Clinical Kidney Journal, 2021, vol. 14, no. 10, 2401–2408

doi: 10.1093/ckj/sfab081
Original Article

ORIGINAL ARTICLE

Sclerostin and DKK1 circulating levels associate with low bone turnover in patients with chronic kidney disease Stages 3 and 4

Ricardo Neto [1,2], Luciano Pereira[1,2], Juliana Magalhães[1], Janete Quelhas-Santos[3], Sandra Martins[4,5], Catarina Carvalho[1] and João Miguel Frazão[1,2,3]

[1]Institute for Innovation and Health Research (I3S), Institute of Biomedical Engineering (INEB), Nephrology and Infectious Diseases Research Group, University of Porto, Porto, Portugal, [2]Department of Nephrology, Centro Hospitalar Universitário São João, Porto, Portugal, [3]Faculty of Medicine, University of Porto, Porto, Portugal, [4]Department of Clinical Pathology, Centro Hospitalar Universitário São João, Porto, Portugal and [5]EPIUnit, Institute of Public Health, University of Porto, Porto, Portugal

Correspondence to: Ricardo Neto; E-mail: ricardoneto76@gmail.com

ABSTRACT

Background. Disordered mineral and bone metabolism is a common complication of chronic kidney disease (CKD). Bone biopsy remains the gold standard tool for evaluating renal osteodystrophy (ROD), but it is an invasive procedure. Despite a growing interest in the ability of newer bone biomarkers to discriminate between different forms of ROD, data on pre-dialysis patients are scarce.

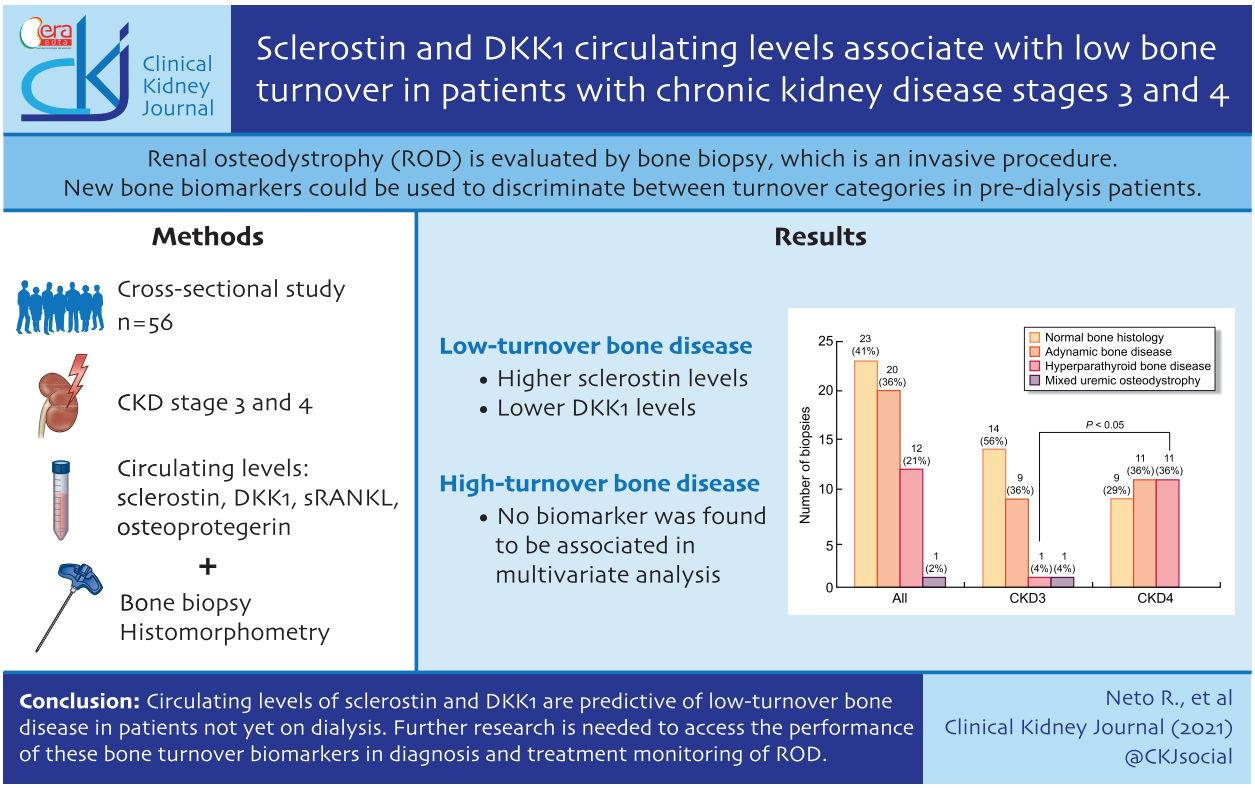
Methods. A cross-sectional study was conducted in a cohort of 56 patients with CKD Stages 3 and 4. Participants underwent a transiliac bone biopsy after a course of double tetracycline labelling. Circulating levels of Wnt signalling inhibitors sclerostin and Dickkopf-1 (DKK1), soluble receptor activator of nuclear factor-κB ligand (sRANKL) and osteoprotegerin were measured and correlated with histomorphometric analysis results.

Results. Most patients had abnormal bone histology and low-turnover bone disease was the predominant form of ROD. Characteristics associated with high bone turnover were worse renal function, lower serum calcium and higher intact parathyroid hormone and fibroblast growth factor-23 levels. Patients with low bone turnover, on the other hand, presented with higher sclerostin along with lower DKK1 and sRANKL levels. In the multivariable logistic regression analysis, sclerostin and DKK1 levels were independently associated with low-turnover bone disease.

