Non-oxidized parathyroid hormone (PTH) measured by current method is not superior to total PTH in assessing bone turnover in chronic kidney disease

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Non-oxidized parathyroid hormone (PTH) measured by current method is not superior to total PTH in assessing bone turnover in chronic kidney disease.

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Running headline: Oxidation of PTH and Bone Turnover in ESKD

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Abstract

Parathyroid hormone (PTH) is a key regulator of bone turnover but can be oxidized in vivo, which impairs biological activity. Variable PTH oxidation may account for the rather poor correlation of PTH with indices of bone turnover in chronic kidney disease. Here, we tested whether non-oxidized PTH is superior to total PTH as a marker of bone turnover in 31 patients with kidney failure included from an ongoing prospective observational bone biopsy study and selected to cover the whole spectrum of bone turnover. Receiver Operating Characteristic (ROC) curves, Spearman correlation and regression analysis of non-oxidized PTH, total PTH and bone turnover markers (bone-specific alkaline phosphatase, procollagen N-terminal pro-peptide and tartrate-resistant acid phosphatase 5b) were used to assess the capability of non-oxidized PTH vs. total PTH to discriminate low from non-low and high from non-high bone turnover, as assessed quantitatively by bone histomorphometry. Serum levels of non-oxidized PTH and total PTH were strongly and significantly correlated. Histomorphometric parameters of bone turnover and the circulating bone turnover markers showed similar correlation coefficients with non-oxidized PTH and total PTH. The area under the ROC (AUROC) values for discriminating between low/non-low turnover for non-oxidized PTH and total PTH were significant and comparable (0.82 and 0.79, respectively). For high/non-high turnover the AUROCs were also significant and of the same magnitude (0.76 and 0.80, respectively). Thus, measuring non-oxidized PTH using the currently available method provides no added value compared to total PTH as an indicator of bone turnover in patients with kidney failure.

Keywords: Parathyroid Hormone, non-oxidized PTH, Bone Turnover, Bone Histomorphometry, Chronic Kidney Disease

Abbreviations: PTH = parathyroid hormone; n-oxPTH = non-oxidized PTH; oxPTH = oxidized PTH; tPTH = total PTH; CKD-MBD = chronic kidney disease – bone and mineral disorder; ESKD = end-stage kidney disease; PINP = total procollagen type 1 N-terminal propeptide; TRACP5b = tartrate-resistant acid phosphatase 5b; Bone ALP = bone-specific alkaline phosphatase; AUROC = area under the receiver operating characteristic; IQR = interquartile range; SD = standard deviation; CV = coefficient of variation; ox(Met8)PTH = PTH oxidized at methionine residue 8; ox(Met18)PTH = PTH oxidized at methionine residue 18; ox(Met8,18)PTH = PTH oxidized at methionine residues 8 and 18

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Introduction

Parathyroid hormone (PTH) is a key diagnostic and prognostic marker in patients with chronic kidney disease and mineral and bone disorders (CKD-MBD).[1] However, the correlation of PTH with underlying bone turnover, as well as mortality and morbidity, is poor, especially in the middle range of KDIGO targets.[1] This poor correlation may partially be explained by variable posttranslational oxidation of the hormone.[2,3]

PTH is a peptide hormone, consisting of 84 amino acids, amongst which two methionine residues on the 8th and 18th position. Methionine is prone to oxidation, resulting in methionine sulfoxide. The methionine residues on PTH can be oxidized *in vivo*.[4] This oxidation results in conformational changes, reducing the biological potency of PTH to activate the PTH-1 receptor.[5] As recently reviewed, *in vivo* and *in vitro* studies in a wide variety of animals have shown that oxidation of both methionine residues diminishes the biological function of PTH.[5] However, contemporary PTH assays have been developed with an emphasis on measuring full-length PTH instead of PTH fragments, but do not discriminate between non-oxidized PTH (n-oxPTH) and oxPTH.

A method to separate n-oxPTH from total PTH (tPTH) and assess n-oxPTH concentrations quantitatively was first described in 2012.[2] First, oxidized PTH is selectively extracted by specific antibodies in an affinity column. Subsequently, n-oxPTH is measured with routine (automated) PTH immunoassays in the remaining sample. With the introduction of the n-oxPTH measurement, intriguing questions have arisen about its added value. Although experimental data clearly reveal reduced biological activity of oxPTH, there is no consensus as to whether measuring n-oxPTH confers diagnostic superiority.

