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Assessment of trabecular and cortical parameters using high-resolution peripheral quantitative computed tomography, histomorphometry and microCT of iliac crest bone core in hemodialysis patients

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ABSTRACT

Patients with end-stage renal disease develop changes in bone quality and quantity, which can be assessed using different methods. This study aimed to compare and to correlate bone parameters obtained *in vivo* using high-resolution peripheral quantitative computed tomography (HR-pQCT) with those obtained by bone biopsy using histomorphometry and microcomputed tomography (microCT) analysis of the iliac crest core, and to evaluate if HR-pQCT is helpful in aiding with categorization of those with high turnover. Twenty hemodialysis patients, 13 females (7 postmenopausal), underwent bone biopsy from 2018 to 2020. The mean age was 48.5 ± 10.6 years, and the mean hemodialysis vintage was 15 years. Histomorphometry identified mineralization defects, low turnover, and high turnover in 65%, 45%, and 35% of the patients, respectively. The highest values of trabecular bone volume (BV/TV) were obtained by histomorphometry, while the highest values of cortical thickness (Ct.Th) were obtained by HR-pQCT at the distal tibia. Moderate correlations were found between BV/TV values obtained by microCT of the bone core and HR-pQCT at the distal radius ($r = 0.531$, $p = 0.016$) and at the distal tibia ($r = 0.536$, $p = 0.015$). BV/TV values obtained from the bone core by histomorphometry and microCT were also significantly correlated ($r = 0.475$, $p = 0.04$). Regarding Ct.Th, there was a strong correlation between the radius and tibia HR-pQCT ($r = 0.800$, $p < 0.001$), between bone core microCT and the distal radius HR-pQCT ($r = 0.610$, $p < 0.01$), as between histomorphometry and microCT ($r = 0.899$, $p < 0.01$). In groups classified by bone turnover, patients with high turnover presented lower BV/TV, Tb.N, Tb.Th, and Ct.Th than those with low turnover in peripheral sites using HR-pQCT. By this method, it was possible to identify low turnover from tibia BV/TV > 12,4% plus Tb.Sp ≤ 0.667 mm (AUC 0.810, 95% CI 0.575 to 0.948) and high turnover from total bone mineral density (BMD) ≤ 154.2 mg HA/cm^3 (AUC 0.860, 95% CI 0.633 to 0.982, $p < 0.001$) and cortical BMD ≤ 691.6 mg HA/cm^3 (AUC 0.840, 95% CI 0.609 to 0.963, $p < 0.001$). In conclusion, HR-pQCT had significant correlation with iliac crest bone in BV/TV and Ct.Th, which are known to provide bone strength. This method is quick and non-invasive and may be helpful in categorizing those with high *versus* low turnover in hemodialysis patients.

1. Introduction

Patients with chronic kidney disease (CKD) are prone to develop changes in bone quantity or quality, or both. These modifications are generally associated with the underlying disease, uremia, osteoporosis, and mineral and bone disorder (CKD-MBD), among others (Yamamoto

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and Fukagawa, 2017; Bover et al., 2018; McNerny and Nickolas, 2017; Zheng et al., 2016). Histomorphometry determines TMV classification, based on turnover (T), mineralization (M), and bone volume (V), thus defining the type of renal osteodystrophy (Moe et al., 2006; Barreto et al., 2018; Ferreira et al., 2021). Bone turnover is classified according to bone formation rate (BFR) in low, normal and high, whereas mineralization is classified in normal or abnormal. Although this method is the gold standard in determine these parameters, many authors have evaluated whether biomarkers or image data can identify bone turnover, since histomorphometry is an invasive procedure and relies on a few specialized centers. In addition, it is based on a two-dimensional analysis, which was extrapolated in 3D structure, based on plate-model (Dempster et al., 2013).

In a large multicenter study that evaluated 630 biopsies, the authors concluded that the TMV classification is relevant but architecture of trabecular and cortical bone should be added to better assess bone status in patients with CKD (TMV/A) (Malluche et al., 2011). Hence, histology can provide a qualitative notion of the structural parameters of cancellous bone, but quantitative studies require additional 3D images with finite element analysis to evaluate load-bearing mechanical competence (Malluche et al., 2013) and "real" microarchitecture.

In the assessment of bone microarchitecture, microcomputed tomography (microCT) of bone fragments was used for many years as the gold standard (Ohs et al., 2020). MicroCT allows true volumetric analysis in a three-dimensional approach through X-ray attenuation with high resolution (small voxels), measuring the trabecular and cortical parameters (Cohen et al., 2010). However, this method can only be performed *ex vivo*, and the projections require more time to be fully acquired. Additionally, the pre-processing methods of these images are not standardized.

In the last two decades, new methods have become available for the assessment of bone microarchitecture, along with X-ray attenuation, with the ability to analyze the cortical and trabecular compartments, such as high-resolution peripheral quantitative computed tomography (HR-pQCT). This method assesses the distal radius and tibia non-invasively with a low level of radiation. Additionally, it provides important information about bone microstructure, and seems to predict outcomes such as fractures (Mikolajewicz et al., 2020). Since this method evaluates bone *in vivo*, it can be affected by motion artifacts. Moreover, the major limitation of the first-generation X-treme CT seems to be its limited isotropic resolution (82 μm), which is near the lower limit of trabeculae. Therefore, it is necessary to derive some trabecular parameters, such as bone volume, trabecular separation, and thickness, all of which depend on the measured density (Boutroy et al., 2005; Agarwal et al., 2016; Manske et al., 2015; Whittier et al., 2020).

In synthesis, patients with CKD have alterations in bone microstructure that can be influenced by bone turnover. We hypothesized that the bone health of these patients can be evaluated by different methods at distinct sites. The present study aimed to evaluate cortical and trabecular parameters, to determine the correlation of peripheral bone data obtained by HR-pQCT with those of histomorphometry and microCT of the iliac crest core obtained through bone biopsy, and to define if HR-pQCT is helpful in aiding with categorization of bone turnover in hemodialysis patients.

2. Patients and methods

2.1. Patients

We included patients with CKD aged 18 years and older, on hemodialysis for at least 6 months, and who had a follow-up at the outpatient clinic for mineral and bone diseases related to CKD. Bone biopsy was indicated as pre-treatment with anti-osteoporotic drugs unexplained bone pain or fracture, or before parathyroidectomy. Patients with known bone diseases (for example, multiple myeloma or Paget) or those using medications that alter bone metabolism, such as glucocorticoids and anticonvulsants, were excluded. Moreover, we did not include patients with diabetes mellitus who may have bone changes due to the underlying disease. Demographic and clinical parameters were noted. Dialysis vintage was defined as the time from the first day of dialysis to bone biopsy.

HR-pQCT and blood sampling for laboratory analysis were performed within seven days prior to bone biopsy. In the interdialytic period, fasting samples were collected, immediately centrifuged, and stored at −70 °C. Biochemistry included serum calcium (range reference [RR]: 8.6 to 10.3 mg/dL), phosphorus (RR: 2.5 to 4.5 mg/dL), intact parathyroid hormone (PTH) using a chemiluminescence assay (RR: 10 to 65 pg/mL), bone and total alkaline phosphatase (RR: 4.9 to 26 μg/L and 35 to 104 U/L, respectively), and 25-hydroxyvitamin D using competitive chemiluminescent immunoassay (RR: 30 to 60 ng/mL).

The study was approved by the Ethics and Research Committee of the Hospital Universitario Clementino Fraga Filho (HUCFF-UFRJ), and all patients signed informed consent forms.

