dr Nigel Toussaint, Department of Nephrology, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia. Tel: +61-03-9594-3072 (w), +61-0418-560-198 (m); E-mail: Nigel.Toussaint@med.monash.edu.au

markedly increased medial calcification (arteriolosclerosis) [17]. This phenomenon is a major determinant of left ventricular pressure overload and of abnormal coronary perfusion [5]. The extent of VC and the degree of arterial stiffening, closely inter-related, are independent predictors of CV mortality in both the general and CKD populations [18–20]. Again, there is limited available data about functional arterial wall properties in mild-to-moderate CKD [21–23].

A relationship also exists between increasing VC and loss of bone mineral density (BMD), with recent experimental studies revealing the mechanisms which link these two processes [24]. In the general population and in CKD there is an association between CV mortality and osteoporosis [25,26] and BMD has been shown to be inversely associated with both VC [8,27,28] and arterial stiffness as measured by pulse wave velocity (PWV) [29].

Few studies in CKD have looked at both structural and functional changes associated with VC and most studies independently addressing VC, arterial stiffness and BMD have looked at ESKD patients, with limited data in the predialysis CKD population. We present cross-sectional data on a cohort of CKD patients, not on dialysis, outlining the prevalence and severity of VC and arterial stiffness. The aims of this study were to explore the relationship between VC, arterial compliance and BMD in CKD and to identify factors potentially related to each.

Methods

Study subjects

Forty-eight patients were recruited between January and June 2007 from outpatient clinics and private consulting rooms by nephrologists at Monash Medical Centre, Clayton, Australia. The cohort selected were those willing to participate in a randomized controlled clinical trial assessing the use of alendronate versus placebo on VC and arterial stiffness in CKD patients over an 18-month period (ClinicalTrials.gov Registration No. NCT00395382). Inclusion criteria were ages 18–80 and reduced GFR between 20 and 60 ml/min/1.73 m^2, as estimated with the Modification of Diet in Renal Disease (MDRD) formula [30]. The protocol was approved by the local Ethics Committee and all patients gave written consent. The trial is currently in progress and results presented in this study are an analysis of baseline data.

Computed tomography

We analysed the non-contrast computed tomography (CT) scans of the abdominal aorta and bilateral superficial femoral arteries (SFAs) of 48 patients, from which the VC scores were determined. The non-contrast CT scans were performed using GE medical systems Lightspeed 16® multi-slice spiral CT scanner (120 kVp, 75 mAs for abdominal aorta, 25–75 mAs for SFAs and 1.375 pitch). Images were acquired in a spiral mode with the patient being supine. The scanning range was from T12 vertebral level to L4 vertebral level for abdominal aorta and from the level of the lesser trochanter to that of the knees for the bilateral SFAs. The images were reconstructed back to 10 mm for viewing on the workstation. Hounsfield units (HU) of any VC in the aorta and SFA were noted by a single radiologist who was blinded to the patient demographics, serum results and arterial compliance and BMD data. The number of calcifications and the highest HU of calcifications in the infra-renal abdominal aorta and SFAs were recorded.

Pulse wave velocity

Arterial stiffness was assessed using a SphygmoCor device (AtCor Medical, PWV Inc., Westmead, Sydney, Australia) to measure PWV and augmentation index (AI), the latter a composite parameter reflecting both large and distal arterial properties. A pencil-type hand-held probe was used to obtain pulse waveforms at radial, carotid and femoral arterial sites. PWV measures the time interval between pulse waves at the carotid and femoral arteries and higher values represent stiffer vessels, as seen in patients with CKD. The AI represents the difference between the early and late systolic peaks of the systolic pulse wave contour, divided by the pulse pressure—again higher values occur with stiffer vessels due to greater reflection of the pulse wave distally. Brachial blood pressure was measured before each PWV determination. A 3-lead electrocardiograph (ECG) was attached to the subject and the surface distance between pulse points was measured using a tape measure while the patient was supine. All measurements were made by a single operator. Determination of PWV on one patient was not possible due to technical difficulty so 47 patients had arterial compliance documented.

Bone mineral density

BMD was assessed by dual-energy X-ray absorptiometry (DEXA) scans. Absolute BMD values, Z-scores and T-scores (number of standard deviations below the BMD of a younger reference group) for lumbar spine and right femoral neck were reported and mean scores for all patients were calculated. The DEXA scan used was a GE-Lunar Prodigy (General Electric Medical Services, Australia) with the same densitometer used for all patients for accurate comparisons.

