NEPHROLOGY

Nephrology **19** (2014) 275–281

Original Article

Vascular calcification in patients undergoing kidney and simultaneous pancreas-kidney transplantation

KATRINA CHAU,[1] GABRIELA MARTINEZ[1] and GRAHAME J ELDER[1,2]

[1]Department of Renal Medicine, Westmead Hospital, and [2]Osteoporosis and Bone Biology Division, Garvan Institute of Medical Research, Sydney, New South Wales, Australia

KEY WORDS:
bone mineral density, cardiovascular disease, renal failure, transplantation, vascular calcification.

Correspondence:
Associate Professor Grahame J Elder, Department of Renal Medicine, Westmead Hospital, Westmead, NSW 2145, Australia.
Email: g.elder@garvan.org.au

Accepted for publication 23 January 2014.
Accepted manuscript online 7 February 2014.

doi:10.1111/nep.12212

SUMMARY AT A GLANCE

This group examined the prevalence and epidemiology of vascular calcification in younger patients undergoing kidney only or kidney–pancreas transplantation. They found it to be common in younger patients and resembled the older patients being associated with age, dialysis vintage and cardiovascular pathology. There was no difference in calcification between the type of transplant done and no significant inverse association of the calcification with bone mineral density.

ABSTRACT:

Aim: **Vascular calcification (VC) is common in patients with chronic kidney disease (CKD) on dialysis, and an inverse relationship of VC to bone mineral density (BMD) has been reported. Because elderly patients are prone to atherosclerosis and BMD artefact, we examined the prevalence and epidemiology of VC in younger patients undergoing transplantation, and its relationship to BMD.**

Methods: **Laboratory testing was performed immediately before kidney or simultaneous pancreas–kidney (SPK) transplantation. Within 4 weeks patients underwent BMD evaluation and lateral abdominal X-ray. Aortic calcification was scored using a validated 24-point scale.**

Results: **Of 650 consecutive patients X-rays were available for 531 (82%). Their median age was 41 years (16–71), 58% were male, dialysis vintage was 20 months (0–402) and 69% had kidney and 31% SPK transplants. VC scores were ≥1 in 47%, with the median score 6 (1–24) and was associated with age, dialysis vintage and presence of cardiovascular, cerebrovascular or peripheral vascular disease. In a multivariate analysis of patients with and without VC, those with VC were older and of longer dialysis vintage (OR 1.07 and 1.17 per 12 months respectively; $P < 0.001$ for both). In that analysis, VC was not significantly associated with gender, transplant type, presence of diabetes, current or former smoking or calcium or calcitriol therapy, and was not inversely related to hip, spine or forearm BMD Z-scores.**

Conclusion: **VC is common in younger patients undergoing transplantation and, similar to older patients, is associated with age, dialysis vintage and cardiovascular pathology. However, in this younger patient group, there was no significant inverse association of VC to BMD.**

Cardiovascular disease (CVD) causes approximately half of all deaths on dialysis. Adjusted for age, sex and diabetes, cardiovascular mortality in patients on dialysis is 10- to 20-fold that of the general population.[1] Vascular calcification (VC) is prevalent in patients with chronic kidney disease (CKD) and is an independent predictor of cardiovascular mortality.[2–4] Medial calcification, or arteriolosclerosis, distinguishes the VC of patients with CKD, as opposed to atherosclerotic intimal calcification in lipid-rich plaque, which is generally responsible for acute coronary events. Medial calcification increases arterial stiffness without causing vascular occlusion, resulting in left ventricular hypertrophy and impaired myocardial perfusion.