Two previous studies, one in CKD stage 5 and one in CKD stage 2-4 patients, have shown that tPTH was more strongly associated with mortality than n-oxPTH.[6,7] It was concluded that oxidative stress, of which total PTH was assumed to be a reflection, is probably a more powerful predictor of mortality than the bioactivity of PTH.[6,8] As the potential clinical added value of n-oxPTH therefore would not reside in predicting mortality and morbidity, it is hypothesized that n-oxPTH may be superior to tPTH in predicting bone turnover.

The purpose of this study was to explore whether n-oxPTH outperforms tPTH as a biomarker of bone turnover in end-stage kidney disease (ESKD).

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Methods

Patients and study protocol

Adult Caucasian patients (age > 18 years) with ESKD were recruited from an ongoing, prospective observational study on the evolution of bone histomorphometry before renal transplantation (clinical trial identifier: NCT01886950) at the University Hospital Leuven. For the present analysis, we selected 10 ESKD patients with low bone turnover, 10 ESKD patients with normal bone turnover and 11 ESKD patients with high bone turnover, as defined by histomorphometric criteria (see below). None of the patients had a history of therapy with anti-resorptive drugs, three patients had a history of parathyroidectomy (time elapsed between procedure and bone biopsy was respectively 7, 23 and 82 months). The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the University Hospital Leuven, Belgium. Informed consent was obtained from all patients.

Biochemical analysis

Blood samples were collected (random, non-fasted) at the time of the bone biopsy. After centrifugation, serum was stored at −80 °C until further analysis.

Total PTH was measured in EDTA plasma using an automated second-generation PTH immunoassay (Cobas, Roche Diagnostics, Rotkreuz, Switzerland) with an intra- and inter-assay CV of < 2.7 and < 6.5%, respectively. n-oxPTH concentrations were measured using an oxPTH affinity column (A1112; Immundiagnostik AG, Bensheim, Germany) as described previously.[4] In short, oxidized PTH is removed by affinity chromatography columns filled with beads coated with antibodies against all oxidized forms of PTH. The columns were filled with 300 µL plasma and incubated end-over-end at room temperature for 1h. Afterwards, we determined n-oxPTH in the eluate by using the same Cobas PTH immunoassay as described above. The inter-assay CV of the n-oxPTH measurement at concentrations of < 2 pmol/L and > 2 pmol/L is 10% and 2.4%, respectively.

25-hydroxy vitamin D (calcidiol) concentrations were measured using in house developed radioimmunoassays as described before.[9] Bone-specific alkaline phosphatase (Bone ALP), tartrate-resistant acid phosphatase 5b (TRACP5b) and intact N-terminal propeptide of type I procollagen (PINP) were measured using the IDS iSYS instrument (IDS, Boldon, UK). Inter- and intra-assay CVs were < 10% for all assays.

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Bone histomorphometry

Transiliac bone biopsies were obtained using a trephine needle with an internal diameter of 4.5 mm (Osteobell [Mirandola, Italy], Biopsybell [Mirandola, Italy]).[10] All but six patients received two 3-day oral tetracycline (2 x 500 mg/day) administration sessions, with in-between an 11-day tetracycline free interval prior to the bone biopsy procedure.

Bone biopsies were fixed in 70% ethanol and embedded in a methylmetacrylate resin. For light-microscopic examination of static bone parameters, the modified Goldner technique was used to stain the non-decalcified 5 µm-thick sections. For fluorescence microscopic examination of dynamic bone parameters, 10 µm thick sections were mounted unstained in 100% glycerol to visualize the tetracycline labels. The results are reported as 2D measurements using nomenclature established by the American Society for Bone and Mineral Research.[11] The following criteria were used to define the turnover category: high turnover: BFR > 613 µm²/mm²/day; low turnover: BFR < 97 µm²/mm²/day and/or absence of cellular activity (ObPm/TtPm and OcPm/TtPM < 1%). Patients not belonging to either group were classified as 'normal bone turnover'.

Statistical analysis

Data following a normal distribution are shown as mean with standard deviation (SD), data following a skewed distribution are shown as median with interquartile range (IQR). Skewed variables were normalized prior to parametric analyses.