Conclusions. Our results suggest that circulating levels of Wnt signalling inhibitors sclerostin and DKK1 are predictive of low-turnover bone disease in patients not yet on dialysis. Further research is needed to assess the performance of these bone turnover biomarkers, compared with histomorphometric analysis, in the diagnosis and treatment monitoring of ROD.

Keywords: bone biopsy, bone histomorphometry, bone turnover, Dickkopf-1, pre-dialysis patients, sclerostin

Received: 9.12.2020; Editorial decision: 17.3.2021



INTRODUCTION

Disordered mineral and bone metabolism is a common complication of chronic kidney disease (CKD) and associates with increased mortality and fracture risk [1]. Renal osteodystrophy (ROD), a component of this complex disorder, is defined as abnormal bone histology in the context of CKD [2]. Bone lesions have classically been described as hyperparathyroid bone disease, adynamic bone disease, osteomalacia or mixed uraemic osteodystrophy [3–5]. In order to standardize the definition and diagnosis of ROD, Kidney Disease: Improving Global Outcomes (KDIGO) has proposed a new classification, based on changes in bone turnover, mineralization and volume (TMV system) [6, 7].

Bone biopsy remains the gold standard for the diagnosis and classification of ROD. However, it is an invasive procedure and histomorphometric analysis requires considerable expertise [8]. Imaging techniques, such as dual-energy X-ray absorptiometry and quantitative computed tomography, can be useful to determine bone mineral density and help predict fracture risk in CKD patients, but they do not distinguish among types of ROD [9]. Therefore, there is great interest in the clinical usefulness of circulating biomarkers to evaluate bone turnover in CKD patients.

Intact parathyroid hormone (iPTH) has long been the most frequently used biomarker for the diagnosis and treatment monitoring of ROD [10]. However, despite iPTH level being associated with bone turnover, it is not sufficiently reliable to predict the underlying bone histology on an individual basis [11]. Several emerging biomarkers have been studied lately, mostly in dialysis patients [12]. Among these are sclerostin and Dickkopf-1 (DKK1), antagonists of the osteoblastic Wnt signalling pathway, and receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin, key mediators of osteoclastogenesis [13, 14] (Figure 1).

The objectives of this study were (i) to characterize bone histology in a cohort of patients not yet on dialysis and (ii) to evaluate whether newer circulating biomarkers were able to discriminate between categories of bone turnover in this population.

MATERIALS AND METHODS

Patients and study design

A cross-sectional study was carried out in a cohort of 56 patients with CKD Stages 3 and 4, consecutively recruited from February 2014 to September 2019. Eligibility criteria included capability to give informed consent, ≥18 years of age and glomerular filtration rate (GFR) between 15 and 60 mL/min/1.73 m^2. We excluded patients with impaired ambulation, previous parathyroidectomy, autoimmune or other chronic inflammatory diseases, and those who had been on drugs that affect bone metabolism, specifically calcium or aluminium salts, native or active vitamin D, steroids or bisphosphonates. Evidence of CKD–mineral and bone disorder, either clinical, biochemical or radiological, was not an inclusion or exclusion criterion. The study protocol was approved by the Ethics Committee of Centro Hospitalar Universitário São João. All patients signed an informed consent form.

Laboratory tests

Blood samples were collected in a fasting state at the time of bone biopsy. Serum calcium, phosphorus, albumin, creatinine and alkaline phosphatase were measured using an Olympus AU5400 analyser (Olympus America, Centre Valley, PA, USA). Serum iPTH and 25-hydroxyvitamin D [25(OH)D] levels were

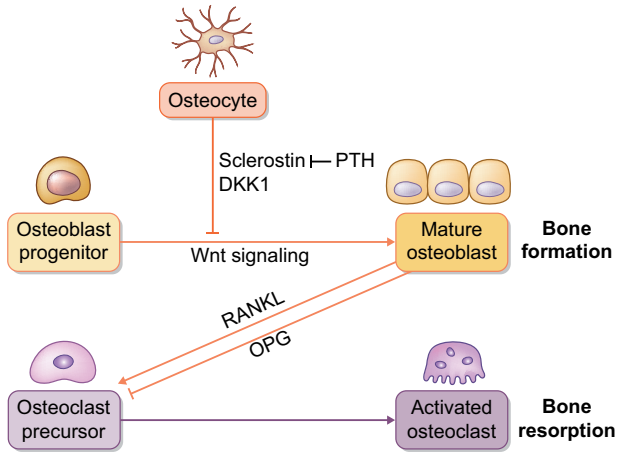


FIGURE 1: Regulatory pathways of bone metabolism. Wnt inhibitors, of which sclerostin is the most studied, are major modulators of bone remodeling. Sclerostin is mainly produced by the osteocyte and blocks osteoblast differentiation, hence inhibiting bone formation. The osteoprotegerin/RANKL axis is another major regulatory system. RANKL is expressed by osteoblasts and induces osteoclast activation, thus promoting bone resorption. Osteoprotegerin, on the other hand, inhibits osteoclastogenesis by binding to RANKL and neutralizing its action. PTH, intact parathyroid hormone; RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin; DKK1, Dickkopf-1