Laboratory values

All patients performed a 24 h urine collection for measurements of creatinine (Cr) clearance (corrected for body surface area), as well as for protein excretion. Serum Cr was analysed using an automated Jaffe rate method and calculation of estimated glomerular filtration rate (eGFR) was performed using the MDRD formula. Other serum markers measured were those addressing mineral metabolism, including calcium (corrected), phosphate, calcium–phosphate product (Ca × *P*), intact parathyroid hormone (PTH) and alkaline phosphatase (ALP), as well as haemoglobin, albumin, ferritin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and lipid profile. Blood samples were drawn in the fasting state and serum was analysed using Synchron LX 20 Pro autoanalyzer (Beckman Coulter Inc., Fullerton, CA, USA). Total

serum calcium was adjusted for albumin levels using the conversion factor; corrected calcium = calcium + 0.02 mmol/L × (40 – albumin) [31]. Intact PTH levels were measured by immunometric assay (Immunolite 1000, Diagnostic Products Corporation, Los Angeles, CA, USA).

Clinical characteristics

Medical charts were reviewed for clinical history and medications, and supplemented with information obtained directly from patients. Weight and height were measured in order to calculate the body mass index (BMI). Patients were considered to have a history of coronary artery disease if there was previous abnormal cardiac investigation or a history of myocardial infarction or angina. Hypertension was defined as having a documented history of high blood pressure and taking, or having taken, blood pressure lowering agents. Patients were considered to have peripheral vascular disease (PVD) if there was a history of intermittent claudication, leg ulceration or previous abnormal peripheral angiography or Doppler ultrasound. Medications were recorded, including calcium-based phosphate binders, anti-hypertensive agents, warfarin and cholesterol-lowering agents.

Statistical analysis

Results are expressed as mean ± SD, median (and range) or frequency (and proportion). Univariate associations between VC, PWV and BMD were explored using linear regression. Multivariate linear regression was performed to determine significant associations between VC, PWV and other variables, adjusted for potential confounders. All variables with *P*-value less than 0.10 in the univariate analysis were included in the multivariate regression model. Stepwise backward elimination was used, beginning with the variable with the highest *P*-value. Inspection of the change in the adjusted R^2 and performance of a likelihood ratio test were both used to confirm that deleted factors did not contribute to the model. The assumptions underlying multiple linear regression were examined. The residuals for each predictor were normally distributed and any individual outliers that excessively biased the model were removed. Comparison of unpaired data for variables related to aortic VC, between two groups divided by the median VC score, was performed using unpaired *t*-tests for parametric data and Mann–Whitney *U*-tests for non-normally distributed data. A *P*-value of <0.05 was considered to be statistically significant. Intercooled Stata 10.0 (StataCorp, College Station, Texas, US) was used for all statistical analysis.

Results

The demographics and clinical characteristics of the patients studied are presented in Table 1. Patients were predominantly male (70.8%) with a median age 64.5 years (range 26–80). Fifty-four percent were diabetic and most patients had a history of hypertension (95.8%), with 60.4% taking ACE (angiotensin-converting enzyme) inhibitors. Diabetes mellitus was the main cause of CKD (47.9%) with hypertensive nephrosclerosis the next most common (27.1%). Renal function and laboratory values, including serum markers of mineral metabolism and inflammation, are displayed in Table 2. Mean serum Cr and eGFR were 185.3 μmol/L and 35.1 ml/min, respectively. Mean 24 h Cr clearance and protein excretion were 51.8 ml/min and 1.19 g respectively. Proteinuria (>0.10 g/day) was found in 82% of patients. Overall the patients had good mineral control and good lipid levels within the normal range, the latter likely related to most patients being administered cholesterol-lowering agents (68.8%).