Spearman's correlation coefficient and univariate linear regressions were used to evaluate the relationship between n-oxPTH, tPTH, bone histomorphometric parameters and biochemical parameters. The receiver operating characteristic (ROC) and area under the ROC (AUROC) were used to assess the diagnostic performance of n-oxPTH vs. tPTH to discriminate low from non-low and high from non-high bone turnover.

p-Values < 0.05 were considered to reflect statistical significance. Statistical analysis was performed using MedCalc Statistical Software version 18.5 (MedCalc Software bvba, Ostend, Belgium).

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Results

Baseline characteristics

Demographic and clinical characteristics of the included participants (n = 31) are depicted in Table 1. The baseline characteristics stratified per bone turnover group are depicted in Supplementary Table 1. The mean age was 52 ± 14 years and 65% of the participants were men. One patient had a history of Type 1 Diabetes Mellitus, one a history of Type 2 Diabetes Mellitus and three previously underwent parathyroidectomy. The median dialysis vintage was 18 months (IQR: 8-41). The median bone formation rate was 342 (IQR: 135-899) $\mu m^2/mm^2/day$. The median n-oxPTH and tPTH concentrations were 2.3 pmol/L (IQR: 1.1-5.4) and 18.3 pmol/L (IQR: 9.1-54.0), respectively. Although there were relatively more patients undergoing hemodialysis than peritoneal dialysis in the high bone turnover group, their serum levels of n-oxPTH and tPTH were of comparable magnitude.

Correlation analysis

The Spearman correlation between n-oxPTH and tPTH, was 0.92 ($p < 0.001$). As Figure 1 shows, the regression analysis equation was linear with the formula: n-oxPTH = 0.12*tPTH + 0.73. Hence, on average n-oxPTH values were 12% (95% CI: 11-14%) of tPTH.

Table 2 shows the correlations coefficients of n-oxPTH and tPTH with serum bone turnover markers, structural histomorphometric parameters and static and dynamic histomorphometric remodeling parameters. Serum bone formation markers, i.e. Bone ALP and PINP, all showed significant correlations with both n-oxPTH and tPTH, with correlation coefficients ranging between 0.61 and 0.71. Structural histomorphometric parameters did not exhibit a significant correlation with n-oxPTH. One of the most frequently used dynamic histomorphometric bone remodeling parameters, the bone formation rate, showed a correlation with n-oxPTH of rho = 0.54 ($p = 0.006$) and with tPTH of rho = 0.61 ($p < 0.001$). Overall, correlations were numerically stronger for tPTH than for n-oxPTH, except for the mineral apposition rate (n-oxPTH: rho = 0.66, $p < 0.001$; tPTH: rho = 0.58, $p < 0.001$).

The diagnostic ability of n-oxPTH vs. tPTH in discriminating between high and low bone turnover was assessed by ROC curves, as depicted in Figure 2 and Table 3. AUROCs for discriminating low vs. non-low turnover, were 0.82 ($p < 0.001$) for n-oxPTH and 0.79 ($p = 0.001$) for tPTH. These AUROCs showed no statistically significant difference ($p = 0.32$). The

high vs. non-high turnover AUROC for n-oxPTH was 0.76 (p = 0.004) and 0.80 (p < 0.001) for tPTH. These AUCs showed no statistically significant difference either (p = 0.32). The AUROC for BAP, PINP and TRAP5b are shown in Table 3. The parameter with the highest AUC for differentiating low from non-low turnover was PINP (AUC = 0.862). For high vs non-high the AUROC was highest for BAP (AUC = 0.909).

Discussion

The key finding of the present study is that n-oxPTH is not superior to total PTH as a biomarker of bone turnover, defined by its gold standard, in patients with ESKD.

Fracture risk is excessively high in patients with advanced CKD.[12] Knowledge of bone turnover is of utmost importance in defining the optimal treatment strategy in patients with advanced CKD and osteoporosis. The gold standard for quantifying bone turnover is the histomorphometric analysis of a tetracycline double-labeled bone biopsy. Taking a bone biopsy, however, is an invasive procedure, and histopathological expertise is laborious and not widely available. As such, quantifying bone turnover by bone histomorphometry is not feasible in many routine clinical practices, and simpler methods to assess bone turnover represent an unmet clinical need.[13]

PTH is a key regulator of bone metabolism and for decades monitoring PTH values has been the *lingua franca* of renal bone disease management. The wide availability of rapid and automated PTH immunoassays contributed to the success of PTH as a biomarker. Data from a cross-sectional retrospective diagnostic test study, including more than 450 patients with bone histomorphometry data, showed an AUC between PTH and BFR/BS ranging between 0.70 and 0.80, indicating only a moderate diagnostic accuracy.[14] This was also demonstrated by another cross-sectional study of 43 patients with CKD, in which the AUC between PTH and BFR/BS was 0.76 for indicating high turnover, but only 0.61 for indicating low turnover.[15]

Oxidation of PTH results in partial or complete loss of its biological activity.[5] Conventional PTH assays cannot distinguish between oxidized and n-oxPTH. This background raised the hypothesis to which extent measuring n-oxPTH confers superior diagnostic accuracy in discriminating between bone turnover categories, also as the majority of research concerning n-oxPTH measurements has been performed by a single research group.