2.2. Bone biopsy and histomorphometry

Percutaneous bone biopsy of the anterior iliac crest was performed with a 7.5 mm trephine (Bonther), 2 cm inferior and 2 cm posterior to the anterosuperior iliac crest, after double-labeling with tetracycline. The protocol included pretreatment with hydrochloride tetracycline (20 mg/kg/day) for three consecutive days, with an interval of 10 days without medication, followed by another three days of tetracycline administration. Biopsy was performed three to five days after the last dose of tetracycline. The samples were fixed in 70% ethanol, and the undecalcified fragment was impregnated in methylmethacrylate according to the standard protocol and analyzed using a microscope (Nikon Labophot II, Tokyo, Japan) equipped with an ultraviolet (UV) high-resolution digital color video camera (Olympus DP71, Center Valley, PA, USA). The image analysis system was a semiautomatic method provided by the Osteomeasure software (Osteometrics Inc., Atlanta, GA, USA). Toluidine blue was the standard stain, that shows mineralized bone (purple/dark blue) and osteoid (pale blue). Solochrome azurine was used to assess aluminum bone deposition. All analyses were performed by the same investigator at the Pro-renal Foundation, and the histomorphometric parameters were described as suggested by the American Society of Bone and Mineral Research Histomorphometry Nomenclature Committee (Dempster et al., 2013). Cortical and trabecular parameters were evaluated: cortical thickness (Ct.Th), trabecular bone volume (BV/TV), mineralized bone volume (Md/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N, derived from [BV/TV]/Tb.Th), trabecular separation (Tb.Sp, derived from [1/Tb.N]–Tb.Th). The range references of these parameters were obtained from healthy Brazilian subjects covering the gender and age range of the study population (Dos Reis et al., 2007). Bone turnover was classified as normal when bone formation rate/bone surface (BFR/BS) was 1.8 to 3.8 mm^3/cm^2/year or 0.05 to 0.10 $\mu m^3/\mu m^2$/day (Malluche et al., 2011). So, low turnover was defined as values less than 1.8 mm^3/cm^2/year and high turnover above 3.8 mm^3/cm^2/year. Mineralization defect was identified when osteoid thickness was above 12.5 μm and mineralization lag time was above 100 days (Dempster et al., 2013).

2.3. MicroCT of iliac crest bone core

Before histomorphometric analysis, the whole methylmethacrylate-impregnated core of the iliac crest was scanned using Skyscan 1173 microtomography (Bruker, Kontich, Belgium), which obtained images with isotropic resolution, source voltage and current 10 μm pixel size, 100 kV and 80 μA, respectively. The reconstruction of microCT projections was performed using the Nrecon software version 1.7.1 (Bruker, Kontich, Belgium). Image analysis was performed using the CTAn software version 1.14.4.1+ (Bruker micro-CT, Kontich, Belgium) to evaluate cortical thickness (Ct.Th, trabecular bone volume (BV/TV), trabecular

thickness (Tb.Th), number of trabeculae (Tb.N) and trabecular separation (Tb.Sp). Volumes of interest (VOI) were manually drawn with the aid of a physician, to separate the cortical and trabecular bone areas. Uniform and unsharp mask filters (both 3D options) were used to highlight bone tissue, and the Otsu threshold method (3D option) was used to binarize the images (to classify what is bone or not). The regions of interest (ROI) shrink wrap was used to embrace the object and refine the initially drawn ROI (for all cortical regions and some trabecular regions with considerable bone tissue), and the despeckle option was selected to remove misclassified bone tissue. Post-processed microCT slices were then used for morphometric measurements.

2.4. HR-pQCT

Volumetric density and bone microarchitecture were measured using a first-generation HR-pQCT system (Xtreme CT I; Scanco Medical AG, Brüttisellen, Switzerland), following the recommendations of a recent consensus (Whittier et al., 2020). The distal radius (of the arm without the arteriovenous fistula) and the ipsilateral distal tibia were used for analysis, except in the case of a previous fracture in either region. Before scan acquisition, the wrist and ankle were immobilized in a carbon-fiber cast. A scout view defined the ROI as follows: the first CT slice was 9.5 mm and 22.5 mm proximal to the reference line positioned at the margin of the distal articular surface, perpendicular to the long axis of the diaphysis of the distal radius and of the distal tibia, respectively. From each site, 110 slices of 82 μm thickness (voxel dimension) were obtained and then digitized to generate a three-dimensional representation of approximately 9 mm in the axial direction. The examinations were performed and analyzed by the same professional and in the case of motion artifacts, the examination was repeated to obtain reliable images. The bone parameters were as follows: cortical thickness ($Ct.Th^d$, derived from cortical volume/outer bone surface), trabecular bone volume fraction (BV/TV^d, derived from trabecular density/1200 mg hydroxyapatite (HA)/cm^3), trabecular number (Tb.N), trabecular separation [$Tb.Sp^d$, derived from (1-BV/TV)/Tb.N], trabecular thickness [$Tb.Th^d$, derived from (BV/TV)/Tb.N], total bone mineral density (Tt.BMD), trabecular bone mineral density (Tb.BMD) and cortical bone mineral density (Ct.BMD). Details for processing the HR-pQCT data have been previously described by Boutroy et al. (2005).

2.5. Statistical analysis

After checking the normal distribution using a Shapiro-Wilk test, we expressed qualitative variables in absolute numbers and percentages and continuous variables as mean ± standard deviation or median (interquartile interval). Group differences were tested using independent t student tests for normal and Mann-Whitney test for non-normal distribution variables. Kruskal-Wallis for non-normal distribution and Analysis of Variance (ANOVA followed by Tukey's test) for normal distribution variables were used to evaluate the differences between three or more groups. We used Pearson's correlation for parametric distributions and Spearman's coefficient for non-parametric distributions. Linear regression was used to determine the R2 coefficient and regression equations to relate the parameters analyzed by the three methods (histomorphometry, microCT, and HR-pQCT). Receiver operator characteristic (ROC) curve was used to discriminate abilities of distal tibia and radius HR-pQCT in identify turnover (low/nonlow and high/nonhigh bone turnover groups). We used logistic regression to combine variables for ROC analysis. All tests were two-sided, and the significant level was fixed at 0.05. Statistical analysis was performed using SPSS version 24 (IBM Corporation, New York, USA).

3. Results

Twenty patients on hemodialysis underwent bone biopsy from 2018 to 2020. The mean age was 48.5 ± 10.6 years (ranging from 28 to 71 years), and the hemodialysis vintage was almost 15 years (ranging from 2 to 30 years). Thirteen were females, and 54% were postmenopausal. Only one patient was an active smoker. Another patient consumed more than three units of alcohol per day at the time of the study. Of the female patients, 54% were postmenopausal. The main clinical and laboratory characteristics of the patients are presented in Table 1.

Histomorphometric analysis of the fragments obtained by bone biopsy identified mineralization defect in 65% of the patients, low turnover in 45%, and high turnover in 35%. None showed aluminum deposit by histomorphometry. All bone cores were also subjected to microCT evaluation, and all patients were evaluated using HR-pQCT. The cortical and trabecular parameters obtained using the three methods are presented in Table 2.

Regarding the trabecular bone, histomorphometry showed a higher BV/TV value compared to those of the other methods. Using this technique, we also evaluated the volume of mineralized bone (Md/TV - %) and found a value of 21.2 ± 11.4%, which was similar to the BV/TV seen in microCT. Unlike trabecular volume, the cortical thickness in the tibia was greater than that in the other sites.

Moderate correlations between bone volume measured *in vivo* by HR-pQCT (radius) and microCT of bone core were observed. Histomorphometry only showed a significant correlation of bone volume with

Table 1
Clinical and laboratorial parameters.

Parameters	Results
Age (years old)	48.5 ± 10.6
Gender (male)	7 (35%)
BMI (kg/m^2)	22.8 ± 3.4
HD vintage (months)	180.6 ± 79.1
Causes of CKD	
Hypertension	8 (40%)
Undetermined	4 (20%)
Chronic glomerulonephritis	3 (15%)
SLE	3 (15%)
Eclampsia	1 (5%)
ADPKD	1 (5%)
CKD-MBD therapy	
Phosphate binder	17 (85%)
Calcitriol/paricalcitol	7 (35%)
Cholecalciferol	6 (30%)
Cinacalcet	6 (30%)
Ca (mg/dL)	9.0 ± 1.9
P (mg/dL)	4.9 ± 2.4
25OHVitD (ng/mL)	28.1 ± 8.0
iPTH (pg/mL)	224 (53,1055)
bALP (UI/L)	35(15,96)
ALP (μg/L)	137 (81,522)

BMI: Body mass index, HD: hemodialysis, CKD: chronic kidney disease, SLE: systemic lupus erythematous, ADPKD: autosomal dominant polycystic disease. Results expressed in absolute number (%) or mean ± SD or median (interquartile interval).