Table 1. Characteristics of patient studied ($n = 48$)

	Median (range) or frequency (%)
Age (years)	64.5 (26–80)
Gender (male)	34 (70.8)
Race (Caucasian)	43 (89.6)
BMI (kg/m^2)	31.3 (18.2–54.5)
DM	26 (54.2)
CAD	15 (31.3)
CVD	5 (10.4)
PVD	6 (12.5)
HT	45 (95.8)
Cause of CKD	
DM	23 (47.9)
HT/renovascular	13 (27.1)
GN	5 (10.4)
Other[a]	7 (14.6)
Smoking	
Current	5 (10.4)
Former	24 (50.0)
Medications	
Vitamin D[b]	8 (16.7)
Phosphate binders	3 (6.3)
ACE inhibitors	29 (60.4)
ARBs	18 (37.5)
Statins[c]	33 (68.8)
Warfarin	2 (4.2)

[a]Other include polycystic kidney disease, reflux nephropathy, vasculitis, interstitial nephritis and obstructive nephropathy.
[b]Active vitamin D, all patients on oral calcitriol.
[c]Cholesterol-lowering HMG-CoA reductase inhibitors.
Abbreviations: BMI, body mass index; DM, diabetes mellitus; CAD, coronary artery disease; CVD, cerebrovascular disease; PVD, peripheral vascular disease; HT, hypertension; CKD, chronic kidney disease; GN, glomerulonephritis; ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers.

Vascular calcification and arterial compliance

Most patients (90%) had evidence of abdominal aortic calcification and 60% had presence of SFA calcification. Mean aortic VC score was 421.5 ± 243.7 HU (range 0–1019.5) and mean PWV 10.0 ± 4.5 m/s (range 5–23) (Table 3). Univariate regression analysis with aortic VC as the dependent variable revealed a significant positive correlation with age, PWV and triglyceride levels and a negative correlation with renal function measured by eGFR (Table 4). Age, PWV and PVD positively correlated with the presence of SFA VC. Although there was a strong association between left and right SFA VC ($P < 0.001$), there was no significant correlation between aortic and SFA VC (data not shown). There was also no association with VC and diabetes, gender, CRP or proteinuria. Increased PWV was associated with age and

Table 2. Renal function, protein excretion and serum markers ($n = 48$)

	Mean ± SD	*Normal range*
Creatinine (μmol/L)	185.3 ± 59.2	*40–120*
eGFR (ml/min)[a]	35.1 ± 10.5	*>60*
Creatinine clearance (ml/min)[b]	51.8 ± 19.9	*90–150*
Proteinuria (g/day)	1.19 ± 1.6	*0.01–0.10*
Urea (mmol/L)	13.8 ± 4.4	*2.5–9.6*
Haemoglobin (g/L)	130.3 ± 14.6	*120–160*
WCC ($\times 10^9$/L)	7.4 ± 1.8	*4–11*
ESR (mm/h)	25.9 ± 29.9	*0–25*
C-reactive protein (mg/L)	6.8 ± 9.9	*0–5*
Albumin (g/L)	37.6 ± 3.3	*35–45*
Calcium (mmol/L)	2.34 ± 0.12	*2.20—2.60*
Phosphate (mmol/L)	1.24 ± 0.19	*0.80—1.50*
Ca × P ($mmol^2/L^2$)	2.89 ± 0.46	*NA*
ALP (U/L)	96.2 ± 46.5	*30–120*
PTH (pmol/L)	14.9 ± 10.5	*1.1–7.7*
Bicarbonate (mmol/L)	24.6 ± 3.2	*22–32*
Glucose (mmol/L)	6.8 ± 3.0	*3.0—5.4*
Ferritin (μg/L)	187.3 ± 193.0	*20–300*
Cholesterol (mmol/L)	4.6 ± 1.2	*<5.5*
Triglycerides (mmol/L)	2.0 ± 1.4	*<2.0*

[a]eGFR, estimated glomerular filtration rate (calculated by the MDRD formula).
[b]Creatinine clearance calculated by 24 h urine collection.
Abbreviations: WCC, white cell count; ESR, erythrocyte sedimentation rate; ALP, alkaline phosphatase; PTH, parathyroid hormone; Ca × P, calcium × phosphate product.