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Our data clearly refute this hypothesis. tPTH and n-oxPTH showed a high and almost identical AUROC, fluctuating around 0.8 for discriminating low from non-low and high from non-high bone turnover. Both tPTH and n-oxPTH showed strong correlation coefficients with histomorphometric and biochemical parameters of bone turnover. These findings were not surprising, acknowledging the very high correlation coefficient between n-oxPTH and tPTH (rho = 0.92; $p < 0.001$). This strong correlation seems to be specific to the hemodialysis setting.[4,6,16] Weaker correlations have indeed been reported in healthy controls and in patients with CKD stage 2-4.[4,7,17] This discrepancy might be related to differences in redox status and metabolic clearance of oxPTH and n-oxPTH across stages of CKD.[18–20]

Theoretically, the ratio of PTH which is not oxidized could be calculated as the percentage of n-oxPTH/tPTH. However, as the affinity of the antibodies used in the tPTH assays may differ for the different PTH species based on oxidation status, this highly influences the results and the n-oxPTH/tPTH, as well as oxPTH values, cannot be calculated.[4] This hinders studies investigating determinants of the oxidizing rate of PTH. In addition, as there is no standard of n-oxPTH or oxPTH yet, n-oxPTH or tPTH concentrations combined with a calculated ratio can only be used to assess longitudinal differences over time using the same PTH immunoassay.[5]

The results of this study should be interpreted in light of its strengths and limitations. A major strength of the present study is the availability of bone biopsy data, the gold standard for assessing bone turnover, covering the spectrum of bone turnover from low to high. Moreover, bone turnover is assessed by a panel of bone formation and resorption biomarkers. However, the sample size, though substantial for a bone biopsy study, is rather small. The high correlation between n-oxPTH and tPTH renders a type 2 statistical error highly unlikely. Hence, we are convinced that extending the study population would not impact conclusions. Caution, however, is warranted when extrapolating the results to non-ESKD patients and to patients with a different ethnic background, as this study included only Caucasians.

The antibodies coated on the beads of the affinity column to capture oxPTH are raised against all forms of oxidized PTH, i.e. PTH oxidized at the 18th, 8th or both methionine residues. Previous studies have shown that the most easily oxidized methionine residue is Met18, due to its location. Met8 is more difficult to become oxidized as it is positioned in a hydrophobic pocket.[21] The amount of oxPTH oxidized in serum at either one or both

residues is currently unknown. What is known is that if these forms are separated and tested for their activity, PTH oxidized solely at Met18 has residual biological activity.[5,22] This was also recently corroborated by a study assessing the FGF23 stimulating effects of several oxPTH forms.[23] As we do not know the amount of ox(Met18)PTH, compared to ox(Met8)PTH or ox(Met8,18)PTH, it could be that the quantification of biologically active PTH by the methods used in the current study, is an underestimation. One could speculate that separation of the fragments may result in more optimal interpretation of PTH results. However, this remains to be investigated as soon as methods are available for specific measurement, such as a LC-MS/MS method.

In conclusion, our data demonstrate that in ESKD patients measuring n-oxPTH was not superior compared to tPTH in discriminating high from non-high and low from non-low turnover. In addition, other histomorphometric and serum parameters of bone turnover also showed no stronger correlation to n-oxPTH than to tPTH. Therefore, using the currently available method, there is at present no added value of measuring n-oxPTH in ESKD.

Disclosure

PE is on scientific advisory boards of Amgen, Vifor FMC, and Medice. PDH is on a scientific advisory board of Vifor Pharma and received research grants from Vifor Pharma, Inositec, Oxthera, Rockwell Medical, Shire (Takeda). MV is on advisory boards of Amgen, Vifor, FMC, Kyowa Kirin and Medice. Research supports from Amgen, FMC and Vifor-OPKO. EC is consultant for DiaSorin, IDS, Fujirebio, bioMérieux, IDS, Menarini and Nittobo. The other authors report no disclosures of interest.

