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**Title**: **Diagnostic accuracy of biomarkers and imaging for bone turnover in renal osteodystrophy.**

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**Significance Statement**

Abnormal bone turnover of renal osteodystrophy in advanced chronic kidney disease can only be diagnosed using bone biopsy (gold standard). However, this is an invasive and painful procedure, thus rarely performed. We found that three bone biomarkers (bALP, intact PINP and TRAP5b) and high resolution bone imaging of distal radius can discriminate patients with low bone turnover from non-low bone turnover as assessed by bone histomorphometry. Hence, the biomarkers and bone imaging may have the potential to replace bone biopsy, particularly in discriminating patients with low bone turnover. They may also be useful in selecting patients for future clinical trials which aim to reduce their fracture risk.

**Abstract**

Renal osteodystrophy is common in advanced chronic kidney disease (CKD) but characterization of bone turnover status can only be done with bone biopsy (gold standard test). We aimed to simultaneously test if bone biomarkers and high resolution peripheral computed tomography (HR-pQCT) can predict bone turnover status on histomorphometry. Fasting blood samples were taken from 69 CKD stages 4-5 including dialysis patients and 68 controls for biomarker analysis (intact parathyroid hormone [iPTH], procollagen type 1 N-terminal propeptide [PINP], bone alkaline phosphatase [bALP], collagen type 1 cross-linked C-telopeptide [CTX] and tartrate-resistant acid phosphatase 5b [TRAP5b]). HR-pQCT of distal radius and tibia were performed. 43 CKD patients had trans-iliac bone biopsy using Jamshidi 4mm trephine for histomorphometry evaluation. All biomarkers were significantly higher in CKD compared to controls. BALP, intact PINP and TRAP5b had area under the receiver operating characteristic curve [AUC] of 0.824, 0.794 and 0.799 respectively to discriminate low bone turnover, significantly better than iPTH AUC of 0.606. Furthermore, radius HR-pQCT total volumetric bone mineral density and cortical bone volume had an AUC 0.811 and 0.802 respectively. iPTH had AUC of 0.760 to discriminate high bone turnover, similar to other biomarkers in this study. In conclusion, bALP, intact PINP, TRAP5b and radius HR-pQCT can discriminate low from non-low bone turnover. Despite poor diagnostic accuracy for low bone turnover, iPTH can discriminate high bone turnover with similar accuracy to other biomarkers.

**Introduction**

Renal osteodystrophy is characterized by abnormal bone turnover, mineralization and volume which can only be assessed on bone histomorphometry (gold standard test).[1] However, bone biopsy is invasive and rarely performed due to patients' reluctance or limited expertise in the procedure and bone histomorphometry. Hence, current clinical practice still relies on intact parathyroid hormone (iPTH) although it has been shown to have low sensitivity and specificity to assess bone turnover.[2-4] The poor diagnostic accuracy of iPTH limits its use to guide therapies that target bone mineral density (BMD) or bone turnover.

Very high levels of both bone alkaline phosphatase (bALP) and iPTH are strongly predictive of high bone turnover in chronic kidney disease (CKD).[4-10] However, bALP has better predictive ability than iPTH for low bone turnover.[4-6, 9] There are other bone turnover biomarkers which are directly released during the process of bone resorption (e.g. collagen type 1 cross-linked C-telopeptide [CTX] and tartrate-resistant acid phosphatase 5b [TRAP5b]) and bone formation (e.g. procollagen type 1 N-terminal propeptide [PINP]), thus are potentially more accurate in assessing bone turnover in CKD.

The changes in BMD and microarchitecture associated with high or low bone turnover cannot be adequately assessed by dual energy X-ray absorptiometry (DXA) which is a 2-dimensional imaging technique.[11] However, high-resolution peripheral quantitative computed tomography (HR-pQCT) can detect microarchitectural changes in both the cortical and trabecular bone compartments. Previous studies using HR-pQCT showed that CKD patients had thinner cortical bone and lower trabecular bone volume compared to healthy controls.[12-14]

The emergence of bone biomarkers and HR-pQCT offer the possibility of replacing bone biopsy, but their diagnostic accuracy for predicting bone turnover in advanced CKD is unknown. We aimed to simultaneously test if Biomarkers and HR-pQCT can identify advanced CKD patients with low and high bone turnover as shown on histomorphometry. We also tested whether these non-invasive tests have better diagnostic accuracy than iPTH.