assessed through an electrochemiluminescence immunoassay with a Cobas E411 analyser (Roche Diagnostics, Mannheim, Germany). Serum bicarbonate was measured with a Siemens RapidLab 1265 gas analyser (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Bioactive sclerostin and free soluble RANKL (sRANKL) were measured in plasma samples by an enzyme immunoassay (Biomedica Medizinprodukte GmbH, Wien, Austria), according to the manufacturer's instructions (detection range of 1.9–320 pmol/L and 0.2–40 pg/mL, respectively). Osteoprotegerin, DKK1 and intact fibroblast growth factor-23 (FGF23) were measured in plasma samples by a multiplex assay (Magnetic Luminex Assay, R&D Systems Inc., Minneapolis, MN, USA), according to the manufacturer's protocol (detection range of 75–18 220 pg/mL, 202–49 060 pg/mL and 11.8–2870 pg/mL, respectively). GFR was estimated using the CKD Epidemiology Collaboration equation [15]. CKD stages were defined according to KDIGO classification guideline [16].

Bone biopsy and histomorphometry

All patients were submitted to a transiliac bone biopsy using a modified Bordier trephine after a course of double-labelling tetracycline (doxycycline 200 mg twice daily for 3 days, repeated after an interval of 12 days, biopsy performed 3 days after the second labelling). Biopsy specimens were 5 mm in diameter by 10 mm in length. Bone was dehydrated in alcohol, cleared with xylene and embedded in methyl methacrylate. Undecalcified 5 μm sections were cut and stained with modified Masson–Goldner trichrome for static histomorphometric evaluation. Unstained 10 μm sections were prepared for fluorescent dynamic analysis. Bone histomorphometry was performed with OsteoMeasure software (OsteoMetrics, Decatur, GA, USA). Static and dynamic parameters were examined according to the standards established by the American Society of Bone and Mineral Research [17]. Reference ranges were obtained from published literature [18–21]. Low bone turnover was defined as bone formation rate/bone surface (BFR/BS) $<10.95\ \mu m^3/\mu m^2$/year and high bone turnover as BFR/BS $>37.5\ \mu m^3/\mu m^2$/year. Mineralization was considered normal if mineralization lag time (Mlt) was <50 days. Bone volume (BV) was defined as low when <16% of tissue volume. Bone histology was categorized according to TMV classification [6]. Biopsies were evaluated by two different observers, blinded to clinical or biochemical features.

Statistical analysis

Continuous data were expressed as mean and standard deviation (SD) or median and interquartile range (IQR), according to variable distribution. Categorical variables were expressed as frequencies and percentages. Mann–Whitney, Kruskal–Wallis and chi-square tests were used to compare differences between groups. Spearman's coefficient was used to assess linear correlations. Multinomial logistic regression was performed in order to identify independent predictors of bone turnover. Receiver operating characteristic (ROC) curves were developed to evaluate cut-off values of biomarkers shown to be predictive of bone turnover. An area under the curve (AUC) was considered good if >0.7 and excellent if >0.8. Youden statistic was used to determine the best cut-off points. Sensitivity, specificity, positive and negative predictive values were subsequently calculated. Data analysis was carried out with SPSS version 26 (IBM SPSS Statistics, Chicago, IL, USA). $P < 0.05$ were considered statistically significant.

RESULTS

Demographic and laboratory characteristics

Clinical and biochemical findings are summarized in Table 1. Most patients were male ($n = 44$, 78.6%) and diabetic ($n = 35$, 62.5%). Mean age was 65.7 ± 9.8 years old, ranging from 32 to 81 years. All subjects were Caucasian. The most frequent cause of CKD was diabetes ($n = 17$, 30.4%), followed by hypertension ($n = 16$, 28.6%) and chronic tubulointerstitial nephritis ($n = 8$, 14.3%).

Mean serum creatinine and estimated GFR were 2.32 ± 0.43 mg/dL and 27.8 ± 6.8 mL/min/1.73 m^2, respectively. According to KDIGO classification, 25 patients (44.6%) had CKD Stage 3b and 31 (55.4%) had CKD Stage 4. Mean serum calcium and phosphorus were 9.3 ± 0.5 and 3.5 ± 0.6 mg/dL, respectively. Noticeably, only four patients (7.7%) had normal vitamin D levels, defined as 25(OH)D ≥30 ng/mL. Vitamin D deficiency (<15 ng/mL) and insufficiency (15–29 ng/mL) were observed in 42.2 and 50.1% of the patients, respectively. Median (IQR) iPTH and FGF23 levels were 93.3 pg/mL (50.6–151.4 pg/mL) and 25.5 pg/mL (16.3–38.4 pg/mL), respectively. Patients with CKD Stage 4 had significantly higher iPTH and FGF23 levels than those with CKD Stage 3. In contrast, measurements of sclerostin, DKK1, sRANKL and osteoprotegerin levels did not differ between CKD stages. Brain natriuretic peptide (BNP) levels were also significantly higher in patients with more advanced CKD. Most patients were on renin–angiotensin–aldosterone system (RAAS) inhibitors, with no difference between CKD stages. There were no patients on other kidney protective medications, such as mineralocorticoid receptor antagonists or sodium–glucose cotransporter 2 inhibitors.

Diabetic patients had significantly higher phosphate, albumin, C-reactive protein, BNP and sclerostin levels than those without diabetes (61.1 versus 39.3 pmol/L, P = 0.035). No other clinical or biochemical difference was noted between the two groups.