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Figure titles and captions

Table 1. Baseline characteristics

Table 2. Spearman correlations n-oxPTH vs. tPTH (n = 31)

Table 3. Area under the receiver operating characteristics for bone turnover (n = 31)

Figure 1. Univariate linear regressions for n-oxPTH and tPTH (n = 31).

Figure 2. Receiver operating characteristics for n-oxPTH and tPTH A) discriminating between low/non-low bone turnover B) discriminating between high/non-high bone turnover

Supplementary Table 1. Baseline characteristics stratified per bone turnover classification

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Table 1. Baseline characteristics (n = 31)	
Parameters	**Mean ± SD or median (IQR)**
Age (years)	52 ± 14
Gender (male)	20/31 (65%)
Systolic blood pressure (mmHg)	143 ± 23
Diastolic blood pressure (mmHg)	85 ± 20
Type 1 Diabetes Mellitus (%)	1/31 (3%)
Type 2 Diabetes Mellitus (%)	1/31 (3%)
Parathyroidectomy	3/31 (10%)
HD/PD	18/13
Dialysis vintage (months)	18 (8-41)
Low bone turnover	10 (32%)
Normal bone turnover	10 (32%)
High bone turnover	11 (35%)
Serum parameters	
tPTH (pmol/L)	26.6 (16.1-66.5)
n-oxPTH (pmol/L)	6.7 (2.3-8.6
Bone ALP (µg/L)	33.3 (16.2-60.8)
PINP (µg/L)	76 (39-165)
TRACP5b (U/L)	5.8 ± 3.0
Calcium (mmol/L); n=26	2.40 (2.28-2.47)
Phosphate (mmol/L); n=26	1.75 (1.39-2.13)
Structural histomorphometric parameters	
Bone Area (%)	17.9 (16.3-25.3)
Trabecular Thickness (µm)	137 (117-152)
Trabecular Number (mm^{-1})	1.85 (1.48-2.17)
Trabecular Spacing (µm)	405 (293-545)
Static histomorphometric remodeling parameters	
Osteoid Area (%)	2.6 (0.97-5.1)
Osteoid Perimeter (%)	20.5 (10.8-35.2)
Osteoid Width (µm)	8.7 (7.0-10.9)
Eroded Perimeter (%)	5.0 (2.1-9.0)
Osteoblast Perimeter/ Total Perimeter (%)	3.0 (0.34-8.6)

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Osteoclast Perimeter/ Total Perimeter (%)	1.0 (0.05-2.0)
Dynamic histomorphometric remodeling parameters	
Mineralized Bone Area (%)	17 (16-24)
Mineral Apposition Rate (µm/day); n=25	1.02 (0.78-1.24)
Bone Formation Rate (µm^2/mm^2/day); n=25	342 (135-899)
Adjusted Apposition Rate (µm/day); n=25	0.56 (0.36-1.39)
Osteoid Maturation Time (days); n=25	9.7 (6.4-11.2)

HD = hemodialysis; PD = peritoneal dialysis; NA = not applicable; tPTH = total PTH; n-oxPTH = non-oxidized PTH; AP = alkaline phosphatase

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Table 2. Spearman correlations n-oxPTH vs. tPTH in ESKD (n=31)

	n-oxPTH		tPTH	
	rho	**p**	**rho**	**p**
Serum parameters				
tPTH	0.92	<0.001		
Bone ALP	0.61	<0.001	0.71	<0.001
PINP	0.69	<0.001	0.68	<0.001
TRACP5b	0.64	<0.001	0.65	<0.001
Structural histomorphometric parameters				
Bone Area	0.03	0.85	0.02	0.91
Trabecular Thickness	0.31	0.09	0.39	0.03
Trabecular Number	-0.09	0.65	-0.15	0.43
Trabecular Spacing	0.08	0.66	0.14	0.45
Static histomorphometric remodeling parameters				
Osteoid Area	0.28	0.13	0.35	0.06
Osteoid Perimeter	0.30	0.10	0.36	0.04
Osteoid Width	0.31	0.09	0.43	0.02
Eroded Perimeter	0.64	<0.001	0.62	<0.001
Osteoblast Perimeter/ Total Perimeter	0.55	0.001	0.53	0.002
Osteoclast Perimeter/ Total Perimeter	0.58	<0.001	0.58	<0.001
Dynamic histomorphometric remodeling parameters				
Mineralized Bone Area	0.02	0.92	-0.01	0.97
Mineral Apposition Rate (n=25)	0.66	<0.001	0.58	<0.001
Bone Formation Rate (n=25)	0.54	0.006	0.61	0.001
Adjusted Apposition Rate (n=25)	0.38	0.06	0.43	0.03
Osteoid Maturation Time (n=25)	-0.09	0.67	0.09	0.69