## Methods

*Study design and population*

This was a cross-sectional study in CKD stages 4-5 (including dialysis) patients, aged 30-80 years old. The exclusion criteria included fracture/orthopaedic surgery in the preceding six months; started/changed the dose of phosphate binders, vitamin D or calcimimetics within four weeks of study entry; and received anti-resorptive, anabolic agent or systemic glucocorticoid in the preceding six months. All patients attending our Nephrology centre who fulfilled the inclusion and exclusion criteria were invited to take part in the study. We also recruited age- and gender-matched controls with estimated glomerular filtration rate (eGFR) ≥60 ml/min/1.73m$^2$. The exclusion criteria were similar to CKD group and we also excluded participants with known osteoporosis. The study adhered to the *Declaration of Helsinki* and was approved by the South Yorkshire Research Ethics Committee. All participants gave written informed consent. All samples and imaging studies were obtained purely for research and summarised below. Complete methods is provided in the supplementary material.

*Bone biomarkers*

Fasting morning blood samples were taken, stored at -80$^0$C and analysed at the end of the study. For haemodialysis patients, blood samples were taken on the day after their haemodialysis session. We measured serum iPTH, bALP, intact PINP, CTX, TRAP5b and 25-hydroxyvitamin D using the IDS-iSYS auto-analyser (Immunodiagnostic Systems, United Kingdom). Total PINP was measured using the Cobas e411 automated immunoassay (Roche Diagnostics, Germany). We measured fasting serum calcium, phosphate, total alkaline phosphatase (total ALP), and creatinine on Roche Cobas c701/702 analyser (Roche Diagnostics, England) on the same day as sample collection. eGFR was calculated using Modification of Diet in Renal Disease equation.

*Bone imaging*

HR-pQCT of the distal radius and tibia were performed using XtremeCT (Scanco Medical AG, Switzerland) using a standard protocol. The images were analysed with standard software (Scanco Medical AG, version 6.0) for volumetric BMD (mg/cm$^3$) and microarchitectural parameters. The extended cortical measurement was also performed.

DXA of the lumbar spine (L1-4), hip and forearm were performed using Hologic Discovery A densitometer (Hologic Inc, USA). Mean areal BMD (g/cm$^2$) was calculated using Hologic APEX software (version 3.4.2).

*Trans-iliac bone biopsy and histomorphometry*

Bone biopsy was only performed in CKD patients using tetracycline bone labelling. A trans-iliac bone biopsy was performed under local anaesthetic using an 8-gauge Jamshidi 4mm trephine and needle. The samples were analysed using the Bioquant Osteo histomorphometry system (Bioquant Image Analysis Corporation) which uses standardised nomenclature.[15] The samples fulfilled the histomorphometry minimum acceptable total section area in the standard analysis region of 30mm$^2$.[16] All histomorphometry analysis was performed by a single operator (OG) and normal bone turnover was defined as bone formation rate/ bone surface (BFR/BS) of 18-38 um$^3$/um$^2$/year.[17]

*Statistical analysis*

Descriptive statistics were presented as mean (standard deviation [SD]) and median (interquartile range [IQR]). We used Student t-test, Mann-Whitney U test and chi-squared test to compare the characteristics of CKD and controls, and Spearman rank analysis to identify the associations between variables.

We selected 43 controls who were age- and gender-matched to the 43 CKD patients with bone histomorphometry to obtain HR-pQCT Z-scores. HR-pQCT Z-scores were used in order to control for the age and gender effects on bone microarchitecture and BMD. The Z-scores were calculated using

the formula: (HR-pQCT CKD measurement – mean of control group)/(SD of control group). DXA BMD Z-scores were obtained from the Hologic software.

For receiver operating characteristic (ROC) analysis, CKD patients were grouped into Low/Non-Low and High/Non-High bone turnover categories based on bone turnover on histomorphometry (BFR/BS). The proportions of low and high bone turnover in this study were used as prevalence of the disease. We classified area under the ROC curve (AUC) of 0.6-0.7 as poor, 0.7-0.8 as fair, 0.8-0.9 as good and 0.9-1.0 as excellent diagnostic accuracy. Combining variables for ROC analysis was performed using regression analysis.

We used $p<0.05$ to indicate statistical significance. All statistical analysis were performed using IBM SPSS Statistics 22 and MedCalc v16.8.4.

**Results**

***Participants and the bone turnover on histomorphometry.***

The demographics of 69 advanced CKD stages 4-5 (including dialysis) patients and 68 age- and gender-matched control participants are shown in Table 1. There were 44 pre-dialysis CKD patients with median (IQR) eGFR of 13 (11-16) ml/min/1.73m$^2$ and 25 dialysis patients (haemodialysis and peritoneal dialysis). Median (IQR) eGFR for controls was 81 (72 to >90) ml/min/1.73m$^2$.

49 bone biopsies were performed but only 43 samples were adequate for histomorphometry analysis. Amongst these 43 patients, 28 (65%) were pre-dialysis CKD, mean (SD) age was 59 (12) years, 77% were male, 26% had diabetes, and 26% had previous fragility fracture. Their current medications are shown in Table 2 but none were taking calcimimetics. Based on bone formation rate/bone surface (BFR/BS) on histomorphometry, 26% of patients had low, 34% had normal and 40% had high bone turnover (Table 2).