Table 1. Clinical and laboratory characteristics of the study population according to CKD stage

	All ($n = 56$)	CKD 3 ($n = 25$, 44.6%)	CKD 4 ($n = 31$, 55.4%)	P-value
Clinical				
Age, years	65.7 (9.8)	63.6 (11.2)	67.6 (8.5)	0.364
Male, %	78.6	88.0	71.0	0.191
Diabetes, %	62.5	56.0	67.7	0.415
Body mass index, kg/m^2	28.4 (4.3)	29.3 (4.5)	28.3 (4.4)	0.499
SBP, mmHg	136 (11)	134 (10)	137 (12)	0.276
DBP, mmHg	78 (8)	79 (8)	81 (8)	0.334
RAAS blockade, %	80.4	80.0	80.6	0.952
Biochemistry				
Creatinine, mg/dL	2.32 (0.43)	1.98 (0.22)	2.57 (0.36)	<0.001
GFR, mL/min/1.73 m^2	27.8 (6.8)	34.3 (3.3)	23.0 (4.0)	<0.001
Haemoglobin, g/dL	13.0 (1.7)	13.1 (2.1)	13.0 (1.4)	0.856
Ferritin, ng/mL	181 (113–340)	235 (137–426)	151 (93–250)	0.076
Albumin, g/dL	4.16 (0.35)	4.12 (0.38)	4.19 (0.33)	0.215
Bicarbonate, mmol/L	25.2 (3.3)	25.1 (3.5)	25.3 (3.2)	0.721
C-reactive protein, mg/L	2.0 (0.8–6.1)	1.3 (0.7–6.4)	3.2 (1.5–5.0)	0.285
LDL cholesterol, mg/dL	94 (30)	98 (27)	91 (32)	0.562
BNP, pg/mL	43 (21–106)	29 (17–54)	54 (43–144)	0.010
Calcium, mg/dL	9.3 (0.5)	9.4 (0.6)	9.3 (0.4)	0.179
Phosphorus, mg/dL	3.5 (0.6)	3.4 (0.7)	3.6 (0.5)	0.173
Magnesium, mEq/L	1.65 (0.24)	1.63 (0.26)	1.67 (0.23)	0.799
25(OH)D, ng/mL	16 (9–22)	16 (11–22)	15 (9–23)	0.611
ALP, U/L	81 (63–102)	68 (55–99)	81 (64–110)	0.432
iPTH, pg/mL	93.3 (50.6–151.4)	67.4 (40.3–101.6)	127.9 (79.4–211.6)	0.001
FGF23, pg/mL	25.5 (16.3–38.4)	18.7 (11.3–27.8)	34.2 (23.3–48.6)	0.002
Sclerostin, pmol/L	57.6 (38.6–72.8)	60.0 (39.1–86.5)	47.7 (37.8–69.2)	0.245
DKK1, pg/mL	800 (276)	851 (356)	753 (170)	0.676
sRANKL, pg/mL	2.66 (1.69–3.58)	2.34 (1.64–3.07)	2.85 (1.95–5.15)	0.214
Osteoprotegerin, pg/mL	1385 (1120–1717)	1374 (1053–1634)	1429 (1132–1783)	0.416
Urinary albumin, mg/day[a]	210 (27–1484)	210 (27–1188)	335 (34–2054)	0.478

Range for the assays used for iPTH, FGF23, sclerostin, DKK1, sRANKL and OPG: 1.20–5000 pg/mL, 0–2200 pg/mL, 2.2–132 pmol/L, 5–20 000 pg/mL, 4.88–20 000 pg/mL and 7–30 000 pg/mL, respectively. Data are reported as mean (SD) for normally distributed variables, median (IQR) for non-normally distributed variables, or percentage for categorical variables. P-values were calculated using Mann–Whitney test for continuous variables and chi-square test for categorical variables.

[a]24-h urine collections were adjusted for adequacy using urinary creatinine excretion.

SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; ALP, alkaline phosphatase.

Bone histomorphometric results

Distribution of bone patterns found by histomorphometric evaluation is depicted in Figure 2. Low bone turnover with normal mineralization (adynamic bone disease) was the most commonly found histological abnormality ($n = 20$, 35.7%). Twelve patients (21.4%) presented with high bone turnover with normal mineralization (hyperparathyroid bone disease) and there was one case (1.8%) of high bone turnover with abnormal mineralization (mixed uraemic osteodystrophy). Normal bone histology was observed in the remaining patients ($n = 23$, 41.1%). No cases of low bone turnover with abnormal mineralization (osteomalacia) were found.

The relative prevalence of bone histological forms differed between CKD stages, as patients with Stage 4 were significantly more likely to present with hyperparathyroid bone disease than those with Stage 3 (35.5% versus 4.0%, P = 0.007). Bone pattern distribution was not statistically different between diabetic and non-diabetic subjects or between male and female gender.

Histomorphometric measurements in relation to bone histology are presented in Table 2. Static formation and resorption parameters, as well as BV, were strongly and positively correlated with BFR. Patients with hyperparathyroid bone disease had a lower Mlt than those in the other groups, but the difference was not statistically significant.