Original Research Article

Table 3. Area under the receiver operating characteristics for bone turnover in ESKD (n=31)

Parameter	AUC	SE	95% CI
Low (n=10) vs. non-low (n=21) turnover			
n-oxPTH	0.824	0.082	0.645 to 0.935
tPTH	0.790	0.086	0.607 to 0.915
BAP	0.833	0.073	0.656 to 0.942
PINP	0.862	0.086	0.691 to 0.959
TRAP5b	0.852	0.075	0.679 to 0.953
High (n=11) vs. non-high (n=20) turnover			
n-oxPTH	0.764	0.091	0.577 to 0.897
tPTH	0.795	0.085	0.613 to 0.918
BAP	0.909	0.052	0.750 to 0.982
PINP	0.864	0.064	0.693 to 0.960
TRAP5b	0.882	0.062	0.715 to 0.969

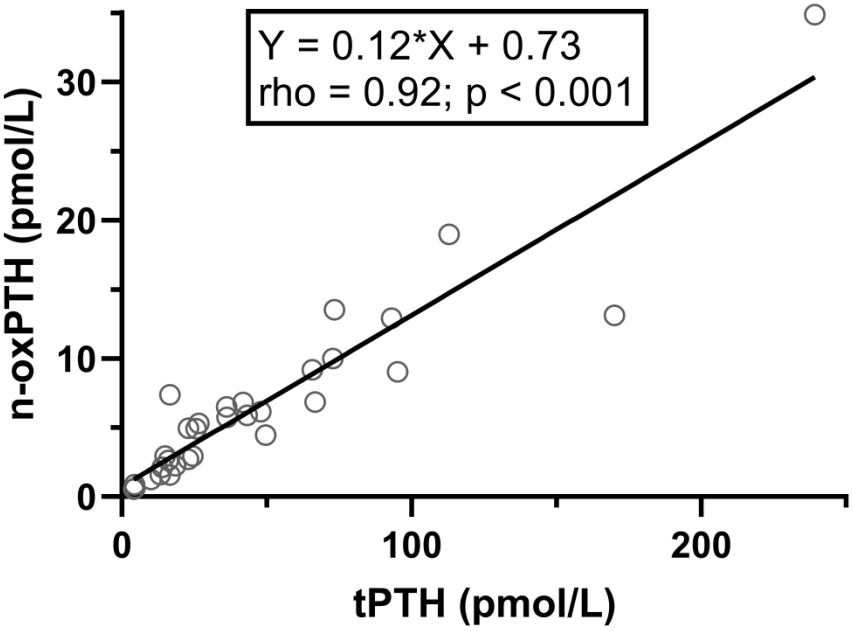


Figure 1. Univariate linear regressions for n-oxPTH and tPTH (n = 31)

105x75mm (1200 x 1200 DPI)