Table 2
Trabecular and cortical parameters.

	HR-pQCT tibia	HR-pQCT radius	MicroCT bone core	Histomorphometry bone core
BV/TV (%)	10.6 ± 4.7	12.7 ± 5.3	19 ± 6.4	25.6 ± 11
Tb.N (1/mm)	1.25 ± 0.42	1.63 ± 0.51	1.40 ± 0.41	2.58 ± 1.33
Tb.Th (mm)	0.087 ± 0.030	0.076 ± 0.020	0.135 ± 0.030	0.103 ± 0.030
Tb.Sp (mm)	0.818 ± 0.330	0.609 ± 0.290	0.480 ± 0.160	0.358 ± 0.200
Ct.Th (mm)	0.751 ± 0.400	0.461 ± 0.310	0.191 ± 0.050	0.641 ± 0.254

BV/TV: bone volume/total volume, Tb.Th: trabecular thickness, Tb.Sp: trabecular separation, Tb.N: number of trabeculae, Ct.Th: cortical thickness.

Table 3
Correlation coefficient between histomorphometry, microCT and HR-pQCT.

	BV/TV	Ct.Th
HR-pQCT radius × tibia	0.656**	0.800***
HR-pQCT radius × microCT	0.531*	0.610**
HR-pQCT radius × histomorphometry	0.322	0.571
HR-pQCT tibia × microCT	0.536*	0.437
HR-pQCT tibia × histomorphometry	0.289	0.338
MicroCT × histomorphometry	0.475*	0.899***

BV/TV: bone volume, Ct.Th: cortical thickness.
* $p < 0.5$.
** $p < 0.01$.
*** $p < 0.001$.

microCT (Table 3). Due to the large number of patients with mineralization defect, we analyzed the association between Md/TV by histomorphometry and BV/TV by microCT and there was no increase in the strength of the correlation ($r = 0.481$, $p = 0.037$). When the correlation was analyzed by turnover and mineralization classification, it did not provide additional information.

With respect to other trabecular parameters, only Tb.N showed a moderate correlation between iliac crest core microCT and

Table 4
Results of linear regression analyses.

	R^2	Intercept	Slope	*p* value
BV/TV (HR-pQCT radius × tibia)	0.430	4.89	0.74	0.002
BV/TV (HR-pQCT radius × microCT)	0.283	4.31	0.44	0.016
BV/TV (HR-pQCT tibia × microCT)	0.289	3.05	0.40	0.014
BV/TV (Histomorphometry × microCT)	0.225	9.49	0.88	0.04
Ct.Th (HR-pQCT radius × tibia)	0.640	0.0	0.62	<0.001
Ct.Th (HR-pQCT radius × microCT)	0.370	−0.29	4.06	0.005
Ct.Th (histomorphometry × microCT)	0.808	−0.22	4.31	<0.001

BV/TV: trabecular bone volume, Ct.Th: cortical thickness.

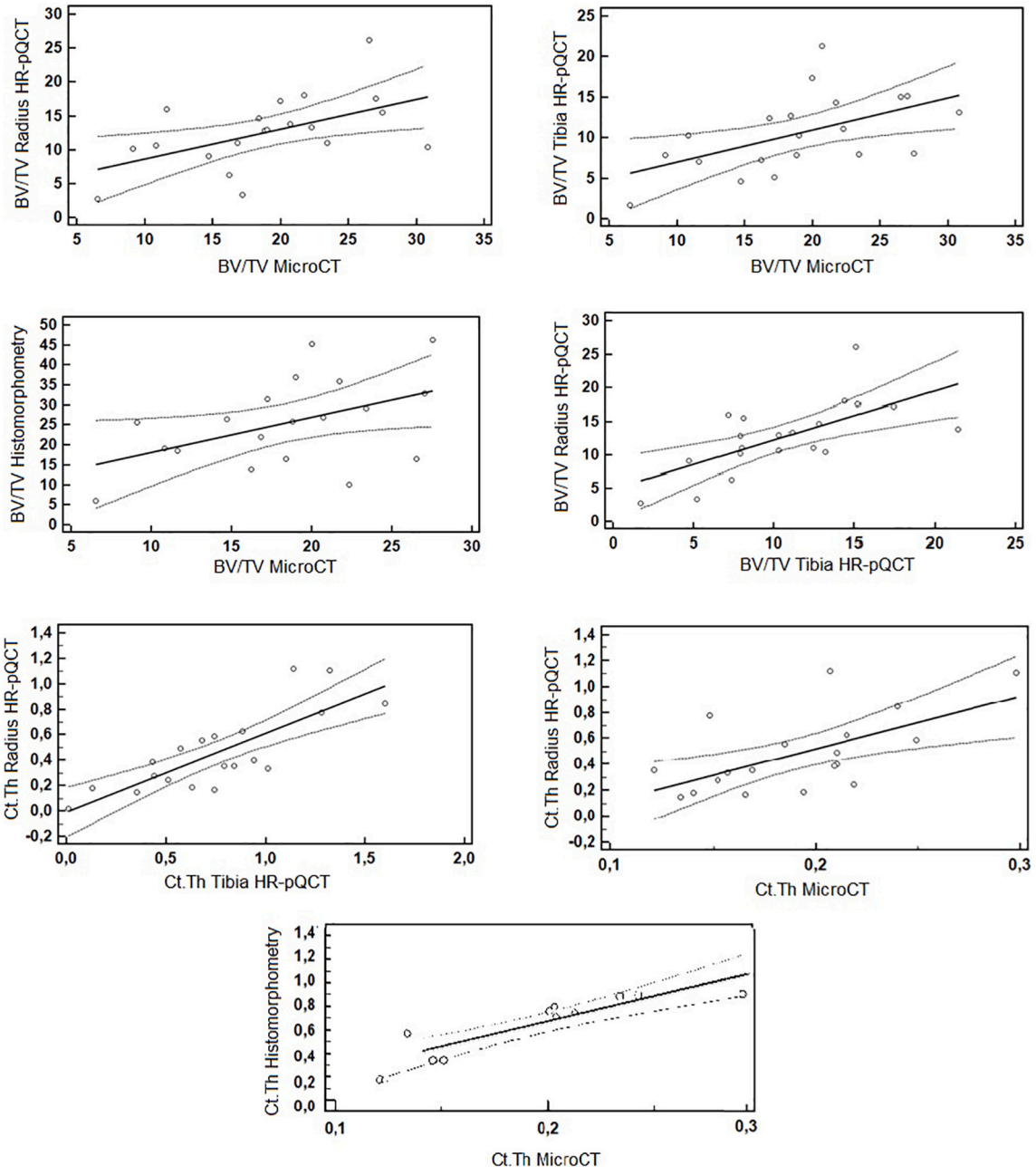

Fig. 1. Linear regression graphics. BV/TV: bone volume, Ct.Th: cortical thickness.