VC in CKD is a complex, active process in which vascular smooth muscle cells (VSMC) undergo phenotypic change with the development of osteoblast characteristics. In CKD stages 4, 5 and 5D (dialysis) and in small transplant cohorts, VC has been consistently associated with age and time on dialysis,[5] and less consistently with diabetes, calcium-based phosphate binders, and values of serum calcium, phosphate and PTH.[6] In addition, a number of studies report an inverse relationship to bone mineral density (BMD) assessed by dual energy X-ray absorptiometry (DXA).[7–10] It is proposed that uncoupling of bone turnover, with a net loss of mineral from bone, may predispose to the development of calcification in blood vessel walls.[11–13]

In studies investigating these associations, the dialysis population has often been elderly, with a high prevalence of vascular pathology. An example is the multicentre calcification outcome in renal disease (CORD) study,[3] which assessed 933 patients with a mean age of 61 years and a 44% prevalence of CVD. Although representative of the general dialysis population, such patients have a high risk of atherosclerosis and age-related degenerative changes affecting the lumbar spine that might obscure predisposing factors to VC that are specific to CKD.[14]

Patients receiving kidney and simultaneous pancreas–kidney (SPK) transplants are younger and fitter than the general dialysis population, and undergo extensive pre-transplant screening to exclude active CVD. We therefore studied associations of VC to epidemiological characteristics, laboratory data and BMD in patients with CKD stages 5 and 5D, who were about to undergo kidney or SPK transplantation.

MATERIALS AND METHODS

In this study, data were gathered prospectively to examine the prevalence of VC in 650 consecutive patients attending Westmead Hospital, Sydney, for kidney or SPK transplantation between 2000 and 2012. Blood was collected for laboratory investigations within the 24 hours preceding transplantation, and routine biochemistry included serum creatinine, corrected calcium, phosphorus and alkaline phosphatase (ALP). Bone-specific ALP (b-ALP) was measured using the Access Ostase® Assay and Unicell DXL 800 immunoassay system (Beckman Coulter, Brea, CA, USA) and intact-parathyroid hormone (PTH) was measured using the Immulite 2000 immunoassay system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Serum 25-hydroxyvitamin D (25(OH)D) was measured using the Liaison assay (DiaSorin Inc., Stillwater, MN, USA) with an 8.2% intra-assay coefficient of variation. The testing laboratory is enrolled in the Royal College of Pathologists Australasia Quality Assurance and the Vitamin D External Quality Assessment Scheme. Levels of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) were measured using the 1,25-dihydroxyvitamin D ^{125}I Radio Immuno Assay Kit with an in-house reference range. Total serum testosterone and sex hormone binding globulin (SHBG) (males) and estradiol levels (females) were measured using the COBAS E immunoassay analyser (Roche) and, prior to 2011, the Immulite 2000 immunoassay system (Siemens), with a sensitivity of 0.52 nmol/L for testosterone, 55 pmol/L for estradiol and 0.02 nmol/L for SHBG, and calculated free testosterone was derived using the Södergård formula.[15] Plasma FSH and LH (females) were measured by Immunoassay using the Elecsys 2010 system, with a sensitivity of 0.1 U/L.

Patients attended the metabolic bone clinic within 4 weeks of surgery, following a lateral X-ray of the thoracic spine and abdomen (including lumbar spine and abdominal aorta) and DXA (Norland XR36 from 2000 to 2007 and Norland XR800 2007 to 2012). Vascular calcification was assessed from the lateral abdominal X-ray, using the validated scoring system described by Kaupilla *et al*.,[16] in which patients are assigned an abdominal aorta calcification score based on the degree of calcification of the anterior and posterior segments of the abdominal aorta corresponding to the first 4 lumbar vertebrae. The calcification severity of each segment was scored from 0 to 3, so that possible scores ranged from 0 to 24. Vascular calcification was defined as any abdominal aorta calcification score ≥ 1. Staff assessing VC scores (GM, GJE) did so without access to other patient information. Fifty VC score sheets were randomly selected for blinded rescoring by the other assessor to check inter-observer consistency. BMD values (g/cm^2) were recorded from the lumbar spine (L2-4), femoral neck, total proximal femur, distal radius and ulna and proximal 1/3 radius and ulna. Z-Scores (adjusted for age and sex) were generated using Australian (Geelong) data for females (lumbar spine and hip), and US data supplied by Norland for forearm measurements in females and for all male BMD measurements.