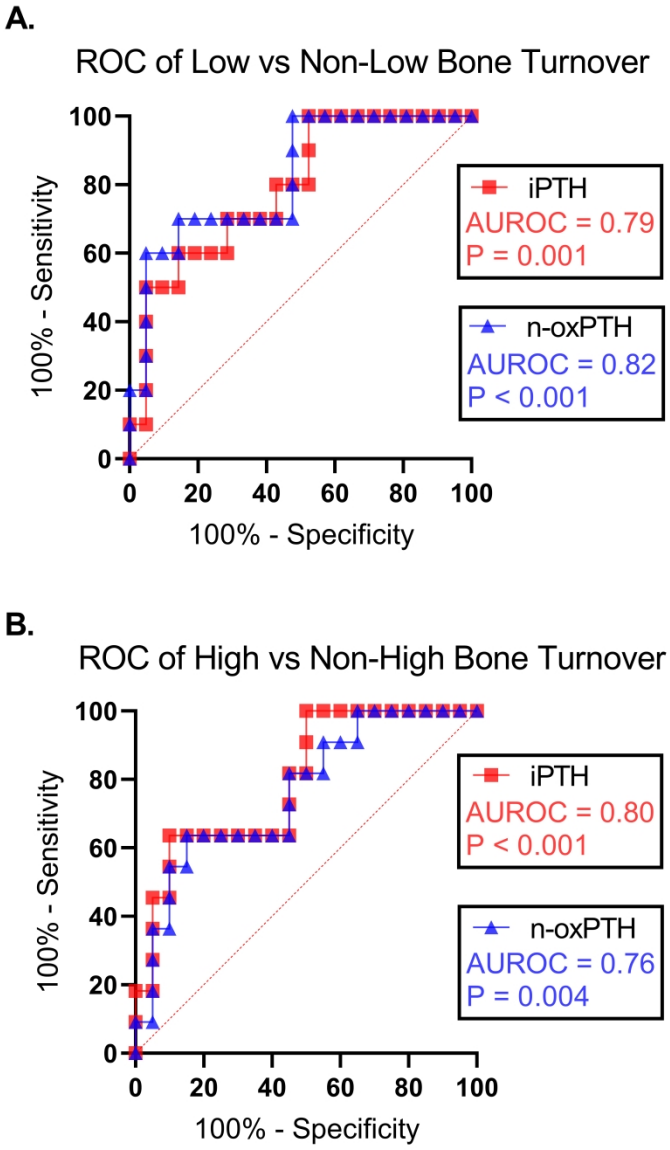


Figure 2. Receiver operating characteristics for n-oxPTH and tPTH A) discriminating between low/non-low bone turnover B) discriminating between high/non-high bone turnover

177x265mm (600 x 600 DPI)

Supplementary Table 1. Baseline characteristics stratified per bone turnover classification

Parameters	Mean ± SD or median (IQR)		
	Low bone turnover	Normal bone turnover	High bone turnover
N	10 (32%)	10 (32%)	11 (35%)
Age (years)	58 ± 9	57 ± 10	43 ± 16
Gender (male)	7/10 (70%)	6/10 (60%)	7/11 (64%)
Systolic blood pressure (mmHg)	132 ± 20	144 ± 20	150 ± 29
Diastolic blood pressure (mmHg)	80 ± 14	77 ± 9	93 ± 28
Type 1 Diabetes Mellitus (%)	0	1/10 (10%)	0
Type 2 Diabetes Mellitus (%)	0	1/10 (10%)	0
Parathyroidectomy	2/11 (18%)	1/10 (10%)	0
HD/PD	5/5	5/5	8/3
Dialysis vintage (months)	14 (8.0-22)	40 (13-60)	15 (7.2-47)
Serum parameters			
tPTH (pmol/L)	15.2 (10.1-36.2)	31.5 (16.0-49.7)	66.7 (24.8-94.7)
n-oxPTH (pmol/L)	2.4 (1.2-5.9)	5.5 (2.9-7.4)	9.0 (4.9-13.1)
Bone ALP (µg/L)	17.8 (11.6-23.6)	24.7 (15.9-40.0)	63.5 (41.0-76.0)
PINP (µg/L)	38 (32-41)	83 (48-136)	166 (101-227)
TRACP5b (U/L)	3.3 (2.4-4.3)	5.8 (3.6-6.5)	7.8 (6.1-10.3)
Calcium (mmol/L)	2.40 (2.29-2.43)	2.43 (2.16-2.50)	2.37 (2.26-2.45)
Phosphate (mmol/L)	2.29 (1.80-2.66)	1.56 (1.37-1.80)	1.66 (1.33-1.91)
Structural histomorphometric parameters			
Bone Area (%)	15 (14-17)	18 (17-25)	25 (19-33)
Trabecular Thickness (µm)	123 (111-132)	145 (117-161)	150 (123-167)
Trabecular Number (mm^{-1})	1.67 (1.46-2.00)	1.85 (1.42-2.12)	2.18 (1.77-2.46)
Trabecular Spacing (µm)	495 (403-558)	413 (302-558)	311 (248-437)
Static histomorphometric remodeling parameters			
Osteoid Area (%)	0.5 (0.0-2.0)	2.0 (1.0-3.0)	4.9 (3.2-7.9)
Osteoid Perimeter (%)	7 (2-11)	18 (14-23)	34 (25-44)
Osteoid Width (µm)	5.2 (3.6-9.5)	8.2 (7.3-9.0)	11.0 (9.4-14.3)
Eroded Perimeter (%)	1.0 (1.0-2.0)	5.5 (4.0-8.0)	10.2 (5.2-12.6)
Osteoblast Perimeter/ Total Perimeter (%)	0.0 (0.0-1.0)	3.0 (2.0-4.0)	9.0 (7.4-13.8)
Osteoclast Perimeter/ Total Perimeter (%)	0.0 (0.0-1.0)	1.0 (0.0-2.0)	2.0 (1.0-4.3)
Dynamic histomorphometric remodeling parameters			
Mineralized Bone Area (%)	15 (13-17)	17 (16-24)	23 (18-31)
Mineral Apposition Rate (µm/day)	0.74 (0.65-0.81)	1.08 (0.78-1.32)	1.13 (0.96-1.40)
Bone Formation Rate ($\mu m^2/mm^2$/day)	110 (82-127)	266 (150-348)	967 (871-1176)
Adjusted Apposition Rate (µm/day)	0.50 (0.14-1.77)	0.41 (0.34-0.51)	1.08 (0.58-1.74)
Osteoid Maturation Time (days)	7.57 (5.78-13.5)	9.19 (5.47-10.6)	10.4 (9.70-11.1)