Table 3. Calcification measurements, pulse wave velocity and bone mineral density ($n = 48$)

	Mean ± SD	Range
Vascular calcification[a]		
Aortic	421.5 ± 243.7	(0–1019.5)
Left SFA	169.5 ± 216.2	(0–1144.9)
Right SFA	169.9 ± 215.0	(0–945.5)
Pulse wave velocity		
PWV (m/s)[b]	10.0 ± 4.5	(5–23)
AI (%)	22.5 ± 10.5	(1–41)
SBP (mmHg)	127.1 ± 23.0	(90–155)
DBP (mmHg)	73.3 ± 11.0	(50–90)
PP (mmHg)	56.1 ± 11.1	(35–80)
Bone mineral density		
BMD spine (g/cm^2)	1.28 ± 0.21	(0.77–1.96)
BMD femoral (g/cm^2)	0.86 ± 0.17	(0.33–1.19)
T-score spine[c]	0.48 ± 1.71	(−3.9–6.0)
T-score femoral[c]	−1.31 ± 1.17	(−3.9–0.9)

[a]Measured in Hounsfield units [median scores: aortic VC 403.4, L SFA 120.4, R SFA 119.7]
(90% had presence of aortic VC, 60% L SFA, 58% R SFA).
[b]Only 47 patients had PWV performed.
[c]T-score: compared to the young-normal reference range.
World Health Organisation (WHO) definitions:
Normal = T-score at or above –1.0.
Osteopenia = T-score between –1.0 and –2.5 SD.
Osteoporosis = T-score at or below –2.5 SD.
Abbreviations: SFA, superficial femoral artery; BMD, bone mineral density; PWV, pulse wave velocity; AI, augmentation index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

triglyceride levels, and there was also a significant positive correlation with phosphate and Ca × P on univariate analysis (Table 4). There was a trend towards greater PWV with reducing eGFR, but this association was not statistically significant ($P = 0.09$).

Table 4. Associations with vascular calcification and arterial stiffness: univariate regression analysis

	Aortic VC		Right SFA		PWV	
	r	*P*	*r*	*P*	*R*	*P*
Age	0.50,	***<0.001***	0.33,	***0.023***	0.31,	***0.044***
PVD	−0.02,	ns	0.39,	***0.008***	0.12,	ns
PWV	0.33,	***0.034***	0.32,	***0.043***	–	–
eGFR	−0.28,	***0.050***	−0.18,	ns	−0.25,	*0.09*
CrCl	−0.23,	ns	−0.29,	*0.058*	−0.13,	ns
Calcium	0.08,	ns	−0.22,	ns	0.07,	ns
Phosphate	0.03,	ns	0.02,	ns	0.35,	***0.024***
Ca x P	0.06,	ns	−0.05,	ns	0.36,	***0.020***
Cholesterol	0.10,	ns	−0.009,	ns	0.04,	ns
Triglycerides	0.47,	***0.002***	−0.19,	ns	0.42,	***0.008***

Statistically significant values $P <$ ***0.05*** in **bold**; all $P <$ *0.10* in *italics*; ns, non-significant ($P \geq 0.10$).
Abbreviations: PWV, pulse wave velocity; VC, vascular calcification; DM, diabetes mellitus; PVD, peripheral vascular disease; eGFR, estimated glomerular filtration rate; CrCl, creatinine clearance; Ca × P, calcium × phosphate product.

Table 5. Multivariate regression analysis with variables entering the equation as correlates of vascular calcification and pulse wave velocity with aortic VC, right SFA VC and PWV as dependent variables

	R^2	Coefficient	95% CI	*P*-value	St β
Aortic VC	0.49				
Age		10.02	4.40–16.07	***0.001***	0.42
eGFR		−5.43	−11.09–0.22	0.059	−0.23
Triglycerides		79.28	36.91–121.64	***0.001***	0.45
R SFA VC	0.35				
Age		8.23	2.00–14.45	***0.011***	0.38
PWV		7.76	−8.11–23.62	0.330	0.14
PVD		226.19	56.71–395.66	***0.010***	0.36
PWV	0.37				
Age		0.13	0.01–0.25	***0.030***	0.30
Triglycerides		1.16	0.30–2.01	***0.009***	0.37
Ca × P		3.19	0.71–5.67	***0.013***	0.35

P-values <***0.05*** in *italics* and ***bold***; St β: standardized coefficient.
Abbreviations: VC, vascular calcification; PWV, pulse wave velocity; eGFR, estimated glomerular filtration rate; PVD, peripheral vascular disease; SFA, superficial femoral artery; Ca × P, calcium phosphate product.

In the multivariate regression model to analyse the independent determinants of aortic VC and PWV, age and triglyceride levels were identified to be significant for both (Table 5). PWV was also significantly associated with Ca × P on multiple linear regression, and SFA VC significantly correlated with age and PVD in the multivariate model. To further investigate factors that predispose patients to develop greater VC, we divided our cohort into two with respect to aortic VC, based on the median score (403 HU), those patients with scores <400 and ≥400. Again we demonstrate that those with greater VC were older, had worse renal function and had faster PWV (Table 6).