Pre-transplant demographic and clinical characteristic data was collected directly from patients and from medical records on admission and at the time of the clinic visit. This included age, sex, racial background, body mass index (BMI), presence of diabetes, menopausal status, cause of kidney failure, dialysis type and duration and both prescribed and non-prescribed medication use and dosage. Data on patient comorbidities was obtained from ANZDATA (the Australian and New Zealand Dialysis and Transplant Registry) from the year of transplantation and confirmed from patient records. ANZDATA provides information on prevalent CVD, including coronary artery, cerebrovascular and peripheral vascular disease, hypertension and information on smoking status Patients are classified as 'yes', 'no' or 'suspected' for CVD and 'current', former' or 'never' for smoking. For the purpose of this study, patients who were 'suspected' to have coronary, cerebrovascular or peripheral vascular disease were deemed to have CVD. Patients who were 'former' smokers were classified as smokers.

The study was approved by the Western Sydney Local Health District Human Research Ethics Committee, and complied with the 1964 Declaration of Helsinki. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the 'Declaration of Istanbul on Organ Trafficking and Transplant Tourism'.

Statistical analysis

Results are presented as median and range or, where indicated, lower quartile to upper quartile range. Variable interdependence was assessed using Spearman's rho, and independent *t*-tests or Mann–Whitney *U*-tests were used to determine between group differences. One-way ANOVA or the

Table 1 Clinical characteristics, laboratory values and dual-energy X-ray absorptiometry Z-scores

	Combined patients	Admitted for kidney transplant	Admitted for SPK transplant	*P*-value
Clinical characteristics				
Age (years)	42 (16, 72)	47 (16, 72)	39 (16, 53)	<0.001
Gender (%)				0.09
Female	41.8	39.6	46.9	
Male	58.2	60.3	53	
Dialysis type (%)				0.003
Haemodialysis	57	61.8	46.2	
Peritoneal dialysis	28.5	25.9	34.9	
Pre-emptive	14.5	12.4	19	
Dialysis vintage (months)	20 (6, 53)	29 (10, 66)	11 (3, 30)	<0.001
Smoker (%)	39.3	40	38.2	0.69
Vascular disease (%)	14.9	12.1	21.5	0.01
Medication use (%)				
Calcium carbonate	74.5	73.9	75.9	0.61
Calcitriol	43.9	42.1	47	0.44
Laboratory values				
Calcium (2.13–2.63 mmol/L)	2.32 (2.19, 2.45)	2.32 (2.19, 2.46)	2.31 (2.2, 2.41)	0.16
Phosphorus (0.81–1.87 mmol/L)	1.61 (1.29, 2.01)	1.58 (1.28, 2.00)	1.71 (1.35, 2.05)	0.047
Parathyroid hormone (9–55 pmol/l)	31.3 (14.1, 59.5)	30.9 (13.2, 63.8)	32.6 (18.4, 53.6)	0.63
Alkaline phosphatase (30–115 U/L)	98 (73, 139)	90 (69, 124)	118 (88, 155)	<0.001
b-ALP (3.7–20.9 μg/L)	20.5 (13, 32.9)	19.9 (13, 30.8)	20.8 (14.5, 37.5)	0.27
25(OH)D (≥50–60 nmol/L)†	58 (40, 80)	63 (43, 90)	50 (35, 70)	<0.001
1,25 $(OH)_2D$ (36–120 pmol/L)	31 (19, 55)	31 (19, 58)	31 (17, 53)	0.16
Calculated free testosterone (120–470 pmol/L)	201 (142, 278)	198 (135, 278)	222 (153, 274)	0.23
BMD Z-scores				
Lumbar spine	–0.2 (–1, 0.80)	0 (–0.80, 0.95)	–0.58 (–1.20, 0.40)	<0.001
Femoral neck	–0.8 (–1.55, 0.20)	–0.40 (–1.1, 0.50)	–1.50 (–2.28, –0.80)	<0.001
Total proximal femur	–1.1 (–1.81, –0.10)	–0.70 (–1.5, 0.10)	–1.82 (–2.8, –1.3)	<0.001
Distal radius and ulna	–0.7 (–0.16, 0.40)	–0.30 (–1.2, 0.65)	–1.50 (–2.1, –0.44)	<0.001
1/3 radius and ulna	–0.6 (–1.64, 0.49)	–0.40 (–1.59, 0.60)	–1.06 (–1.84, 0.21)	0.046