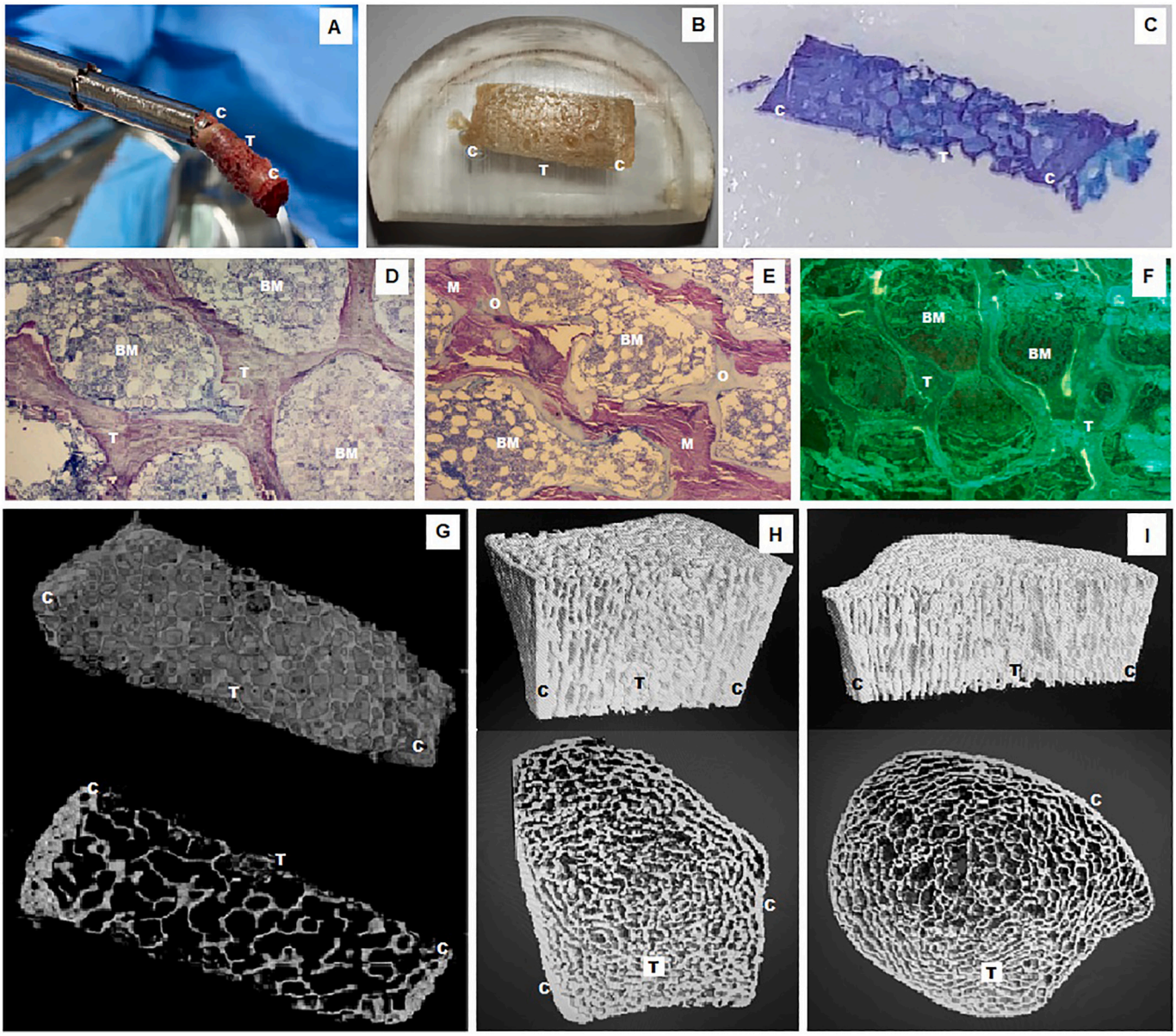

Fig. 2. Bone imaging visualized by different methods. A: Transiliac crest core extracted by bone biopsy; B: Bone core embedded in methylmethacrylate; C: Histology of transiliac crest core using toluidine blue stain, D: Histology showing trabeculae and bone marrow; E: Histology in high magnification, using toluidine blue stain where purple/dark blue indicates mineralized bone and unmineralized osteoid stains pale blue; F: Tetracycline double-labeled areas (yellow) under fluorescent microscopy; G: microCT of transiliac crest bone core (3D and 2D); H: Coronal and axial radius HR-pQCT; I: Coronal and axial tibia HR-pQCT.
C: Cortical bone; T: trabecular bone; M: mineralized bone; O: osteoid; BM: bone marrow.

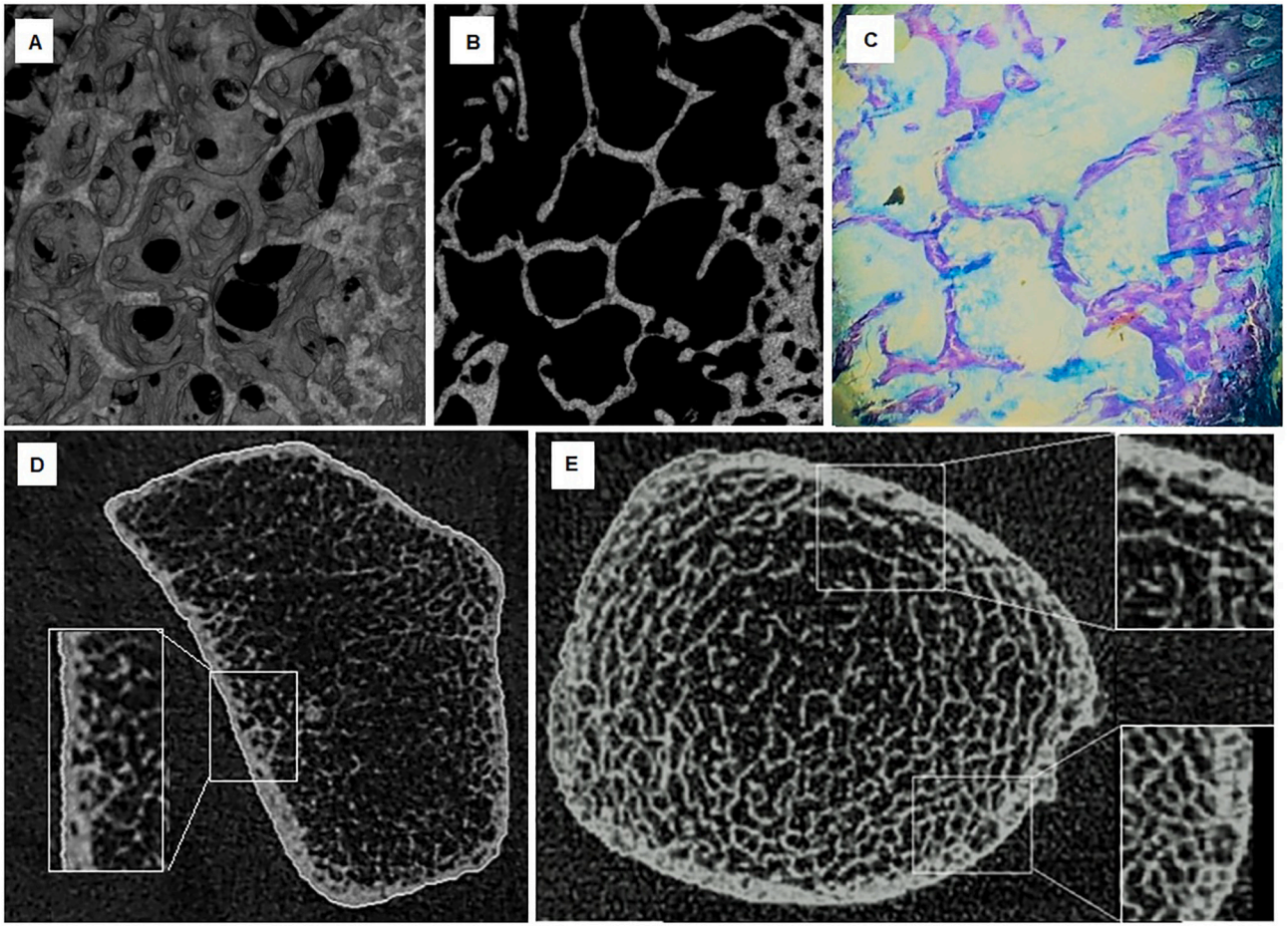

Fig. 3. Bone imaging in high magnification to assess similarities among different methods. A: 3D MicroCT of iliac crest core; B: 2D MicroCT of iliac crest core; C: Histology of iliac crest; D: Radius HR-pQCT; E: Tibia HR-pQCT.