Bone mineral density

Mean T-scores on DEXA were 0.48 ± 1.71 and −1.31 ± 1.17 for lumbar spine and femoral regions respectively,

Table 6. Variables shown with respect to aortic calcification scores (VC)

Variable	VC < 400	VC ≥ 400	*P*
Age (years)	56.6	68.3	***0.0001***
eGFR (ml/min)	38.6	32.0	***0.02***
BMI (kg/m^2)	30.1	32.5	ns
CRP (mg/L)	5.4	8.3	ns
Cholesterol (mmol/L)	4.6	4.5	ns
Triglycerides (mmol/L)	1.76	2.27	ns
Calcium (mmol/L)	2.33	2.34	ns
Phosphate (mmol/L)	1.21	1.26	ns
Ca × P (mmol^2/L^2)	2.83	2.95	ns
PTH (pmol/L)	11.8	18.1	***0.05***
PWV (m/s)	8.58	11.48	***0.03***

P-values <**0.05** in *italics* and **bold**.
Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; PTH, parathyroid hormone; PWV, pulse wave velocity; Ca × P, calcium × phosphate product.

the latter falling within the World Health Organisation (WHO) definition of osteopenia (Table 3). Twenty-three patients (47.9%) had femoral T-scores less than −1.0, with 6 (12.5%) having T-scores less than −2.5 (WHO osteoporotic range). Only five patients had vertebral T-scores less than −1.0 (one patient less than −2.5). There was a trend towards a positive association between renal function, measured by Cr clearance, and femoral BMD and T-scores, but not for vertebral BMD (Table 7).

BMD and T-scores of the femoral region showed a strong inverse correlation with the presence of SFA calcification ($r -0.34, P = 0.022, r -0.43, P = 0.004$) (Table 7). In multivariate analysis, this finding remained significant (data not shown) and is consistent with reports in the literature of an inverse relationship between osteopenia and VC. However, correlation between BMD and T-scores of the lumbar spine and aortic VC revealed a significantly, positive (but likely false) association ($P = 0.012$ and $P = 0.016$, respectively), which may represent a limitation in reporting of DEXA for patients with CKD and aortic VC. Z-scores were also determined from DEXA, and on multivariate analysis there was a similar positive association with aortic VC and inverse relationship with SFA VC for vertebral and femoral neck Z-scores respectively, as with T-scores (data not shown).

Discussion

Although our observational study was limited by its cross-sectional design, the results indicate that the prevalence of VC and arterial stiffness in pre-dialysis patients is high. VC induces arterial stiffness and increases PWV measured in large elastic arteries and it has been previously demonstrated that VC scores measured using CT are strongly correlated to arterial stiffness [7,12]. In our study we also found a positive correlation between VC and PWV, although in multivariate analysis this was no longer statistically significant, perhaps related to a small sample size. Studies in CKD report a 1.9 times increase in mortality hazard ratio for each unit increment of VC [6] and also mortality being increased by 39% with every 1 m/s increase in PWV [32], therefore detection of one or both sub-clinical markers of arterial disease may provide useful tools to gain an appreciation of the burden of CV disease in CKD.

VC is now recognized as an active process involving a complex interaction of inducers and inhibitors [33] and studies in ESKD reveal that 80–85% of prevalent dialysis patients and 60% of incident patients have some degree of coronary artery or aortic VC [10,34]. Few data are currently available, however, comparing VC and its functional CV consequences in the pre-dialysis CKD population. Studies in CKD Stages 3 and 4 show that 40–70% of patients have coronary artery VC [11,14] and in one recent study 47% of CKD Stage 4 patients demonstrated SFA VC [15]. Our study revealed that 90% of the 48 CKD patients assessed (mean eGFR 35 ml/min) had some degree of measurable VC in the abdominal aorta, with 60% having SFA VC. The presence of VC in our study is greater than previous reports which may be related to differences in the sample selection because other studies have not included diabetic patients [11] or have assessed younger patients with all stages of CKD (including Stage 1) [13]. Also, our CT measurement of VC involved the abdominal aorta and not coronary arteries like most reports and there may potentially be more VC in the aorta. Although this may be one explanation, a previous study of 33 dialysis patients reported that only 61% had aortic VC scores [35]. The most likely reason for greater VC prevalence in our study population is probably differences in calcification scoring as we have documented all detectable VC in our study, as opposed to measurements >130 HU like previous reports [15,36].