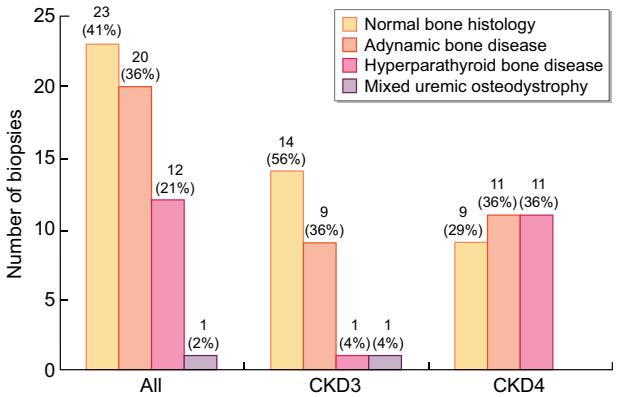


FIGURE 2: Distribution of bone histological patterns in relation to CKD stage

Relationship between circulating biomarkers and bone histomorphometry

In the univariate analysis, patients with high bone turnover had significantly lower serum calcium and higher iPTH and FGF23 levels than those in the other groups (Table 3). On the other side, low bone turnover group presented with significantly

Table 2. Histomorphometric parameters according to bone histological pattern

	All ($n = 56$) ($n = 23$, 41.1%)	Normal bone histology ($n = 20$, 35.7%)	Low-turnover bone disease ($n = 12$, 21.4%)	High-turnover bone disease	P-value
BFR, $\mu m^3/\mu m^2$/year	20.26 (15.4)	18.31 (5.12)*,**	5.71 (3.77)*	41.32 (4.34)	<0.001
Ob.S, %	0.41 (0.11–0.84)	0.53 (0.27–0.80)*,**	0.04 (0–0.17)*	2.06 (0.97–2.39)	<0.001
Oc.S, %	0.23 (0–0.49)	0.26 (0.07–0.45)*,**	0 (0–0.09)*	0.72 (0.33–0.97)	<0.001
OS, %	16.77 (13.42)	16.89 (11.01)*,**	6.74 (5.22)*	29.68 (12.61)	<0.001
ES, %	2.74 (1.47–4.15)	2.79 (2.16–4.02)*,**	1.10 (0.62–2.21)*	8.19 (3.73–11.04)	<0.001
Mlt, day	22.56 (12.47)	24.49 (12.58)	25.17 (15.12)	17.03 (6.45)	0.274
ES, %	2.54 (1.14–3.27)	2.94 (2.19–3.35)**	0.99 (0.52–2.32)*	3.69 (2.64–6.18)	<0.001
O.Th, μm	6.45 (1.73)	7.41 (1.31)**	5.48 (2.06)*	6.64 (0.95)	0.001
BV, %	19.09 (6.81)	21.95 (6.22)	13.65 (5.28)*	23.02 (4.45)	0.005

Data are reported as mean (SD) for normally distributed variables or median (IQR) for non-normally distributed variables. P-values were calculated using Kruskal–Wallis test.

*$P < 0.05$ versus high-turnover bone disease.

**$P < 0.05$ versus low-turnover bone disease.

Ob.S/BS, osteoblast surface/bone surface; Oc.S/BS, osteoclast surface/bone surface; OS/BS, osteoid surface/bone surface; ES/BS, eroded surface/bone surface; OV/BV, osteoid volume/BV; O.Th, osteoid thickness; BV/TV, BV/total volume.

Table 3. Clinical and laboratory data according to bone histological pattern

	Normal bone histology ($n = 23$, 41.1%)	Low-turnover bone disease ($n = 20$, 35.7%)	High-turnover bone disease ($n = 12$, 21.4%)	P-value
Clinical				
Age, years	67.3 (10.3)	66.4 (9.9)	63.3 (9.6)	0.215
Male, %	69.6	90.0	75.0	0.278
Diabetes, %	56.5	75.0	50.0	0.328
Body mass index, kg/m^2	28.9 (2.5)	29.4 (4.7)	26.7 (5.1)	0.163
SBP, mmHg	134 (9)	137 (12)	136 (14)	0.523
DBP, mmHg	80 (8)	80 (10)	79 (8)	0.893
Biochemistry				
Creatinine, mg/dL	2.10 (0.34)*	2.30 (0.33)	2.71 (0.46)	0.001
GFR, mL/min/1.73 m^2	29.9 (7.7)*	28.5 (5.5)	22.9 (5.3)	0.005
Haemoglobin, g/dL	12.5 (2.1)	13.6 (1.4)	13.2 (1.3)	0.054
Ferritin, ng/mL	169 (93–336)	238 (147–424)	163 (91–249)	0.215
Albumin, g/dL	4.08 (0.27)	4.22 (0.34)	4.28 (0.29)	0.099
Bicarbonate, mmol/L	25.6 (3.3)	25.3 (3.4)	24.8 (3.4)	0.691
C-reactive protein, mg/L	1.6 (0.9–6.5)	3.4 (0.9–7.5)	1.7 (0.8–3.4)	0.406
LDL cholesterol, mg/dL	106 (28)	97 (26)	73 (32)	0.056
BNP, pg/mL	44 (21–126)	36 (16–105)	48 (26–93)	0.691
Calcium, mg/dL	9.4 (0.5)*	9.4 (0.3)*	9.1 (0.4)	0.021
Phosphorus, mg/dL	3.6 (0.7)	3.4 (0.5)	3.7 (0.4)	0.202
Magnesium, mEq/L	1.7 (0.2)	1.6 (0.2)	1.6 (0.3)	0.749
25(OH)D, ng/mL	15 (11–22)	16 (13–23)	15 (9–22)	0.887
ALP, U/L	79 (64–91)	74 (52–98)	99 (66–144)	0.138
iPTH, pg/mL	79.4 (45.2–116.9)*	83.9 (51.3–125.9)*	207.4 (126.3–274.2)	0.012
FGF23, pg/mL	22.7 (9.4–34.2)*	23.3 (16.3–31.6)*	43.5 (28.2–75.4)	0.010
Sclerostin, pmol/L	51.7 (34.1–67.9)	65.2 (52.7–91.7)*	40.7 (30.7–52.8)	0.039
DKK1, pg/mL	867 (290)**	657 (180)	820 (196)	0.016
sRANKL, pg/mL	2.83 (2.26–4.26)	1.98 (1.68–2.64)*	3.14 (2.44–7.09)	0.014
Osteoprotegerin, pg/mL	1434 (1292–1618)	1270 (1033–1535)	1520 (1027–1813)	0.332