Table 5
Clinical, laboratory, dynamic data, trabecular and cortical parameters divided by turnover.

	Low turnover	Normal turnover	High turnover	p value
Gender (F)	6 (66%)	2 (50%)	5 (71%)	0.540
Age	47.1 ± 11.3	50 ± 1.2	49.1 ± 11.8	0.908
Ca	8.2 ± 2	9.6 ± 0.5	9.2 ± 1	0.088
P	5 ± 1.9	5.5 ± 2.4	5.3 ± 2.1	0.855
ALP	100 (74,137)	97.5 (81,125.3)	530 (438,653)	0.001*
bALP	19.8 (11.9,35.1)	19.5 (16.1,28.7)	121 (90.4,181)	0.002*
iPTH	55 (17,308)	187.5 (170,210)	1242 (1042,1716)	0.001*
Dynamic data				
BFR/BS	0.009 ± 0.005	0.076 ± 0.032	0.455 ± 0.332	0.003*
MS/BS	3.2 ± 1.5	13.9 ± 10.7	24.5 ± 1.4	0.001*
MAR	0.33 ± 0.08	0.71 ± 0.11	1.82 ± 1.23	0.006*
Histomorphometry				
BV/TV	26.5 ± 3.6	24.2 ± 4.6	25.4 ± 6.4	0.682
TbN	2.70 ± 0.60	2.30 ± 0.30	2.70 ± 0.50	0.353
TbTh	0.106 ± 0.010	0.105 ± 0.070	0.093 ± 0.020	0.808
TbSp	0.345 ± 0.050	0.372 ± 0.090	0.365 ± 0.120	0.821
CtTh	0.619. ± 0.259	0.822 ± 0.055	0.539 ± 0.200	0.552
MicroCT				
BV/TV	21 ± 1.8	16.9 ± 2.9	19.4 ± 2.8	0.018*
TbN	1.60 ± 0.10	1.20 ± 0.20	1.40 ± 0.10	<0.001*
TbTh	0.135 ± 0.008	0.137 ± 0.0160	0.136 ± 0.008	0.941
TbSp	0.420 ± 0.030	0.584 ± 0.100	0.463 ± 0.070	<0.001*
CtTh	0.193 ± 0.020	0.200 ± 0.010	0.173 ± 0.020	0.099
Radius HR-pQCT				
BV/TV	14.4 ± 2	11.7 ± 0.6	10.3 ± 2.1	0.002*
TbN	1.70 ± 0.20	1.60 ± 0.10	1.40 ± 0.10	0.026*
TbTh	0.082 ± 0.007	0.072 ± 0.006	0.07 ± −0.0130	0.034*
TbSp	0.619 ± 0.120	0.543 ± 0.030	0.656 ± 0.090	0.206
CtTh	0.517 ± 0.090	0.546 ± 0.160	0.264 ± 0.090	<0.001*
Tt.BMD	272.9 ± 84.1	276.5 ± 98.3	175.4 ± 60.8	0.367
Ct.BMD	693.6 ± 91.3	738.8 ± 180	555.2 ± 89.6	0.135
Tb.BMD	167.3 ± 76	136 ± 15.1	141.2 ± 54.4	0.643
Tibia HR-pQCT				
BV/TV	12.9 ± 1.6	9.5 ± 0.6	6.9 ± 1.8	<0.001*
TbN	1.40 ± 0.10	1.10 ± 0.20	1.10 ± 0.20	0.002*
TbTh	0.093 ± 0.009	0.101 ± 0.02	0.062 ± 0.010	<0.001*
TbSp	0.692 ± 0.100	0.927 ± 0.140	1.108 ± 0.230	<0.001*
CtTh	0.844 ± 0.140	0.806 ± 0.090	0.512 ± 0.160	<0.001*
Tt.BMD	233 ± 71.4	211.9 ± 36.6	133.3 ± 58.4	0.339
Ct.BMD	708.2 ± 71.8	757.8 ± 126.2	599.4 ± 110	0.122
Tb.BMD	152.9 ± 60.2	112 ± 17	101.9 ± 48.4	0.176

Data are presented as absolute number (%), mean ± SD, median (interquartile range). F: female, Age (years old), Ca: calcium (mg/dL), phosphorus (mg/dL), ALP: alkaline phosphatase (U/L), bALP: bone alkaline phosphatase (μg/L), iPTH: intact PTH, BFR/BS ($\mu m^3/\mu m^2$/day): Bone Formation Rate per unit of Bone surface, MS/BS (%): mineralizing surface, MAR (μm/day): Mineral apposition Rate BV/TV (%): bone volume, Tb.N (1/mm): number of trabeculae, Tb.Th (mm): trabecular thickness, Tb.Sp (mm): trabecular separation, Ct.Th (mm): cortical thickness. Tt.BMD: Total bone mineral density (mg HA/cm^3), Ct.BMD: cortical bone mineral density (mg HA/cm^3), Tb.BMD: trabecular bone mineral density (mg HA/cm^3).

* $p < 0.05$.

histomorphometry ($r = 0.460$, $p = 0.047$). The Tb.Th had a significant correlation only between the radius and tibia using HR-pQCT ($r = 0.566$, $p = 0.009$).

Regarding cortical thickness, there was a strong correlation between the radius and tibia, and between microCT of the iliac crest core and the distal radius using HR-pQCT, as well as between microCT and histomorphometry (Table 3). Additionally, we found a trend toward a positive correlation ($p = 0.06$) in Ct.Th between distal tibia using HRpQCT and the bone core microCT, as between Ct.Th by radius HRpQCT and Ct.Th using histomorphometry of iliac crest.

The regression graphs are shown in Fig. 1, followed by the regression analyses in Table 4.

Figs. 2 and 3 show examples of the images found in microCT, histomorphometry, and HR-pQCT of the radius and tibia.

Among three methods, we used HR-pQCT to determine volumetric bone mineral density. In our study, total, cortical and trabecular bone mineral density were 249.4 ± 93.3 mg HA/cm^3, 670.3 ± 137.9 mg HA/cm^3, 151.9 ± 62.3 mg HA/cm^3, respectively, by radius HR-pQCT. In distal tibia, Tt.BMD was 202.8 ± 73.6 mg HA/cm^3, Ct.BMD was 693.4 ± 113.7 mg HA/cm^3, and Tb.BMD was 126.9 ± 55.5 mg HA/cm^3.

Clinical and laboratory parameters, dynamic data, trabecular and cortical parameters and density divided by turnover were presented in Table 5. When we compared the trabecular and cortical parameters in groups classified by bone turnover, patients with high turnover presented lower BV/TV, Tb.N, Tb.Th, and Ct.Th than those with low turnover in peripheral sites using radius and tibia HRpQCT. There was no