***Biomarkers and imaging in CKD and controls.***

CKD patients had significantly higher biomarker levels compared to controls (Table 1). On HR-pQCT, CKD patients had lower volumetric BMD, and lower trabecular thickness and trabecular bone volume at the distal radius and distal tibia compared to controls (Table 1). Additionally, CKD patients had a thinner cortical bone at the tibia. Figure 1 shows examples of 3-dimensional reconstruction of HR-pQCT images at both sites in this study.

Areal BMD Z-score by DXA at the forearm and total hip were also lower in CKD compared to controls (Table 1). 59% of the CKD group had osteopaenia (T score -1.0 to -2.5), and 25% had osteoporosis (T score<-2.5) as assessed by BMD at the three sites. In the control group, 51% had osteopaenia, and 12% had osteoporosis.

***Relationship between bone turnover on histomorphometry and biomarkers and imaging in CKD***

In 43 CKD patients with bone histomorphometry data, all biomarkers were significantly correlated with each other (Supplementary Table 1). iPTH was positively associated with bone turnover [BFR/BS] (rho= 0.42, p<0.01) but the other biomarkers showed higher correlations with bone turnover (Figure 2). There were significant differences for all the biomarkers between low, normal and high bone turnover categories (Table 2).

Bone turnover on histomorphometry was negatively associated with radius Z-scores for BMD and microarchitecture (Figure 3) but no significant associations were found with tibia HR-pQCT Z-scores. Differences were only significant for radius HR-pQCT total BMD and cortical bone volume (p<0.05) between the three bone turnover categories (Table 2). On DXA, only the forearm BMD Z-score was significantly associated with bone turnover (rho= -0.307, p<0.05). No significant differences were found for DXA BMD Z-scores between the bone turnover categories (Table 2).

***Diagnostic accuracy of biomarkers and imaging for low bone turnover***

In ROC analysis for discriminating low from non-low bone turnover, AUC for bALP was 0.824, intact PINP was 0.794 and TRAP5b were 0.799 (Table 3). These AUCs were significantly better ($p<0.05$) than AUC for iPTH which was 0.606 (Figure 4a). Combining biomarkers did not improve the AUC.

Radius HR-pQCT Z-scores for total BMD and cortical bone volume had AUC of 0.811 and 0.802 respectively for discriminating low bone turnover (Figure 4b, Table 3). However, these AUCs were not significantly better than iPTH AUC. Tibia HR-pQCT Z-scores only had AUCs ≤0.70. All three sites DXA BMD Z-scores also had non-significant AUCs (Supplementary Table 2). Combining bALP and radius total BMD Z-score did not improve the AUC (Table 3).

***Diagnostic accuracy of biomarkers and imaging for high bone turnover***

In ROC analysis for discriminating high from non-high bone turnover, iPTH had an AUC of 0.76 which was similar to AUCs for the other biomarkers (Table 4). Combining biomarkers did not improve the AUC. All bone imaging parameters also had non-significant AUCs (Supplementary Table 2 & 3).

**Discussion**

This is the first study to simultaneously compare biomarkers and HR-pQCT to bone histomorphometry to determine their diagnostic accuracy in discriminating bone turnover status in advanced CKD patients. BALP and radius HR-pQCT can discriminate low bone turnover, with their AUCs being >0.80. We also found that iPTH, all the biomarkers and bone imaging had similarly suboptimal diagnostic accuracy for discriminating high bone turnover.

BALP, intact PINP and TRAP5b can discriminate patients with low bone turnover better than iPTH, most likely because they do not accumulate in advanced CKD.[18, 19] Secondary hyperparathyroidism is

a common complication in advanced CKD and has a major role in CKD mineral bone disorder (CKD-MBD).[1] iPTH is a poor diagnostic test to discriminate low bone turnover in advanced CKD patients, partly due to the assay used. A second generation iPTH assay measures the whole (1-84) PTH molecule and the (7-84) PTH fragment. The fragment accumulates in CKD and has an antagonistic effect on bone turnover.[20] Despite those limitations, iPTH can still discriminate high bone turnover with similar accuracy as other biomarkers used in this study. iPTH has 90% positive predictive value (PPV) for high bone turnover which is consistent with previous studies.[6, 7, 10] The optimal cut off value for discriminating high bone turnover in this study is five times the upper limit of normal, whereas KDIGO CKD-MBD guideline recommends that iPTH level is maintained 2-9 times the upper limit of normal in dialysis patients.[1] The number of dialysis patients in our study was too small for further analysis to make a comparison.