Values are expressed as percent, or median plus lower and upper quartile ranges. *P*-values represent differences between patients admitted for kidney and SPK transplants. Smokers include current or previous smokers. Vascular disease includes definite or suspected coronary artery disease, peripheral vascular disease and cerebrovascular disease. Calculated free testosterone values are for males. b-ALP, bone-specific alkaline phosphatise; SPK, simultaneous pancreas–kidney transplant.

†Values represent 25(OH)D sufficiency.

Kruskal–Wallis test was used to determine differences in a continuous variable between independent groups. When two or more independent variables were associated in univariate analyses with VC scores, backward stepwise regression was used for exploratory analyses. Significance was determined by *P*-values <0.05. Data were analysed using SPSS version 20 (IL, USA).

RESULTS

Demographic information

Of 650 consecutive patients admitted for transplantation, 531 (82%) had lateral abdominal X-rays available for evaluation, comprising 81% of those undergoing kidney only and 85% of those undergoing SPK transplants. Transplant type was kidney only in 69% and SPK in 31%. Patient demographics and clinical characteristics are presented in Table 1. Calcium carbonate was generally taken as a phosphate binder, with a median elemental calcium dose of 1800 mg daily (3 tablets). The median dose of calcitriol was 1.75 μg weekly (7 capsules). Causes of CKD for patients receiving a kidney only transplant are listed in Table 2, and are compared with causes of CKD for all Australian dialysis patients in 2010.[17]

Laboratory values and BMD Z-scores

Relevant laboratory values and BMD Z-scores are shown in Table 1. The table does not include pre-transplant values of FSH or LH, which were higher ($P < 0.001$) or estradiol, which was lower ($P = 0.02$) in older women undergoing kidney transplants, or 4 week post transplant values of the bone resorption marker deoxypyridinoline to creatinine ratio, which did not differ between groups. BMD Z-scores were significantly lower at all sites for patients with Type 1 diabetes receiving SPK transplants.

Table 2 Causes of CKD in patients undergoing kidney only transplants, and comparison with the general dialysis population[17]

Diagnosis	Admitted for kidney transplant, % (*n*)	General dialysis patients (%)
Diabetes	7† (30)	35
Glomerulonephritis	45 (196)	22
Hypertension	4 (18)	14
Polycystic kidney disease	12 (53)	7
Vesicoureteric reflux	12 (52)	3
Analgesic nephropathy	0	2
Miscellaneous	17 (74)	12
Unknown	3 (13)	5

†Diabetes caused CKD in 7% of study patients but was present in 10.6%.

Vascular calcification scores

Vascular calcification was present in 48% of patients admitted for kidney only and 46% of patients admitted for SPK transplants, with a median score of 6 (0–24) and no significant differences in VC between transplant types ($P = 0.64$). For the 50 rescored X-rays, the correlation between assessors' scores was high at 0.91 ($P < 0.001$). One of 32 X-rays initially given a zero VC score was rescored as 2.