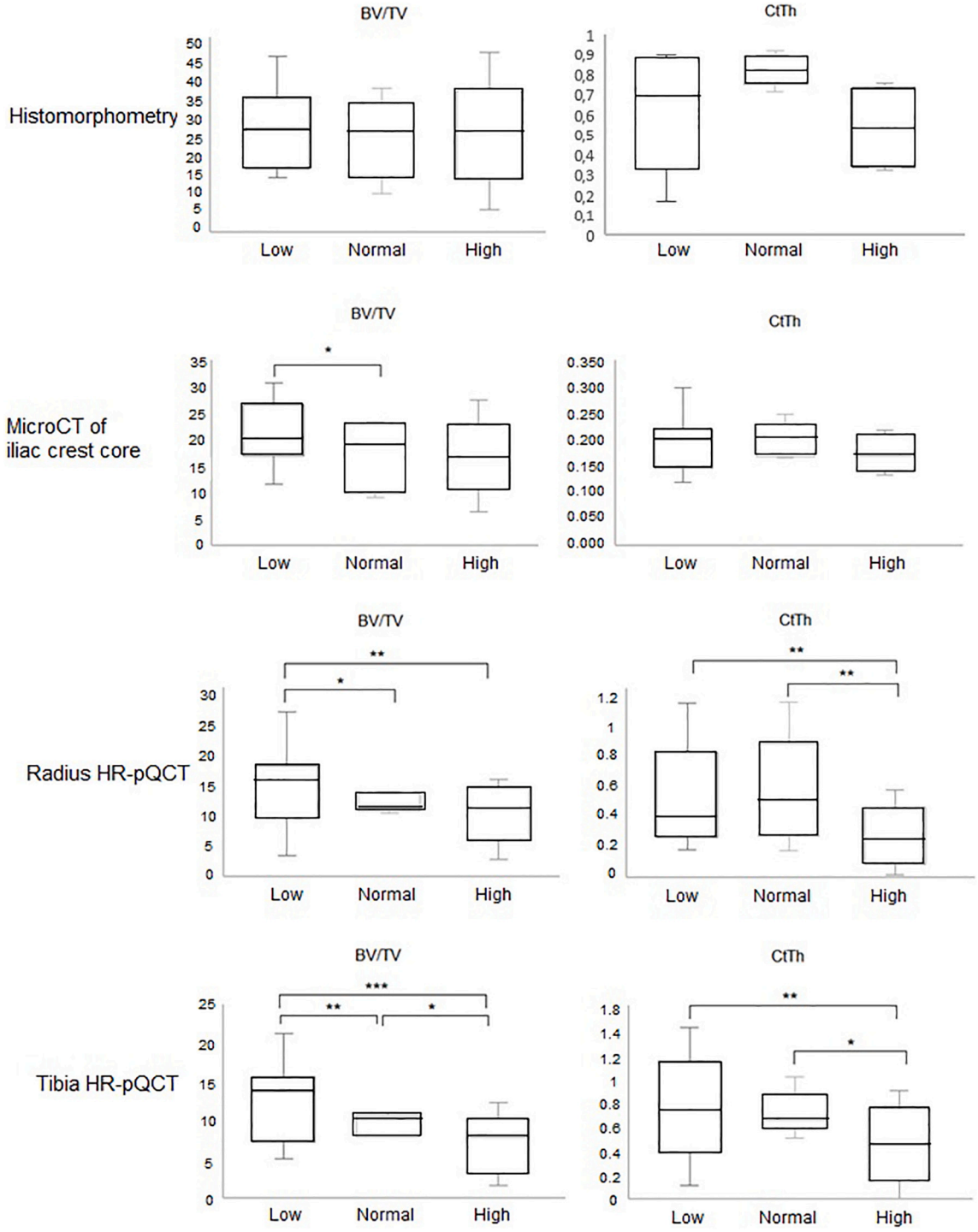

Fig. 4. Trabecular and cortical parameters divided by turnover. Tukey test for multiple comparison analysis. BV/TV: bone volume; Ct.Th: cortical thickness. *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$.

significant difference between the turnover classes regarding cortical thickness on microCT and histomorphometry, as well as trabecular parameters in histomorphometric evaluation (Table 5 and Fig. 4). There was no significant difference in structural parameters between groups with and without mineralization defect. Furthermore, HR-pQCT was not able to classify patients with normal or abnormal mineralization.

In order to discriminate low from nonlow turnover using ROC curve, the AUC for tibia BV/TV > 12.4% was 0.760 and tibia Tb.Sp ≤ 0.667 mg HA/cm^3 was 0.750 (Table 6). When we combine these parameters, AUC improved to 0.810 (95% CI 0.575 to 0.948, p 0.046). The ROC curve used to differentiate high from nonhigh bone turnover, determined the AUC for tibia BV/TV ≤ 8.1% of 0.780 and radius Ct.Th ≤ 0.340 mm of 0.787. Combining these data, AUC increased to 0.827 (95% CI 0.594 to 0.957, p 0,46) (Table 6). The parameters of density were better than structural parameters to identify patients with high turnover. AUC from total bone mineral density (Tt.BMD) and AUC from cortical bone mineral density (Ct.BMD) were presented in Table 6. Combining these variables at tibia HR-pQCT, AUC improved to 0.867 ((95% CI 0.641 to 0.975, p 0.028). The ROC curves were showed in Fig. 5 and all AUC (95% confidence interval) were presented in Supplementary Table 1.

Table 6
Diagnostic accuracy of radius and tíbia HR-pQCT for identifying patients with low and high bone turnover.

Variables	AUC (95% CI)	Cut-off	Sens (%)	Spec (%)	PPV (%)	NPV (%)	p value
Low turnover							
Tibia BV/TV	0.760 (0.520 to 0.920)	>12.4	70	100	100	80.3	0.043
Tibia Tb.Sp	0.750 (0.509 to 0.913)	≤0.667	70	80	74.1	76.5	0.034
High turnover							
Tibia BV/TV	0.780 (0.541 to 0.931)	≤8.1	80	66.7	56	86	0.021
Tibia Tt.BMD	0.860 (0.633 to 0.982)	≤154.2	86.7	80	70	92	<0.001
Tibia Ct.BMD	0.840 (0.609 to 0.963)	≤691.6	100	66.7	62	100	<0.001
Radius Ct.Th	0.787 (0.549 to 0.935)	≤0.340	80	73.3	61.5	87	0.023
Radius Tt.BMD	0.800 (0.563 to 0.943)	≤203.2	80	80	68	88	0.005
Radius Ct.BMD	0.827 (0.594 to 0.957)	≤593.1	80	80	68	88	0.002

AUC: Area under curve, 95% CI: 95% confidence interval, Sens: sensitivity, Spec: Specificity, PPV: positive predictive value, NPV: negative predictive value, BV/TV: trabecular bone volume (%), Tb.Sp: trabecular separation (mm), Tt.BMD: total bone mineral density (mg HA/cm^3), Ct.BMD: cortical bone mineral density (mg HA/cm^3).

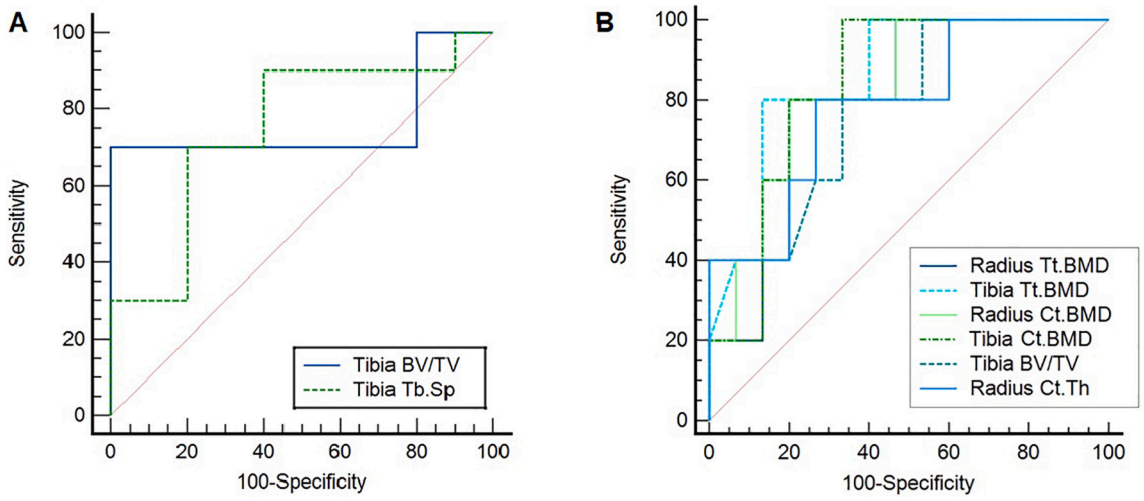

Fig. 5. ROC curve. A: for discriminating low from nonlow bone turnover, B: for discriminating high from nonhigh turnover.