Data are reported as mean (SD) for normally distributed variables, median (IQR) for non-normally distributed variables, or percentage for categorical variables. P-values were calculated using Kruskal–Wallis test for continuous variables and Chi-square test for categorical variables.

*$P < 0.05$ versus high-turnover bone disease.

**$P < 0.05$ versus low-turnover bone disease.

SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; ALP, alkaline phosphatase.

higher sclerostin, as well as lower DKK1 and sRANKL levels, in comparison with other histological classes.

Multinomial logistic regression, adjusted for variables found to be significant in the univariate analysis, showed that high sclerostin and low DKK1 levels were predictive of low bone turnover [$P = 0.026$, odds ratio (OR) $= 1.053$, 95% confidence interval (CI) 1.006–1.103; $P = 0.046$ and OR $= 0.993$, 95% CI 0.987–0.999, respectively] (Table 4). In contrast, no biomarker

was found to be independently associated with high bone turnover.

An ROC curve analysis was conducted to evaluate the diagnostic accuracy of circulating biomarkers to predict bone histological class (Table 5). Sclerostin and DKK1 showed a good performance in predicting low-turnover bone disease, with an AUC of 0.70 and 0.76, respectively. A sclerostin cut-off of 55.5 pmol/L revealed a sensitivity and specificity of 76.5 and 64.5%, respectively, while a DKK1 cut-off of 615.2 pg/mL had a sensitivity and specificity of 68.8 and 86.2%, respectively, in the diagnosis of low bone turnover. Discrimination ability was significantly improved by the combination of both biomarkers, with an AUC of 0.86, providing a sensitivity and specificity of 75.0 and 85.7%, respectively. In contrast, neither biomarker on its own nor their combination could adequately predict high from non-high bone turnover.

There was no correlation between Mlt and 25(OH)D levels. BV did not associate with any clinical or biochemical parameter, in either the univariate or the multivariate analysis.

DISCUSSION

In this study, we sought to characterize bone histological abnormalities in a cohort of pre-dialysis patients and to determine whether newer biomarkers could discriminate between different groups of bone turnover.

Bone biopsy is considered the gold standard tool in the differential diagnosis of ROD, but it is uncommonly performed in clinical practice, given its invasive nature and overall complexity [8]. Moreover, there is a paucity of bone biopsy studies in CKD Stages 3 and 4, since most research on ROD has been conducted in dialysis patients [9].

In our cohort, most patients had normal bone morphology and low-turnover bone disease was the most common form of ROD, followed by high-turnover bone disease. The relative prevalence of histological types shifted with CKD progression, as Stage 4 patients were significantly more likely to present with high-turnover bone disease than those with Stage 3. Historically, hyperparathyroid bone disease and mixed uraemic osteodystrophy were considered the predominant forms of ROD in CKD patients. More recently, however, an increasing prevalence of adynamic bone disease has been reported [22–24]. Our results are consistent with these studies, suggesting that low bone turnover prevails in the earlier stages of CKD, with high bone turnover becoming increasingly important as renal function declines [25]. The changing of bone pattern with progression of CKD is likely to be multifactorial [9]. Recent evidence suggests the contribution of uraemic toxins, such as indoxyl sulphate, and antagonism of the osteocyte Wnt signalling pathway in the early development of adynamic bone disease [25].

Soluble Wnt inhibitors sclerostin and DKK1 are increasingly recognized as major modulators of bone metabolism [26].

Table 4. Multivariable logistical regression analysis for prediction of bone histological class

	β	OR	95% CI	P-value
Low versus normal bone turnover				
GFR, mL/min/1.73 m^2	−0.157	0.855	0.672–1.087	0.201
Calcium, mg/dL	1.834	6.260	0.132–297.7	0.352
iPTH, pg/mL	− 0.005	0.995	0.971–1.019	0.673
FGF23, pg/mL	−0.020	0.980	0.892–1.077	0.670
Sclerostin, pmol/L	0.052	1.053	1.006–1.103	0.026
DKK1, pg/mL	−0.007	0.993	0.987–0.999	0.046
sRANKL, pg/mL	−0.278	0.757	0.335–1.711	0.504
High versus normal bone turnover				
GFR, mL/min/1.73 m^2	−0.169	0.844	0.677–1.052	0.132
Calcium, mg/dL	−4.744	0.009	0.000–2.901	0.109
iPTH, pg/mL	−0.009	0.991	0.967–1.017	0.502
FGF23, pg/mL	0.037	1.037	0.928–1.159	0.520
Sclerostin, pmol/L	−0.085	0.919	0.820–1.028	0.140
DKK1, pg/mL	0.002	1.002	0.996–1.008	0.466
sRANKL, pg/mL	0.349	1.417	0.791–2.537	0.241

Table 5. ROC curve analysis using circulating biomarkers for discrimination between bone phenotypes