Vascular calcification, demographic factors and laboratory values

Associations of VC to patient demographics, laboratory values and BMD Z-scores are shown in Table 3. For the combined patients, factors associated with VC were age, dialysis vintage, dialysis type, smoking and vascular disease. For patients undergoing kidney only transplants, VC was also associated with the presence of diabetes. VC was not consistent across dialysis modes, but the dialysis vintage of patients on hemodialysis was significantly longer (Table 1). For patients undergoing SPK transplantation, VC was only significantly associated with age and dialysis vintage. There were no significant associations between VC scores and values of PTH, ALP, b-ALP, 25(OH)D or $1,25(OH)_2D$. Serum calcium and phosphate values were positively associated with VC in patients admitted for SPK transplantation, and for males admitted for kidney transplants, there was a positive association between VC and values of calculated free testosterone.

Vascular calcification and bone mineral density Z-scores

In patients admitted for kidney transplants, VC scores correlated positively to lumbar spine Z-scores, with no significant associations at other sites. No significant associations between VC and BMD Z-scores were identified in younger patients admitted for SPK transplants.

Comparison of patients with and without vascular calcification

As VC was not present in a large proportion of patients, a multivariate analysis was performed to compare all patients without identifiable VC to all those patients with VC scores ≥ 1. The initial model included age, dialysis vintage, dialysis type, smoking and vascular disease. By backward stepwise regression, factors independently associated with VC were age (OR = 1.072 per year of age, $P < 0.001$) and dialysis vintage (OR = 1.167 per year of dialysis, $P < 0.001$). Analysing patients admitted for kidney transplantation and including presence of diabetes in the model, independent factors associated with VC were once again age (OR = 1.083 per year of age, $P < 0.001$) and dialysis vintage (OR = 1.192 per year of dialysis, $P < 0.001$).

In order to identify which factors were associated with a higher AAC score, patients with calcification scores ≥ 1 were assessed by linear stepwise regression. Independent predictors of VC score were age ($\beta = 0.26$, $P < 0.001$), dialysis vintage ($\beta = 0.21$, $P = 0.003$) and documented vascular disease ($\beta = 0.38$, $P < 0.001$), with an adjusted R square of 0.25.

DISCUSSION

This study is, to the authors' knowledge, the largest observational study to assess epidemiological and laboratory variables associated with vascular calcification in patients with stages 5 and 5D CKD, considered fit to undergo kidney transplantation. It is also the only study to assess these associations in patients with type 1 diabetes about to undergo SPK transplantation. As part of routine pre-transplantation screening, these patients underwent a myocardial perfusion study and proceeded to coronary angiography if there was suspicion of significant CVD. As a consequence, compared with other studies of patients on dialysis with VC, patients in the current study were younger, with a low (15.9%), prevalence of CVD. Nevertheless their VC prevalence was high at 47.7%. This percentage is higher than a study using conventional radiographs of the aorto-iliac region and a different scoring technique, in which VC was identified in 24.4% of 1117 kidney transplant recipients.[18] However, other studies using electron-beam computed tomography (EBCT)[19] and spiral CT[20] have reported up to 65% of patients with VC, and a prevalence rate of 81% was reported in the CORD study.[3]

Although patients in this study did not have active CVD and were younger than the general dialysis population, VC was associated with the same factors reported elsewhere: age, dialysis vintage and prior CVD.[2,3,20] However, these variables explained a relatively small percentage of VC causation, indicating the important role of other factors related to CKD that we did not identify.

As in other studies,[19,21] we detected no significant associations between VC and pre-transplant values of serum

Table 3 Association of vascular calcification to demographic factors, laboratory values and bone mineral density Z-scores