In this study, bALP had AUC>0.80 and has better diagnostic accuracy for low bone turnover than iPTH which are consistent with previous studies. [5, 6, 8, 9] BALP and other biomarkers in this study had consistently low PPV but high negative predictive value for the optimal threshold (criterion) for low bone turnover. We also found that bALP only has 69% PPV for discriminating high bone turnover whereas previous studies found >90% PPV.[6, 7, 10]

Sprague et al. recently published diagnostic accuracy of biomarkers in predicting bone turnover in 492 dialysis patients from four countries.[4] They also showed that iPTH had similar diagnostic accuracy to bALP and PINP in predicting high bone turnover. They found that bALP and iPTH had the highest diagnostic accuracy for low bone turnover but we found that iPTH to be a poor diagnostic test for low bone turnover. They also found that combining iPTH and bALP improved the discrimination of both low and high bone turnover but this is not the case in our study. There are differences between the study and ours; firstly, we included pre-dialysis and dialysis patients which may account for the different proportion of patients with low bone turnover. Although our sample size was smaller, the proportion of patients with low bone turnover was similar to other studies.[5, 9]

In contrast, Sprague et al. reported that around 60% of patients had low bone turnover but most of their biopsies were performed for clinical indication whereas ours were collected purely for research. Our histomorphometry analysis was performed by a single operator to reduce intra-observer variability whereas Sprague et al. had bone histomorphometry analysed in several centres with different ranges of bone turnover defined as normal. The assays used for iPTH and bALP were also different from ours and Sprague et al used total PINP whereas we evaluated total and intact PINP separately.

PINP is cleaved off from type 1 collagen during bone formation process. Total PINP assay measures the trimeric propeptide and its monomeric fragments, whilst the intact PINP assay only measures the trimeric propeptide.[19] The trimeric propeptide is cleared from circulation by liver endothelial cells,[21] whereas the monomeric fragments are cleared by the kidneys, hence the fragments accumulate in advanced CKD.[19] We have shown that in advanced CKD, (1) only intact PINP can discriminate low bone turnover better than iPTH and (2) both have suboptimal diagnostic accuracy for high bone turnover. Although total PINP is often used in the field of osteoporosis, we do not recommend its use to assess bone turnover in advanced CKD.

Biomarker profile in dialysis patients may differ from that in non-dialysis CKD even with similar bone turnover status because biomarkers, such as CTX, may be dialysed.[22] Hence, our haemodialysis patients had blood sample taken the day after a dialysis session but we did not assess residual renal function. These issues may have introduced bias in our study. However, the number of pre-dialysis patients was small with <10 patients each in the low and high bone turnover categories which makes further analysis in this subgroup questionable.

CKD patients in our study had lower BMD on HR-pQCT compared to controls which is mostly due to cortical bone impairment. Additionally, CKD patients also had thinner cortical bone at the tibia. Previous studies also found that CKD had lower BMD on HR-pQCT compared to controls due to both trabecular and cortical bone impairment.[12-14] However, we did not match our control participants'

BMD to CKD patients and we excluded participants with known osteoporosis which may have introduced bias.

We found that radius BMD and microarchitecture were negatively associated with bone turnover in advanced CKD. Negri et al. showed similar trend on HR-pQCT in female dialysis patients but they used biomarkers as measures of bone turnover rather than bone histomorphometry.[14] We also found that normal and high bone turnover CKD patients had significantly lower radius BMD compared to those with low bone turnover, mostly due to lower cortical bone volume. Gerakis et al. described similar finding using DXA in haemodialysis patients who had a bone biopsy.[23] We did not find any difference in DXA BMD between bone turnover categories. Importantly, DXA is unable to discriminate bone turnover status in advanced CKD.

Distal radius HR-pQCT can discriminate low bone turnover from non-low bone turnover patients in this study. However, it is important to recognise that the effects of bone turnover on microarchitecture are dynamic whereas HR-pQCT is a static test. Thus the cross-sectional use of HR-pQCT is perhaps more appropriate in assessing bone volume (static measurement) rather than bone turnover (dynamic measurement).[24] Nevertheless, the use of bone imaging could be complementary to biomarkers in discriminating bone turnover status and deciding treatment decisions, for example, in osteoporotic CKD.

We included pre-dialysis and dialysis CKD patients and we assessed bone turnover using the gold standard bone biopsy but there were several limitations to our study. This was a single centre observational study with a small number of participants. However, the proportion of patients with low/high bone turnover was similar to previous studies, and we had a broad range of bone turnover which is important in assessing diagnostic test accuracy. We were unable to assess pre-dialysis and dialysis patients separately. Hence, our results must be interpreted in the context of CKD stages 4-5 and dialysis.