	AUC (95% CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	P-value
Low versus non-low bone turnover						
Sclerostin >55.5 pmol/L	0.70 (0.54–0.86)	76.5	64.5	54.5	83.2	0.023
DKK1 <615.2 pg/mL	0.76 (0.60–0.92)	68.8	86.2	73.5	83.3	0.004
Combined sclerostin + DKK1	0.86 (0.74–0.98)	75.0	85.7	74.4	86.1	<0.001
High versus non-high bone turnover						
Sclerostin <51.1 pmol/L	0.70 (0.54–0.86)	80.0	63.2	37.2	92.1	0.054
DKK1 >755.3 pg/mL	0.62 (0.45–0.79)	72.7	58.8	32.5	88.8	0.229
Combined sclerostin + DKK1	0.69 (0.52–0.87)	90.0	47.1	31.7	94.5	0.065

Sclerostin is mainly produced by the osteocyte and its major role is the blockade of osteoblast differentiation, thus inhibiting bone formation [13].

In our study, patients with high bone turnover presented with worse renal function, lower serum calcium and higher iPTH and FGF23 levels, whereas patients with low bone turnover showed higher sclerostin, lower DKK1 and sRANKL levels, in comparison with the other groups. After adjusting for significant variables in the univariate model, we found that sclerostin and DKK1 levels were able to discriminate low from non-low bone turnover condition with fair accuracy. To our knowledge, this is the first study reporting an independent association between sclerostin and DKK1 with bone histomorphometric parameters in a non-dialysis population. The regression model did not identify independent associations with high-turnover bone disease, which could be partly explained by the limited number of patients presenting with this type of ROD. Of note, the difference in serum calcium between patients with high bone turnover and those in the other groups was lost in the adjusted analysis.

In a study on 60 haemodialysis patients submitted to bone biopsy, Cejka *et al.* [27] reported a negative correlation between serum sclerostin levels and histomorphometric parameters of bone turnover. Sclerostin was a better predictor of high bone turnover than iPTH, but no biomarker was helpful to predict low bone turnover. Noticeably, DKK1 showed no association with parameters of bone turnover. Another biopsy study, performed by de Oliveira *et al.* [28] in a cohort of 41 patients on peritoneal dialysis, also reported an inverse association between serum sclerostin and BFR, pointing to a role of increased sclerostin circulating levels in the pathophysiology of adynamic bone disease. Recently, Graciolli *et al.* [29] presented bone data from 148 adult patients across the spectrum of CKD, 66% of them undergoing haemodialysis. The authors quantified both serum and bone sclerostin, which was shown to correlate weakly. Serum sclerostin was found to be a good predictor of low bone turnover and low BV. Moreover, a lower bone expression of sclerostin was seen in patients with high turnover in comparison with those with low bone turnover.

Despite the differences between assays, sclerostin circulating levels are generally increased in CKD patients [30, 31]. However, whether this results from increased osteocyte production, decreased renal elimination, or both, is not yet clear [32, 33]. Interestingly, despite the fact that a correlation between serum sclerostin levels and renal function has been reported [34], in our cohort, plasma sclerostin levels did not differ between CKD stages.

Contrary to sclerostin, DKK1 levels are lower in CKD patients as compared with controls [30]. In our population, DKK1 levels did not differ between CKD stages. On the other hand, we found that the concentration of both biomarkers diverged significantly between bone histological categories, as sclerostin levels were highest and DKK1 lowest in patients with low bone turnover.

The osteoprotegerin/RANKL system is another major regulator of bone metabolism [14]. Osteoprotegerin, a glycoprotein expressed by osteoblasts, inhibits osteoclastogenesis by binding to RANKL, in opposition to sclerostin and DKK1, which produce their inhibitory effects on osteoblastogenesis [35]. In our study, osteoprotegerin levels did not differ between bone turnover categories. On the other hand, patients with high compared with low bone turnover had significantly higher soluble RANKL levels, but this association was lost in the regression model.

Limitations of our study include its cross-sectional design, which does not allow cause–effect relationships to be established. In addition, the cohort size was relatively modest, potentially reducing the ability to detect statistically significant associations. It was not possible to perform a regression analysis separately for each gender, which would have been interesting. On the other hand, patients had no previous exposure to drugs known to interfere with bone metabolism, such as calcium salts or vitamin D analogues, thus removing an important confounding factor.