Table 7. Associations with bone mineral density: univariate regression analysis

	BMD spine		T-score spine		BMD femoral		T-score femoral	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Age	0.28,	*0.063*	0.28,	*0.062*	−0.16,	ns	−0.13,s	ns
CrCl	−0.04,	ns	−0.03,	ns	0.30,	*0.053*	0.28,	*0.068*
Aortic VC	0.37,	***0.012***	0.36,	***0.016***	0.16,	ns	0.20,	ns
R SFA VC	0.09,	ns	0.08,	ns	−0.34,	***0.022***	−0.43,	***0.004***
PWV	0.24,	ns	0.24,	ns	0.07,	ns	−0.005,	ns

Statistically significant values $P <$ ***0.05*** in **bold**; all $P <$ *0.10* in *italics*; ns, non-significant ($P \geq 0.10$).
Abbreviations: BMD, bone mineral density; VC, vascular calcification; PWV, pulse wave velocity; Cr, creatinine; SFA, superficial femoral artery; CrCl, creatinine clearance.

Similar to previous studies, we found a strong correlation with VC and increasing age but no association between calcification scores and abnormalities of mineral metabolism (such as hypercalcaemia, hyperphosphatemia, raised Ca × P and hyperparathyroidism) or diabetes. Factors reported to be associated with increasing severity and prevalence of VC in ESKD have included age, duration of dialysis and diabetes as well as abnormal mineral metabolism and administration of calcium-based phosphate binders, although increasing age and dialysis duration are the only consistent risk factors [6,7,9,10,20,37]. In the literature, coronary arteries are the commonest site for VC to be measured by CT but many studies have also used CT to assess the aorta and a few have looked at the SFAs [15,35,38]. We found a significant correlation with both aortic and SFA VC and increasing age, but not with gender. Not surprisingly, there was also an association with SFA VC and documented PVD in our study. Previous studies using semi-quantitative methods to assess VC in peripheral vessels have found VC to be functionally significant in dialysis patients [20].

Patients with CKD have stiffer arteries compared with the non-renal population, and abnormal PWV is apparent early in the development of CKD, with progression as renal impairment deteriorates [16,23,39]. Briet *et al* reported on arterial stiffness of 95 CKD patients (mean GFR 31 ml/min), compared to 121 hypertensive and 57 normotensive subjects, showing that PWV was significantly higher in CKD (11.3 versus 10.6 and 9.5 m/s respectively) [23]. Previous reports have also shown that increased PWV in CKD is consistently associated with elevated serum phosphate and Ca × P and the total dose of calcium-based phosphate binders, all important risk factors for VC [40]. We also report a positive correlation between PWV and elevated phosphate and Ca × P, as well as with age and triglycerides, in univariate regression analysis, and a significant association with age, triglyceride levels and Ca × P in multivariate analysis.

CKD has been individualized as an independent risk factor for CV disease, proportionally with the decline in renal function [41]. Limitations with the use of indirect renal function measurement, using both estimation of GFR (with MDRD formula) and 24 h urine Cr clearance, however have resulted in a paucity of available data on VC and arterial wall properties in pre-dialysis CKD. One recent report of CKD patients not on dialysis used nuclear isotope GFR determination, the gold standard, with assessment of arterial stiffness [23] and prior to this study, associations between GFR and arterial stiffness had not been adequately detected. In our study we examined VC and PWV in relation to renal function measured by both eGFR and Cr clearance (strong correlation between these, r 0.77, $P < 0.001$). We showed that there was a negative correlation between VC and PWV and eGFR (r −0.28, $P = 0.05$, r −0.25, $P = 0.09$ respectively) in our patients with mild-to-moderate CKD, although not quite statistically significant and only seen in univariate analysis. Cr clearance was not significantly associated with either VC or PWV, but this may be explained by small sample size or perhaps because Cr clearance is less reliable when GFR <60 ml/min, and can over-estimate the true renal function at this level.