HD = hemodialysis; PD = peritoneal dialysis; NA = not applicable; tPTH = total PTH; n-oxPTH = non-oxidized PTH; AP = alkaline phosphatase

Non-oxidized parathyroid hormone (PTH) measured by current method is not superior to total PTH in assessing bone turnover in chronic kidney disease.

Population

31 patients with CKD G5D

Bone biopsy

Blood sampling

Measurement

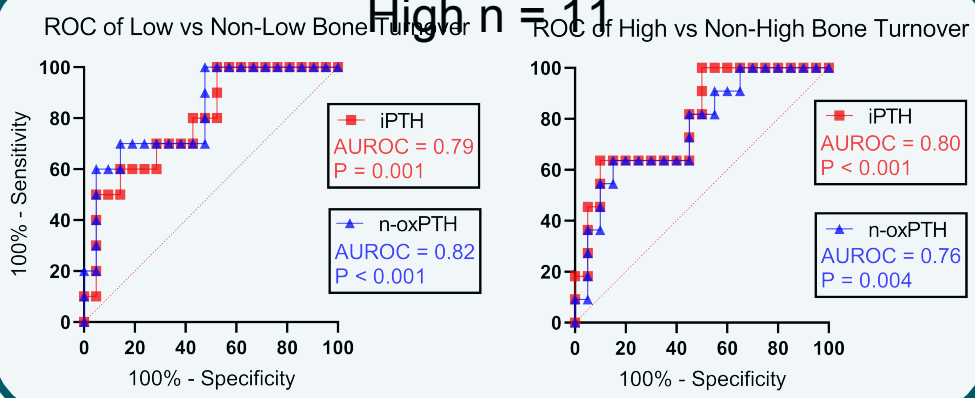
- Non-oxidized PTH
- Total PTH (iPTH)
- Histomorphometric parameters of bone turnover
- Bone turnover markers: Bone ALP, PINP, TRACP5b

Analysis

ROC curves, Spearman correlation and regression analysis

Primary Outcome

Low bone turnover n = 10; Normal n = 10; High n = 11



CONCLUSION:

Measuring non-oxidized PTH provides no added value compared to total PTH as indicator of bone turnover in ESRD

Ursem et al, 2020

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Modified STROBE Statement—checklist of items that should be included in reports of observational studies (Cohort/Cross-sectional and case-control studies)

	Item No	Recommendation	Checked
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Yes
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Yes
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Yes
Objectives	3	State specific objectives, including any prespecified hypotheses	Yes
Methods			
Study design	4	Present key elements of study design early in the paper	Yes
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Yes
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Yes
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Yes
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement).	Yes
Bias	9	Describe any efforts to address potential sources of bias	Yes
Study size	10	Explain how the study size was arrived at (if applicable)	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Yes

Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Yes
		(*b*) Describe any methods used to examine subgroups and interactions	Yes
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	Yes
		(c) Use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Yes
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*NA*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	Yes
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence	Yes

		interval). Make clear which confounders were adjusted for and why they were included	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Yes
Discussion			
Key results	18	Summarise key results with reference to study objectives	Yes
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Yes
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Yes
Generalisability	21	Discuss the generalisability (external validity) of the study results	Yes

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.