	Combined patients	Admitted for kidney transplant	Admitted for SPK transplant
Age	$r = 0.46$, $P < 0.001$	$r = 0.52$, $P < 0.001$	$r = 0.35$, $P < 0.001$
Diabetes	NS	$P = 0.005$	N/A
Dialysis type	$P < 0.001$	$P < 0.001$	NS
Dialysis vintage	$r = 0.29$, $P < 0.001$	$r = 0.32$, $P < 0.001$	$r = 0.23$, $P = 0.003$
Smoker	$P = 0.012$	$P = 0.009$	NS
Vascular disease	$P = 0.006$	$P = 0.01$	NS
Serum calcium	NS	NS	$r = 0.17$, $P = 0.032$
Serum phosphate	$r = 0.1$, $P = 0.023$	NS	$r = 0.15$, $P = 0.049$
Serum calculated free testosterone (males)	$r = 0.2$, $P < 0.001$	$r = 0.18$, $P = 0.009$	NS
Lumbar spine Z-score	$r = 0.1$, $P = 0.02$	$r = 0.12$, $P = 0.024$	NS

Vascular calcification (VC) was not significantly associated to gender, values of total or bone specific alkaline phosphatase, PTH, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, use of calcium carbonate or calcitriol, or to bone mineral density Z-scores at the femoral neck, total proximal femur, distal radius and ulna or 1/3 radius and ulna. N/A, not applicable; NS, non-significant.

calcium or phosphate in patients admitted for kidney transplants, and only weak associations in patients admitted for SPK transplantation. While hyperphosphataemia is a strong inducer of VC *in vivo*, and has been associated with higher mortality in both CKD and general populations,[22] serum phosphate values may not be indicative of phosphate balance, or the effect of phosphate at the cellular level. In fact, patients undergoing long and intensive dialysis with excellent phosphate control have been reported to have prevalent VC rates similar to other dialysis patients at 83%,[23] suggesting that once patients are on dialysis, VC may develop despite phosphate lowering strategies, including those not reliant upon phosphate binders.

VC was not associated with the use of calcium-based phosphate binders or calcitriol supplementation in this study. Although some studies suggest that non-calcium based phosphate binder use attenuates VC development,[24] other studies report conflicting results.[25,26] Factors associated with VC such as dialysis vintage may also be surrogates for long-term calcium and active vitamin D exposure, and a recent randomized controlled trial and meta-analysis of observational and randomized studies suggested a survival advantage for patients treated with non-calcium-based phosphate binders.[27,28]

Various techniques have been used to assess VC, including EBCT and multi slice CT, echocardiography, and pulse wave velocity. The assessment of VC using lateral abdominal X-ray is low-cost, non-invasive, and correlates closely to coronary artery calcification identified by CT.[29] VC identified by this technique was also predictive of cardiovascular risk and outcome in the Framingham heart study population and after transplantation.[18]

BMD evaluation by DXA does not identify abnormalities of bone quality and microarchitecture that characterize renal osteodystrophy, and consequently the utility of DXA for fracture prediction in CKD stages 5 and 5D is controversial. However, uncoupling of balanced bone turnover is a major component of renal osteodystrophy, and either increased bone resorption or reduced formation may reduce mineral deposition in bone and predispose to medial calcification, supporting the inverse relationship of VC to BMD reported in a number of studies.[9,10,12] In this study, we did not detect inverse relationships of VC to age-adjusted Z-scores, or to values of PTH or bone turnover markers. In fact the only significant association was the weakly positive association of VC to lumbar spine Z-scores that was likely to be caused by artefact from aortic VC and degenerative vertebral changes. Despite this lack of an inverse association, reduced BMD due to abnormal bone turnover or age-related osteoporosis may certainly exacerbate VC in older individuals with CKD.

Patients in this study receiving SPK transplants were younger, with shorter dialysis vintage than kidney only transplant patients, but their VC prevalence and VC associations were similar. Due to inflammation and oxidative stress, diabetes may predispose to medial VC. Somewhat surprisingly, diabetes has not been consistently reported as a predisposing factor to VC,[5,6] possibly because patients on haemodialysis have high levels of advanced glycation end products regardless of diabetes status.