In conclusion, bALP, intact PINP, TRAP5b and radius HR-pQCT were able to discriminate low bone turnover in advanced CKD patients. Despite poor diagnostic accuracy for low bone turnover, iPTH can discriminate high bone turnover with similar accuracy to other biomarkers in this study. In clinical practice, iPTH and bALP remain the diagnostic tests of choice to discriminate high and low bone turnover. However, we believe that all four biomarkers and radius HR-pQCT can potentially be used for patient selection in clinical trials in advanced CKD as we continue to search for bone-specific treatment to reduce fracture risk in this population.

**Author contributions**

AK, RE and SS designed the study. SS was jointly supervised by AK and RE. SS oversaw the study visits, performed bone biopsy, interpreted the data and wrote the manuscript. OG performed bone histomorphometry, FG performed biomarker analysis, and MP performed bone imaging analysis. All authors reviewed, edited and approved the final version of the manuscript.

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All the authors worked independently from the funders. The funders were not involved in the study design; data collection, analysis or interpretation; writing of this manuscript or in the decision to

submit for publication. Abstracts relating to this manuscript were presented at the European Calcified Tissue Society Conference 2017 and at the UK Kidney Week Conference 2017.

**Statement of competing financial interests** – OG, FG, MP and AK have no financial conflict of interest to declare. SS has received research grant and support from Immunodiagnostics Systems and Biomedica. RE has received grants and consulting fees from Immunodiagnostics Systems and Roche Diagnostics.

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**Table 1:** Demographics, biomarkers and imaging parameters in CKD patients and control participants.

| Variables | CKD (N=69) | Control (N=68) | p values |
|---|---|---|---|
| Age, years | 62 (12) | 62 (12) | |
| Male, N | 53 | 53 | |
| BMI (kg/m$^2$) | 27 (4.1) | 28 (4.3) | 0.3 |
| Diabetes | 28% | 0% | **<0.001** |
| Previous fragility fracture | 22% | 7% | **<0.05** |
| | | | |
| **Biomarkers** | | | |
| iPTH (pg/mL) | 188 (121 – 280) | 32 (27 – 45) | **<0.001** |
| Intact PINP (ng/mL) | 67.5 (42.8 – 107.7) | 38.5 (31.8 – 55.3) | **<0.001** |
| Total PINP (ng/mL) | 125 (72.8 – 237.2) | 41.4 (33.7 – 56.7) | **<0.001** |
| bALP (µg/L) | 22.3 (16.6 – 33.3) | 17 (12.9 – 20.2) | **<0.001** |
| tALP (IU/L) | 88 (73 – 126) | 65.5 (55.3 – 78) | **<0.001** |
| CTX (ng/mL) | 1.49 (0.76 – 2.39) | 0.27 (0.19 – 0.5) | **<0.001** |
| TRAP5b (U/L) | 4.9 (3.2 – 6.9) | 3.8 (3.3 – 4.5) | **0.001** |
| Adjusted calcium (mmol/L) | 2.28 (0.15) | 2.28 (0.07) | 0.9 |
| Phosphate (mmol/L) | 1.53 (0.3) | 1.06 (0.15) | **<0.001** |
| 25-hydroxyvitamin D (ng/mL) | 22.9 (9.4) | 23.9 (7.0) | 0.5 |
| | | | |
| **HRpQCT distal radius** | | | |
| Total vBMD (mg/cm$^3$) | 266.2 (75.56) | 308.47 (74.2) | **0.003** |
| Cortical vBMD (mg/cm$^3$) | 782.58 (110.66) | 821.04 (88.7) | **0.04** |
| Trabecular vBMD (mg/cm$^3$) | 156.69 (46.17) | 184.6 (41.42) | **0.001** |
| Cortical thickness (mm) | 0.61 (0.27) | 0.71 (0.26) | 0.06 |
| Cortical porosity (%) | 3.0 (2.3 – 4.2) | 3.2 (2.0 – 3.8) | 0.4 |
| Cortical BV/TV (%) | 90.0 (85.4 – 92.1) | 90.7 (88.6 – 93.4) | 0.1 |
| Trabecular thickness (mm) | 0.064 (0.012) | 0.073 (0.013) | **<0.001** |
| Trabecular number (1/mm) | 2.01 (0.363) | 2.11 (0.296) | 0.14 |
| Trabecular separation (mm) | 0.434 (0.371 – 0.496) | 0.4 (0.349 – 0.443) | 0.06 |
| Trabecular BV/TV (%) | 13.1 (3.8) | 15.4 (3.5) | **0.001** |
| | | | |
| **HRpQCT distal tibia** | | | |
| Total vBMD (mg/cm$^3$) | 276.99 (63.67) | 314.97 (61.2) | **0.001** |
| Cortical vBMD (mg/cm$^3$) | 819.85 (88.67) | 858.7 (67.89) | **0.005** |
| Trabecular vBMD (mg/cm$^3$) | 172.12 (41.06) | 189.99 (41.12) | **0.01** |
| Cortical thickness (mm) | 1.05 (0.36) | 1.25 (0.35) | **0.001** |
| Cortical porosity (%) | 7.1 (5.7 – 10.4) | 6.8 (4.7 – 10.3) | 0.2 |
| Cortical BV/TV (%) | 86.2 (6.0) | 88.1 (4.9) | 0.05 |
| Trabecular thickness (mm) | 0.075 (0.014) | 0.081 (0.013) | **0.01** |
| Trabecular number (1/mm) | 1.92 (0.35) | 1.97 (0.4) | 0.4 |
| Trabecular separation (mm) | 0.444 (0.395 – 0.522) | 0.425 (0.359 – 0.523) | 0.2 |
| Trabecular BV/TV (%) | 14.3 (3.4) | 15.8 (3.4) | **0.01** |
| | | | |
| **DXA BMD Z-score** | | | |
| Forearm | -0.4 (1.5) | 0.2 (1.4) | **0.02** |
| Total hip | -0.2 (1.0) | 0.6 (1.1) | **<0.001** |