4. Discussion

To our knowledge, this is the first study to evaluate trabecular and cortical parameters in hemodialysis patients using three different methods (HR-pQCT, microCT, and histomorphometry) and to describe a significant correlation among them. Patients with end-stage renal disease (ESRD) develop changes in bone quality and quantity (McNerny and Nickolas, 2017; Pimentel et al., 2017) and it is possible to assess these characteristics at different sites through different methods. Histomorphometry is a two-dimensional, invasive technique capable of determining the rate of bone remodeling and mineralization, in addition to bone volume. However, it depends on expertise in execution, processing, and reading, which can be time consuming (Moe et al., 2006; Dempster et al., 2013). MicroCT analyzes the microarchitecture in a three-dimensional manner, but it also requires an invasive biopsy and detects only the mineralized matrix (Ohs et al., 2020). HR-pQCT has characteristics similar to those of microCT in terms of the method used (X-ray attenuation) and the analyzed parameters of microarchitecture in a three-dimensional manner. Nevertheless, it has the great advantage of being non-invasive and more comfortable for the patient and provides fast results (Boutroy et al., 2005; Whittier et al., 2020). In our study, we observed patients from a wide range of ages (from 28 to 71 years) and hemodialysis vintage ranging from 2 to 30 years. In addition, we observed patients with low (45%), normal (20%), and high turnover (35%), and PTH levels ranging from 10 to 3107 pg/mL with normal and altered mineralization, and wide variation in bone volume (10.1 to 46.3%). Therefore, we evaluated a sample of all changes likely to be found in the hemodialysis population using three methods.

4.1. Comparison among three methods

4.1.1. Trabecular parameters

Some authors evaluated populations with CKD that were under conservative management or on hemodialysis in age groups comparable to that in our study. In these studies, the values of BV/TV by histomorphometry (Sharma et al., 2018; Marques et al., 2017), microCT (Sharma et al., 2018) and HR-pQCT were similar to those in our study (Marques et al., 2017; Negri et al., 2012). In our study, bone volume assessed by microtomography was 24% lower than the values measured by histomorphometry, which directly measures both mineralized and non-mineralized bone. Conversely, both microCT and HR-pQCT use X-ray attenuation, which quantifies only the mineralized bone matrix. Sharma et al. (Sharma et al., 2018) compared microCT and histomorphometry in patients with ESRD and observed greater BV/TV (25%) in the histological evaluation than in microtomography. Another study also carried out in patients undergoing dialysis found similar BV/TV values in microCT and histomorphometry, but reduced values by tibial HR-pQCT (Benillouche et al., 2020). Lower BV/TV values in our patients were also found in HR-pQCT, mainly in the tibia. The BV/TV measured by the first-generation HR-pQCT used in this study, was calculated from the average bone density within the trabecular volume of interest divided by 1200 mg HA/cm^3 (density of fully mineralized bone)

(Whittier et al., 2020) and is underestimated when compared with the second-generation Xtreme CT, which makes the direct measurement (Manske et al., 2017). Using the regression equation proposed by Manske et al. (2017) to estimate BV/TV in the second-generation HR-pQCT from the value found in the first-generation HR-pQCT, the radius BV/TV is quite similar (18.4%) to that found by microCT (19%), as both methods make direct measurements.

Trabecular thickness is also derived from trabecular bone density and bone volume. Therefore, this value may be underestimated in the first-generation HR-pQCT analysis. As found by Cohen et al. (Cohen et al., 2010), the trabecular thickness seen in microCT is greater than that in histomorphometry and HR-pQCT.

4.1.2. Cortical parameters

In relation to cortical thickness, the values of Ct.Th measured by microCT in our patients were also similar to those described in the literature (Sharma et al., 2018) but our patients had decreased cortical thickness as evaluated by HR-pQCT (Marques et al., 2017; Negri et al., 2012; Manske et al., 2017; Cejka et al., 2011). The HR-pQCT method demonstrated higher values than the microCT method. Studies that compared bone using a first-generation device (Ct.Th derived from cortical volume and outer bone surface) and second-generation device HR-pQCT (direct measurement of Ct.Th) demonstrated that the derived analysis underestimates cortical thickness both in the radius and tibia (Agarwal et al., 2016; Manske et al., 2017).

4.1.3. Trabecular and cortical parameters evaluated in different sites

It is likely there are differences in BV/TV and Ct.Th values between microCT and HR-pQCT since we analyzed different sites. We compared different bones, with unequal ratios of cortical to trabecular bone that support different loads. Nevertheless, a significant correlation was found between Ct.Th and BV/TV, especially between the iliac crest and the radius in our study. A review evaluating the validation of HR-pQCT against microCT reinforces the importance of matching anatomical sites when performing such comparisons (Ohs et al., 2020). Maquer et al. (Maquer et al., 2015) evaluated radius, iliac crest, femur, and spine fragments by microtomography and observed that the iliac crest BV/TV was greater than the radius BV/TV, as seen in our study. Hildebrand et al. (1999) compared five distinct sites by microtomography (iliac crest, lumbar vertebrae L2 and L4, femoral head, and calcaneus) and found significant differences between these sites and individuals. Of note, they observed that in the iliac crest, some patients had a pattern similar to that of the lumbar spine (rod-like), while in other patients, it was similar to that of the femur (plate-like). We did not find studies that evaluated whether clinical conditions, such as CKD, can determine the predominance of a specific pattern (rod × plate). While previous studies compared the various sites using only microtomography, Hiller et al. (2017) performed this analysis using histomorphometry and microCT of the iliac crest, proximal tibia, and lumbar vertebra, wherein they observed variations in BV/TV and no correlation between them. The authors highlighted intra-individual and inter-individual differences.

4.2. Correlations between methods

4.2.1. Correlation between histomorphometry and microCT

In our study, histomorphometry showed a moderate correlation between trabecular bone volume and microtomography of the iliac crest core. Some authors have analyzed different conditions in populations, such as osteoporosis, hypoparathyroidism, and primary hyperparathyroidism, and observed a positive correlation (Cohen et al., 2010; Chappard et al., 2005; Müller et al., 1998). However, authors who compared microCT and histomorphometry among individuals with ESRD or renal osteodystrophy did not find a significant correlation (Benillouche et al., 2020; Tamminen et al., 2011).

A study carried out in pediatric patients with renal osteodystrophy revealed that in those with osteomalacia, the BV/TV observed in histomorphometry was higher than that in microtomography and the osteoid accumulation in histomorphometry was negatively correlated with the trabecular density observed in microCT (Pereira et al., 2016). In our study, there was a high prevalence of mineralization defect (65%). However, we found a significant correlation in Md/TV between histomorphometry and microCT, similar to what was observed in BV/TV.

Regarding cortical thickness, there was a strong correlation between histomorphometry and microCT of iliac crest core, due these methods analyzed the same site. This result was similar that found by Benillouche et al. (2020), while Sharma et al. (2018) did not observe significant correlation between these methods.

4.2.2. Correlation between histomorphometry and HR-pQCT

Similar to our study, other studies compared histomorphometry and HR-pQCT and did not show a correlation in BV/TV between these methods (Marques et al., 2017; Benillouche et al., 2020). It is important to note that although BV/TV can be assessed by these methods, it is analyzed and calculated in different ways. In histomorphometry, it is necessary to extrapolate the two-dimensional measurements to three dimensions and to derive assuming a fixed structural model (plate type), while in HR-pQCT, this assumption about the nature of the trabecular structure (rod or plate) is not necessary Correlation between microCT and HR-pQCT (Cohen et al., 2010). Furthermore, as mentioned above, distinct sites were compared.

Although there was a trend toward a positive correlation between radius and iliac crest core, we did not find correlation in Ct.Th between these methods. Another group that evaluated ESRD patients also showed no correlation in cortical thickness between histomorphometry and tibia HRpQCT (Benillouche et al., 2020), while other authors that observed patients without CKD found weak but significant correlation by these methods (Cohen et al., 2010).