Bone quality is impaired in CKD and renal bone disease has a wide spectrum from osteitis fibrosa (high bone turnover) to adynamic bone disease (low bone turnover), with both cases leading to excessive minerals in the circulation. Although bone biopsy is the gold standard for determination of bone abnormalities, serum values of mineral metabolism and non-invasive imaging are more often used. In our study we utilized DEXA scans to measure BMD and revealed a strong inverse correlation with femoral BMD and VC measured at the SFA, highlighting the link between osteopenia and calcification in CKD, similar to the general population. Aortic VC, however, was not inversely associated with BMD, and instead showed a positive (likely false) correlation with vertebral BMD. One potential reason for this is that DEXA scans measure the absorption of dual-energy X-ray beams projected blindly through the body which may be absorbed by dense VC in the aorta rather than the spine, therefore leading to falsely elevated BMD readings. Results from our study support the lack of reliability of DEXA in CKD reported in the literature and it had been suggested that quantitative CT should probably be used to assess spine BMD instead [29,42]. Another potential reason for the positive association between aortic VC and BMD may be the presence of osteophytes in the spine which could also falsely increase BMD. CT scans therefore may be useful to not only measure aortic VC but to more accurately determine vertebral BMD in CKD compared to DEXA. Measurement of BMD in CKD patients may however be of limited value as results do not necessarily reflect classic osteopenia and osteoporosis due to the complexity of renal bone disease.

Increased arterial stiffness, like VC, is also associated with low BMD and a recent study of 110 haemodialysis patients revealed that PWV increased as BMD, measured by quantitative CT, decreased [29]. In contrast in this study, there was no relationship between DEXA-measured vertebral BMD and PWV. We found no correlation between BMD (femoral or vertebral) and PWV in our cohort, perhaps because DEXA scans, and not CT, were used to measure BMD. There is also a widely appreciated association between increasing osteopenia and declining renal function [43,44] and, although we found a trend towards decreased femoral neck BMD with worsening renal function measured by Cr clearance (but not eGFR), this was not statistically significant.

There was no association between blood pressure parameters and VC or arterial stiffness in our study, although only one blood pressure reading was taken for each individual. Studies have shown systolic blood pressure to correlate with VC [6,20] and PWV [38,39], although this is not a consistent finding, and a negative finding in our study may be related to more aggressive hypertensive treatment with most patients taking renin–angiotensin–aldosterone antagonists. Dyslipidemia has also been reported to be associated with VC [45,46] and PWV [21], although again not consistently. There was no correlation with VC or PWV and hypercholesterolaemia in the present study (most study patients were on lipid-lowering therapy) but triglyceride levels were an independent determinate of both VC and PWV on multivariate analysis. This finding was unrelated to the presence of diabetes, and diabetes itself was not significantly

associated with VC or arterial compliance. Despite further analysis we were unable to determine any differing characteristics between patients with low or high triglycerides that may have confounded the positive association between VC, PWV and triglyceride levels. Whether reduction of triglyceride levels through pharmacological measures results in a reduction in VC however is yet to be determined.

There were a few limitations in our study. The study was observational and there were small patient numbers. No intra-observer variability was determined by repeated measures of VC or PWV parameters. However, both right and left SFA VC scores were calculated with a high correlation between these. Also, individual investigators reported each of VC, PWV and BMD measurements independently without knowledge of results of the other tests. We did not quantify dietary calcium intake in our study patients, although the amount of exogenous calcium ingested may be as important as serum levels with regards to calcium balance in advanced CKD and we also did not measure 25-hydroxy vitamin D levels, although this would be useful in the CKD population when assessing VC and BMD.

Conclusion

Our study reports a high incidence of VC in pre-dialysis CKD patients, worse with increasing age, higher triglycerides and deteriorating renal function. There is a positive correlation between VC and arterial stiffness measured by PWV, and both have been previously shown to be associated with CV mortality in the CKD population. Screening methods to assess the degree of both could potentially be worthwhile in general clinical practice given the high prevalence and functional significance of these structural changes. Detection may allow accurate risk stratification and changes in treatment, as VC rarely regresses once developed. There is also an inverse relationship between VC and bone mineralization and we demonstrated that femoral BMD is inversely associated with SFA VC. DEXA scans of the femoral neck may be a reliable measure of BMD in CKD, however measurement of spine BMD by DEXA may be less accurate in CKD patients with aortic VC.

Conflict of interest statement. None declared.

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