| | | | |
|---|---|---|---|
| Lumbar spine | 0.4 (1.7) | 0.5 (1.6) | 0.7 |

Data presented as mean (standard deviation) for normal distribution variables and median (inter-quartile range) for non-normal distribution variables. Group differences were tested using independent t-test for normal distribution variables, Mann-Whitney U test for non-normal distribution variables and chi squared test for categorical variables.

Abbreviations: eGFR; estimated glomerular filtration rate; BMI, body mass index; iPTH, intact parathyroid hormone; PINP, procollagen type 1 N-terminal propeptide; bALP, bone alkaline phosphatase; tALP, total alkaline phosphatase CTX, collagen type 1 cross-linked C-telopeptide; TRAP5b, tartrate-resistant acid phosphatase 5b; HR-pQCT, high resolution peripheral quantitative computed tomography; vBMD, volumetric bone mineral density; BV/TV, bone volume/tissue volume; DXA, dual energy X-ray absorptiometry; BMD, bone mineral density.

**Table 2:** Biomarkers and imaging parameters for low, normal and high bone turnover categories in advanced chronic kidney disease patients (N=43).

| Variables | Low (N=11) | Normal (N=15) | High (N=17) | p value |
|---|---|---|---|---|
| BFR/BS ($um^3/um^2$/day) | 13.8 (3.6 – 15.5) | 27.5 (22.5 – 32.2) | 67.4 (46.5 – 112.5) | **<0.001** |
| **Medications** | | | | |
| Vitamin D | 45% | 13% | 47% | |
| Calcium based phosphate binder | 9% | 20% | 41% | |
| Non-Calcium based phosphate binder | 0% | 7% | 6% | |
| **Biomarkers** | | | | |
| iPTH (pg/mL) | 172 (119 – 292) | 172 (86 – 194) | 347 (161 – 381) | **<0.05** |
| Intact PINP (ng/mL) | 44.1 (29.2 -68.4) | 81.1 (54.3 – 92.4) | 107.9 (63.5 – 182) | **<0.005** |
| Total PINP (ng/mL) | 76.3 (51.7 – 159.3) | 127.3 (68.4 – 221.7) | 214 (110.6 – 403) | **<0.05** |
| bALP (µg/L) | 17.7 (5.6) | 25.9 (8.7) | 34.4 (13.3) | **<0.005** |
| tALP (IU/L) | 82 (53 – 86) | 94 (82 – 127) | 115 (82 – 156) | **<0.05** |
| CTX (ng/mL) | 1.01 (0.68) | 1.46 (0.67) | 2.65 (1.68) | **<0.005** |
| TRAP5b (U/L) | 3.2 (2.9 – 4.3) | 5.2 (3.2 – 7.4 ) | 5.8 (4.8 – 8.5) | **<0.05** |
| Adjusted calcium (mmol/L) | 2.27 (2.22 – 2.33) | 2.32 (2.26 – 2.35) | 2.24 (2.14 – 2.40) | 0.6 |
| Phosphate (mmol/L) | 1.48 (1.30 – 1.77) | 1.61 (1.43 – 1.83) | 1.30 (1.25 – 1.55) | 0.1 |
| 25-hydroxyvitamin D (ng/mL) | 23 (9.9) | 23.7 (8.2) | 22.6 (10.3) | 0.95 |
| **HR-pQCT radius Z-score** | | | | |
| Total vBMD | -0.11 (0.63) | -1.06 (0.66) | -0.97 (1.08) | **<0.05** |
| Cortical vBMD | 0.49 (-0.47 to 0.9) | -0.63 (-1.4 to -0.14) | -0.84 (-1.67 to 0.15) | 0.09 |
| Trabecular vBMD | -0.31 (0.83) | -1.03 (0.57) | -1.11 (1.31) | 0.16 |