This study has a number of limitations. Because VC scores were assigned by different investigators there is a possibility of inconsistency. However, inter-observer reproducibility of scores has been reported to be excellent[16] and in a subgroup of patients in this study, inter-observer scoring correlated closely. In addition, laboratory values were measured at only one time point. Nevertheless, another study that analysed time averaged phosphate values, reported that these were not significantly associated with VC.[20] Because these patients were relatively young, BMD values were generally above the osteoporotic range. However, for the combined groups, Z-scores at the hip were > 1 SD below age adjusted means, and for SPK patients > 1 SD below age adjusted means at the hip and forearm sites, so it remains possible that mineral flux from bone could have impacted VC prevalence. Finally,

dialysis vintage may be a surrogate for a variety of exogenous influences (such as calcium exposure and accumulation) and endogenous factors that predispose to VC.

The prevalence of VC amongst younger patients awaiting kidney and SPK transplantation is high, despite lower prevalent CVD than the general dialysis population, and for patients awaiting kidney only transplants, a lower prevalence of diabetes and hypertension. Nevertheless, epidemiological factors predisposing these patients to VC were similar to those reported in studies of older patients on dialysis, with the exception of an inverse relationship of VC to BMD. Of course, this does not exclude altered bone turnover as a potential contributor to VC in younger patients, but does imply that the calcifying process is driven by CKD-related factors that do not impact on age adjusted bone mineral density.

ACKNOWLEDGEMENTS

The authors thank Tanya Sykes and Abhijit Patekar for their assistance with data collection and Karen Byth for providing statistical advice.

Disclosures

GJE has acted on the advisory boards of Amgen, Sanofi and Shire Australia. KC and GM have no disclosures.