In conclusion, this study suggests that circulating sclerostin and DKK1 are predictive of low bone turnover in pre-dialysis patients. Our results add to accumulating evidence that disrupted osteocyte function and Wnt pathway inhibition are major events in the initial development of ROD. Prospective studies are required to further assess the usefulness of emerging bone biomarkers in CKD patients.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Toussaint ND. The burden of fractures, vascular pathology and mortality in chronic kidney disease-mineral and bone disorders. *Nephrology (Carlton)* 2017; 22 (Suppl 2): 9–10
2. Moe S, Drüeke T, Cunningham J *et al.* Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2006; 69: 1945–1953
3. Malluche HH, Faugere MC. *Atlas of Mineralized Bone Histology*. Basel, Switzerland: Karger AG, 1986
4. Hruska KA, Teitelbaum SL. Renal osteodystrophy. *N Engl J Med* 1995; 333: 166–174
5. Lehmann G, Ott U, Kaemmerer D *et al.* Bone histomorphometry and biochemical markers of bone turnover in patients with chronic kidney disease stages 3-5. *Clin Nephrol* 2008; 70: 296–305
6. 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 Suppl* 2009; 7 (Suppl 113): S1–S130
7. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Update Work Group. KDIGO 2017 clinical practice guideline update for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). *Kidney Int Suppl* 2017; 7: 1–59
8. Covic A, Voroneanu L, Apetrii M. PTH and/or bone histology: are we still waiting for a verdict from the CKD-MBD grand jury? *Am J Kidney Dis* 2016; 67: 535–538
9. Drüeke TB, Massy ZA. Changing bone patterns with progression of chronic kidney disease. *Kidney Int* 2016; 89: 289–302
10. Barreto FC, Barreto DV, Moysés RM *et al.* K/DOQI-recommended intact PTH levels do not prevent low-turnover bone disease in hemodialysis patients. *Kidney Int* 2008; 73: 771–777
11. Sprague SM, Bellorin-Font E, Jorgetti V *et al.* Diagnostic accuracy of bone turnover markers and bone histology in patients with CKD treated by dialysis. *Am J Kidney Dis* 2016; 67: 559–566
12. Evenepoel P, Cavalier E, D'Haese PC. Biomarkers predicting bone turnover in the setting of CKD. *Curr Osteoporos Rep* 2017; 15: 178–186

13. Brandenburg VM, Verhulst A, Babler A *et al*. Sclerostin in chronic kidney disease-mineral bone disorder think first before you block it!. *Nephrol Dial Transplant* 2019; 34: 408–414
14. Znorko B, Oksztulska-Kolanek E, Michałowska M *et al*. Does the OPG/RANKL system contribute to the bone-vascular axis in chronic kidney disease? A systematic review. *Adv Med Sci* 2017; 62: 52–64
15. Levey AS, Stevens LA, Schmid CH *et al*.; for the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150: 604–612
16. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl* 2013; 3: 1–150
17. Dempster DW, Compston JE, Drezner MK *et al*. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res* 2013; 28: 2–17
18. Glorieux FH, Travers R, Taylor A *et al*. Normative data for iliac bone histomorphometry in growing children. *Bone* 2000; 26: 103–109
19. Coen G, Mazzaferro S, Ballanti P *et al*. Renal bone disease in 76 patients with varying degrees of predialysis chronic renal failure: a cross-sectional study. *Nephrol Dial Transplant* 1996; 11: 813–819
20. Dos Reis LM, Batalha JR, Muñoz DR *et al*. Brazilian normal static bone histomorphometry: effects of age, sex, and race. *J Bone Miner Metab* 2007; 25: 400–406
21. National Kidney Foundation: K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis* 2003; 42: S1–S202
22. Gal-Moscovici A, Sprague SM. Role of bone biopsy in stages 3 to 4 chronic kidney disease. *Clin J Am Soc Nephrol* 2008; 3 (Suppl 3): S170–S174
23. Spasovski GB, Bervoets AR, Behets GJ *et al*. Spectrum of renal bone disease in end-stage renal failure patients not yet on dialysis. *Nephrol Dial Transplant* 2003; 18: 1159–1166
24. Barreto FC, Barreto DV, Canziani ME *et al*. Association between indoxyl sulfate and bone histomorphometry in predialysis chronic kidney disease patients. *J Bras Nefrol* 2014; 36: 289–296
25. Massy Z, Drueke T. Adynamic bone disease is a predominant bone pattern in early stages of chronic kidney disease. *J Nephrol* 2017; 30: 629–634
26. Ke HZ, Richards WG, Li X *et al*. Sclerostin and dickkopf-1 as therapeutic targets in bone diseases. *Endocr Rev* 2012; 33: 747–783
27. Cejka D, Herberth J, Branscum AJ *et al*. Sclerostin and Dickkopf-1 in renal osteodystrophy. *Clin J Am Soc Nephrol* 2011; 6: 877–882
28. de Oliveira RA, Barreto FC, Mendes M *et al*. Peritoneal dialysis per se is a risk factor for sclerostin-associated adynamic bone disease. *Kidney Int* 2015; 87: 1039–1045
29. Graciolli FG, Neves KR, Barreto F *et al*. The complexity of chronic kidney disease-mineral and bone disorder across stages of chronic kidney disease. *Kidney Int* 2017; 91: 1436–1446
30. Behets GJ, Viaene L, Meijers B *et al*. Circulating levels of sclerostin but not DKK1 associate with laboratory parameters of CKD-MBD. *PLoS One* 2017; 12: e0176411
31. Delanaye P, Paquot F, Bouquegneau A *et al*. Sclerostin and chronic kidney disease: the assay impacts what we (thought to) know. *Nephrol Dial Transplant* 2018; 33: 1404–1410
32. Vervloet MG, Massy ZA, Brandenburg VM *et al*. Bone: a new endocrine organ at the heart of chronic kidney disease and mineral and bone disorders. *Lancet Diabetes Endocrinol* 2014; 2: 427–436
33. Figurek A, Rroji M, Spasovski G. Sclerostin: a new biomarker of CKD-MBD. *Int Urol Nephrol* 2020; 52: 107–113
34. Pelletier S, Dubourg L, Carlier M-C *et al*. The relation between renal function and serum sclerostin in adult patients with CKD. *Clin J Am Soc Nephrol* 2013; 8: 819–823
35. Morena M, Jaussent I, Dupuy AM *et al*. Osteoprotegerin and sclerostin in chronic kidney disease prior to dialysis: potential partners in vascular calcifications. *Nephrol Dial Transplant* 2015; 30: 1345–1356