| Cortical thickness | 0.07 (0.8) | -0.76 (0.82) | -0.64 (0.98) | 0.1 |
| Cortical porosity | -0.51 (-0.95 to -0.02) | -0.12 (-0.94 to 1.58) | 0.10 (-0.47 to 0.90) | 0.15 |
| Cortical BV/TV | 0.72 (-0.08 to 0.97) | -0.2 (-1.48 to 0.27) | -0.23 (-1.46 to 0.27) | **<0.05** |
| Trabecular thickness | -0.43 (-0.82 to -0.18) | -1.14 (-1.57 to -0.79) | -1.21 (-1.77 to 0.11) | 0.07 |
| Trabecular number | -0.03 (1.01) | -0.11 (0.84) | -0.66 (1.58) | 0.39 |
| Trabecular separation | -0.13 (-0.5 to 0.71) | 0.30 (-0.39 to 0.55) | 0.59 (-0.50 to 1.18) | 0.5 |
| Trabecular BV/TV | -0.30 (0.82) | -1.01 (0.57) | -1.09 (1.31) | 0.17 |
| **HR-pQCT tibia Z-score** | | | | |
| Total vBMD | -0.34 (0.91) | -1.05 (0.90) | -0.92 (1.13) | 0.21 |
| Cortical vBMD | 0.26 (-0.93 to 0.44) | -0.60 (-2.0 to 0.31) | -0.21 (-2.0 to 0.13) | 0.13 |
| Trabecular vBMD | -0.48 (0.92) | -0.63 (0.88) | -0.70 (1.11) | 0.86 |
| Cortical thickness | -0.18 (0.92) | -1.1 (0.86) | -0.86 (1.16) | 0.1 |
| Cortical porosity | -0.31 (0.52) | 0.43 (1.26) | 0.31 (1.17) | 0.23 |
| Cortical BV/TV | 0.36 (-0.15 to 0.73) | -0.43 (-1.82 to 0.70) | 0.14 (-2.12 to 0.37) | 0.17 |
| Trabecular thickness | -0.70 (-0.87 to 0.23) | -0.93 (-1.62 to 0.21) | -0.78 (-1.66 to 0.14) | 0.44 |
| Trabecular number | -0.31 (1.23) | -0.03 (0.76) | -0.07 (0.92) | 0.74 |
| Trabecular separation | 0.56 (-0.75 to 0.84) | -0.09 (-0.30 to 0.55) | -0.04 (-0.44 to 0.52) | 0.66 |
| Trabecular BV/TV | -0.46 (0.91) | -0.60 (0.88) | -0.67 (1.10) | 0.86 |
| **DXA BMD Z-score** | | | | |
| Forearm | -0.24 (0.88) | -0.31 (0.99) | -0.94 (1.33) | 0.18 |

| | | | | |
|---|---|---|---|---|
| Total hip | -0.07 (0.76) | -0.35 (0.96) | -0.38 (1.05) | 0.68 |
| Lumbar spine | -0.10 (-0.5 to 0.6) | 0.2 (-0.8 to 1.0) | 0 (-0.8 to 1.3) | 0.86 |

Data is presented as mean (standard deviation) for normal distribution variables or median (interquartile range) for non-normal distribution variables. Group differences were tested using one-way ANOVA or Kruskal-Wallis test depending on the distribution of the variables.

Abbreviations: BFR/BS, bone formation rate/bone surface; eGFR; estimated glomerular filtration rate; BMI, body mass index; iPTH, intact parathyroid hormone; PINP, procollagen type 1 N-terminal propetide; bALP, bone alkaline phospahatse; tALP, total alkaline phosphatase; CTX, collagen type 1 cross-linked C-telopeptide; TRAP5b, tartrate-resistant acid phosphatase 5b; HR-pQCT, high resolution peripheral quantitative computed tomography; vBMD, volumetric bone mineral density; BV/TV, bone volume/tissue volume; DXA, dual energy X-ray absorptiometry; BMD, bone mineral density.