REFERENCES

1. Roberts MA, Polkinghorne KR, McDonald SP, Ierino FL. Secular trends in cardiovascular mortality rates of patients receiving dialysis compared with the general population. *Am. J. Kidney Dis.* 2011; **58**: 64–72.
2. Adragao T, Pires A, Lucas C, Birne R. A simple vascular calcification score predicts cardiovascular risk in haemodialysis patients. *Nephrol. Dial. Transplant.* 2004; **19**: 1480–88.
3. Honkanen E, Kaupilla L, Wikstrom B *et al*. Abdominal aortic calcification in dialysis patients: results of the CORD study. *Nephrol. Dial. Transplant.* 2008; **23**: 4009–15.
4. Okuno S, Ishimura E, Kitatani K *et al*. Presence of abdominal aortic calcification is significantly associated with all-cause and cardiovascular mortality in maintenance hemodialysis patients. *Am. J. Kidney Dis.* 2007; **49**: 417–25.
5. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO Clinical Practice Guideline for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int.* 2009; **113** (Suppl): S1–130.
6. Caplin B, Wheeler DC. Arterial calcification in dialysis patients and transplant recipients. *Semin. Dial.* 2007; **20**: 144–9.
7. Banks LM, Lees B, MacSweeney JE, Stevenson JC. Effect of degenerative spinal and aortic calcification on bone density measurements in postmenopausal women: Links between osteoporosis and cardiovascular disease? *Eur. J. Clin. Invest.* 1994; **24**: 813–17.
8. Marcovitz PA, Tran HH, Franklin BA *et al*. Usefulness of bone mineral density to predict significant coronary artery disease. *Am. J. Cardiol.* 2005; **96**: 1059–63.
9. 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.* 1996; **27**: 394–401.
10. Toussaint ND, Lau KK, Strauss BJ, Polkinghorne KR, Kerr PG. Associations between vascular calcification, arterial stiffness and bone mineral density in chronic kidney disease. *Nephrol. Dial. Transplant.* 2008; **23**: 586–93.
11. London GM, Marchais SJ, Guérin AP, Boutouyrie P, Métivier F, de Vernejoul MC. Association of bone activity, calcium load, aortic stiffness, and calcifications in ESRD. *J. Am. Soc. Nephrol.* 2008; **19**: 1827–35.
12. London GM, Marty C, Marchais SJ, Guerin AP, Metivier F, de Vernejoul MC. Arterial calcifications and bone histomorphometry in end-stage renal disease. *J. Am. Soc. Nephrol.* 2004; **15**: 1943–51.
13. Adragao T, Herberth J, Monier-Faugere M-C *et al*. Low bone volume – a risk factor for coronary calcifications in hemodialysis patients. *Clin. J. Am. Soc. Nephrol.* 2009; **4**: 450–55.
14. Yu W, Gluer C, Fuerst T *et al*. Influence of degenerative joint disease on spinal bone mineral measurements in postmenopausal women. *Calcif. Tissue Int.* 1995; **57**: 169–74.
15. Södergård R, Bäckström T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *J. Steroid Biochem.* 1982; **16**: 801–10.
16. Kauppila L, Polak J, Cupples LA, Hannan MT, Kiel DP, Wilson PWF. New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: A 25 year follow-up study. *Atherosclerosis* 1997; **132**: 245–50.
17. Grace B, Dent H, Hurst K, McDonald S. New patients 2-10. ANZDATA 34th Registry Report 2010. Australia and New Zealand Dialysis and Transplant Registry, Adelaide, South Australia.
18. Hernández D, Rufino M, Bartolomei S *et al*. Clinical impact of preexisting vascular calcifications on mortality after renal transplantation. *Kidney Int.* 2005; **67**: 2015–20.
19. Rosas SE, Mensah K, Weinstein RB, Bellamy SL, Rader DJ. Coronary artery calcification in renal transplant recipients. *Am. J. Transplant.* 2005; **5**: 1942– 7.
20. Toussaint ND, Pedagogos E, Lau KK *et al*. Lateral lumbar x-ray assessment of abdominal aortic calcification in Australian haemodialysis patients. *Nephrology* 2001; **16**: 389–95.
21. Moe SM, O'Neill KD, Fineberg N *et al*. Assessment of vascular calcification in ESRD patients using spiral CT. *Nephrol. Dial. Transplant.* 2003; **18**: 1152–8.
22. Floege J, Kim J, Ireland E *et al*. Serum iPTH, calcium and phosphate, and the risk of mortality in a European haemodialysis population. *Nephrol. Dial. Transplant.* 2011; **26**: 1948–55.
23. Jean G, Bresson E, Jean-Claude T *et al*. Peripheral vascular calcification in long-haemodialysis patients: Associated factors and survival consequences. *Nephrol. Dial. Transplant.* 2009; **24**: 948–55.
24. Chertow GM, Burke SK, Raggi P. Sevelamer attenuates the progression of coronary and aortic calcification in hemodialysis patients. *Kidney Int.* 2002; **62**: 245–52.
25. St Peter WL, Liu J, Weinhandl E, Fan Q. A comparison of sevelamer and calcium-based phosphate binders on mortality, hospitalization, and morbidity in hemodialysis: A secondary analysis of the Dialysis Clinical Outcomes Revisited (DCOR) randomized trial using claims data. *Am. J. Kidney Dis.* 2008; **51**: 445–54.
26. Block GA, Wheeler DC, Persky MS *et al*. Effects of phosphate binders in moderate CKD. *J. Am. Soc. Nephrol.* 2012; **23**: 1407–15.

27. Di Iorio B, Molony D, Bell C *et al*. Sevelamer versus calcium carbonate in incident hemodialysis patients: Results of an open-label 24-month randomized clinical trial. *Am. J. Kidney Dis.* 2013; **62**: 771–8.
28. Jamal SA, Vandermeer B, Raggi P *et al*. Effect of calcium-based versus non-calcium-based phosphate binder on mortality in patients with chronic kidney disease: An updated systematic review and meta-analysis. *Lancet* 2013; **382**: 1268–77.
29. Bellasi A, Ferramosca E, Muntner P *et al*. Correlation of simple imaging tests and coronary artery calcium measured by computed tomography in hemodialysis patients. *Kidney Int.* 2006; **70**: 1623–8.