4.2.3. Correlation between microCT and HR-pQCT

In our study, there were significant correlations between trabecular bone volumes assessed by fragment microCT, radius HR-pQCT, and tibia HR-pQCT. Despite the evaluation of different sites, these methods used X-ray attenuation and performed volumetric measurements (3D). Our results were similar those observed by Cohen et al. (2010). A study that evaluated a small number of dialysis patients with fractures found no correlation in trabecular parameters between microtomography and HR-pQCT. However, only the tibia was evaluated (Benillouche et al., 2020).

Similar to other studies, Tb.Th measurement did not appear to have a correlation between the various methods. It was observed only between the radius and tibia HR-pQCT, despite the low resolution (82 μm) for this evaluation. Furthermore, this parameter is derived from BV/TV and Tb.N in first-generation devices, while it is measured directly by microCT and histomorphometry. Tb.Sp had no correlation among the methods, while Tb.N had a moderate correlation between histomorphometry and microCT. Maquer et al. (2015) demonstrated that of all trabecular parameters, BV/TV is the best determinant of trabecular bone stiffness.

Cortical bone is an important determinant of bone strength and quality. When the cortical thickness was evaluated, we found a strong correlation between HR-pQCT of the radius and tibia, similar found by Cohen et al. (2010), as well as microCT of the iliac crest core with the radius. We did not find a correlation of Ct.Th between microCT and HR-pQCT at the tibia, which was similar to the findings in two other studies (Cohen et al., 2010; Benillouche et al., 2020).

4.3. Bone parameters evaluated by turnover classification

4.3.1. Comparison between methods

It is known that density and microarchitecture parameters, both in cortical and trabecular bone, could be influenced by age and gender (van den Bergh et al., 2021; Burghardt et al., 2010). In our study, there was no significant difference in age or gender among turnover groups.

In a large study, Malluche et al. (2011) showed no relationship between mineralization defects, turnover, trabecular bone volume or cortical thickness by histomorphometry in agreement with our findings. Furthermore, similar to the study by Pereira et al. (2016) and unlike what was observed by Malluche et al. (2012), we did not detect a significant difference in BV/TV when three groups of turnovers were compared by histomorphometry.

When we evaluated the structural parameters according to turnover classification using HR-pQCT, we found results comparable to those seen by Salam et al. (2018), in which BV/TV, Tb.Th, and Ct.Th assessed by the HR-pQCT decreased as the turnover increased. In the assessment of Ct.Th by HR-pQCT according to turnover, we observed that cortical thickness decreased as bone turnover increased, similar to what was found by other authors (Negri et al., 2012; Nickolas et al., 2013).

4.3.2. Diagnostic accuracy of imaging for bone turnover

Bone histomorphometry is the gold standard to classify bone turnover. In our study, microCT was unable to discriminate turnover, but HRpQCT identified patients with low and high bone turnover. Several studies (Sprague et al., 2016; Laowalert et al., 2020; Delanaye et al., 2014; Vervloet and Brandenburg, 2017; Chavassieux et al., 2015) evaluated if biomarkers were capable to discriminate turnover, however few groups evaluated the use of HR-pQCT with this objective in patients with CKD. Negri et al. (2012) found significant negative correlations both at the radius and tibia between iPTH and Ct.Th and Ct.BMD, while Nickolas et al. (2013) suggested higher PTH predicted radius thinner cortical thickness. In comparison with histomorphometry, Marques et al. (2017) showed radius Ct.BMD can be used to identify high bone turnover unlike Salam et al. (2018), who found that radius Tt.BMD and Ct.BMD identified low bone turnover. Our study demonstrated that combining tibia BV/TV and Tb.Sp identifying low turnover while Tt.BMD or Ct.BMD identifying high turnover. It was noteworthy that we found better results and more parameters probably because these groups recruited people in research protocol, with narrow range of biochemical parameters and short time of HD vintage. Besides that, the last group included patients with CKD without dialysis, that could present less bone alterations. We selected hemodialysis patients with clinical bone biopsy indication, who could reflect a sample of this population, with large range of HD vintage and mineral and hormonal disorders.

In our study, we were able to observe more alterations in tibia than in radius, which are in variance with other groups (Marques et al., 2017; Negri et al., 2012; Salam et al., 2018; Nickolas et al., 2013). Of note, intermittent PTH is a hormone with anabolic effects in trabecular bone, while it has catabolic effects in cortical bone in continuous and excessive levels, as in hyperparathyroidism (Hong et al., 2019; Rejnmark and Ejlsmark-Svensson, 2020), leading to thinner cortical thickness, increased eroded surface in endocortical (cortical trabecularisation) and intracortical (cortical porosity) (Kulak and Dempster, 2010). One study that compared peripheral and central sites, demonstrated that distal tibia as examined by HR-pQCT was comparable with direct measurements of the proximal femur by QCT, known to be a cortical bone (Liu et al., 2010). As our patients had high level of PTH (including severe secondary hyperparathyroidism) unlike other groups, we found thinner thickness and lower cortical density in tibia.

Bone biomarkers reflect cell activity in a given moment, and can be influenced by renal metabolism and clearance (Vervloet and Brandenburg, 2017). Evaluation of bone images could demonstrate the effect of this alteration in long-term. It is likely that the combination of these data can contribute to the understanding of bone turnover in hemodialysis patients.

4.4. Final considerations

Our study has some limitations. It was performed in a single center, and only a small number of patients were evaluated. Despite this, we were able to find a correlation between trabecular and cortical parameters using several methods. Furthermore, our population is relatively homogeneous, in which all patients are at the same stage of kidney disease and undergoing the same method of dialysis.

In summary, we found a significant correlation between HR-pQCT at the distal radius and microCT of the iliac crest core with respect to trabecular (BV/TV) and cortical (Ct.Th) bones in hemodialysis patients. Bone volume (Cejka et al., 2011; Maquer et al., 2015; Qiu et al., 2006) and cortical thickness (Qiu et al., 2006; Nickolas et al., 2010) are known determinants of bone quantity and strength. Indeed, the reduction in bone volume associated with the reduction in cortical thickness appears to have a synergistic effect on bone fragility (Qiu et al., 2006). Furthermore, there was a significant difference in microstructural parameters between turnover groups using HR-pQCT. This method was also capable to identifying bone turnover. This fact is important because not all patients have access to biopsy, which depends on experts and specialized centers. Hence, these data combined with biomarkers can assess bone turnover in fast and noninvasive way, facilitating clinical practice. The determination of bone turnover allows to decide anti-osteoporotic therapies, when we should start or stop the treatment of hyperparathyroidism, evaluation of fracture risk or vascular calcification in dialysis patients. (Sprague et al., 2016; Ott et al., 2021; London et al., 2015; Barreto et al., 2008).

5. Conclusion

HR-pQCT is noninvasive and fast imaging method that allows the assessment of microstructure parameters that provide bone strength, such as trabecular bone volume and cortical thickness, which are significantly correlated with microCT of iliac crest bone core. Our findings also showed HR-pQCT may be helpful in classifying hemodialysis patients with high *vs.* low bone turnover.

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CRediT authorship contribution statement

Alinie Pichone: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Carlos Perez Gomes:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision. **Luis Felipe Cardoso Lima:** Investigation, Formal analysis, Writing – review & editing. **Carolina Aguiar Moreira:** Investigation, Formal analysis, Writing – review & editing. **Francisco de Paula Paranhos-Neto:** Investigation, Formal analysis, Writing – review & editing. **Miguel Madeira:** Investigation, Formal analysis, Writing – review & editing.

Ricardo Tadeu Lopes: Investigation, Formal analysis, Writing – review & editing. **Maria Lucia Fleiuss Farias:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Maurilo Leite:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

All authors declare no competing interests. We don't have any financial and personal relationships with other people or organizations that could inappropriately influence (bias) the study entitled: "Assessment of trabecular and cortical parameters using high-resolution peripheral quantitative computed tomography, histomorphometry and microCT of iliac crest bone core in hemodialysis patients."

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