**Table 3:** Diagnostic accuracy of biomarkers and radius HR-pQCT for identifying patients with low bone turnover

| Variables | AUC (95% CI) | Criterion | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|
| **Biomarkers** | | | | | | |
| iPTH | 0.563 (0.401 to 0.715) | ≤183 pg/mL | 70 | 53 | 32 | 85 |
| Intact PINP | 0.794 (0.641 to 0.903) | ≤57 ng/mL | 80 | 75 | 50 | 92 |
| Total PINP | 0.719 (0.557 to 0.848) | ≤124 ng/mL | 80 | 68 | 44 | 91 |
| bALP | 0.824 (0.671 to 0.926) | ≤21 µg/L | 89 | 77 | 53 | 96 |
| tALP | 0.753 (0.598 to 0.871) | ≤88 IU/L | 91 | 63 | 46 | 95 |
| CTX | 0.766 (0.610 to 0.882) | ≤0.84 ng/mL | 60 | 84 | 55 | 87 |
| TRAP5b | 0.799 (0.643 to 0.909) | ≤4.6 U/L | 89 | 71 | 47 | 96 |
| **Radius HR-pQCT Z-score** | | | | | | |
| Total vBMD | 0.811 (0.646 – 0.922) | >-1.0 | 100 | 59 | 45 | 100 |
| Cortical BV/TV | 0.802 (0.636 – 0.916) | > -0.2 | 89 | 63 | 44 | 94 |
| **Combined variables** | | | | | | |
| bALP & radius total vBMD Z-score | 0.797 (0.621 – 0.916) | Not available | 100 | 58 | 39 | 100 |

Abbreviations: HR-pQCT, high resolution peripheral quantitative computed tomography; AUC, area under the receiver operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; iPTH, intact parathyroid hormone; PINP, procollagen type 1 N-terminal propeptide ; bALP, bone alkaline phosphatase; tALP, total alkaline phosphatase; CTX, collagen type 1 cross-linked C-telopeptide; TRAP5b, tartrate-resistant acid phosphatase 5b; vBMD, volumetric bone mineral density; BV/TV, bone volume/tissue volume.

**Table 4:** Diagnostic accuracy of biomarkers for identifying patients with high bone turnover.

| Biomarkers | AUC (95% CI) | Criterion | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|
| iPTH | 0.760 (0.603 to 0.878) | >327 pg/mL | 53 | 96 | 90 | 75 |
| Intact PINP | 0.765 (0.609 to 0.882) | >107 ng/mL | 53 | 92 | 82 | 74 |
| Total PINP | 0.725 (0.563 to 0.853) | >142ng/mL | 75 | 68 | 60 | 81 |
| bALP | 0.750 (0.588 to 0.873) | >31 µg/L | 56 | 83 | 69 | 74 |
| tALP | 0.670 (0.510 to 0.805) | >102 IU/L | 65 | 73 | 61 | 76 |
| CTX | 0.762 (0.606 to 0.880) | >2.39 ng/mL | 53 | 96 | 90 | 75 |
| TRAP5b | 0.710 (0.545 to 0.842) | >4.6 U/L | 81 | 58 | 57 | 82 |

Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; iPTH, intact parathyroid hormone; PINP, procollagen type 1 N-terminal propeptide ; bALP, bone alkaline phosphatase; tALP, total alkaline phosphatase; CTX, collagen type 1 cross-linked C-telopeptide; TRAP5b, tartrate-resistant acid phosphatase 5b.

**Figure legends**

**Figure 1:** Examples of HR-pQCT 3-dimensional images of distal radius and distal tibia from a control participant and a CKD patient in this study. This CKD patient demonstrated trabecular bone impairment whereas the control participant had normal bone microarchitecture.

**Figure 2:** All biomarkers showed positive correlations with bone turnover on histomorphometry (N=43).

Abbreviations: BFR/BS, bone formation rate/bone surface; PINP, procollagen type 1 N-terminal propeptide; ALP, alkaline phosphatase; CTX, collagen type 1 cross-linked C-telopeptide; TRAP5b, tartrate-resistant acid phosphatase 5b.

**Figure 3:** Distal radius HR-pQCT parameters showed negative correlations with bone turnover on histomorphometry (N=43).

Abbreviations: BFR/BS, bone formation rate/bone surface; HR-pQCT, high resolution peripheral quantitative computed tomography; vBMD, volumetric bone mineral density; BV/TV, bone volume/tissue volume.

**Figure 4:** Biomarkers and distal radius HR-pQCT parameters can discriminate CKD patients with low bone turnover. Receiver operating characteristic curves show that the biomarkers bALP, intact PINP and TRAP5b (panel A) performed significantly better than iPTH in discriminating patients with low bone turnover. Distal radius HR-pQCT parameters (panel B) were not significantly better than iPTH despite area under the curves being >0.80.

Abbreviations: iPTH, intact parathyroid hormone; bALP, bone alkaline phosphatase; PINP, procollagen type 1 N-terminal propeptide; TRAP5b, tartrate-resistant acid phosphatase 5b; HR-pQCT, high resolution peripheral quantitative computed tomography; vBMD, volumetric bone mineral density; BV/TV, bone volume/tissue volume.