Static histomorphometry allows for a diagnosis of bone turnover in renal osteodystrophy in the absence of tetracycline labels

STATIC HISTOMORPHOMETRY ALLOWS FOR A DIAGNOSIS OF BONE TURNOVER IN RENAL OSTEODYSTROPHY IN THE ABSENCE OF TETRACYCLINE LABELS

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Abstract

A bone biopsy with prior tetracycline labeling is the gold standard to diagnose renal osteodystrophy. In cases of missing tetracycline labels, it is still paramount to gain clinically relevant information from the extracted bone sample, by evaluating the static histomorphometry. This study investigates the diagnostic performance of static histomorphometry for the evaluation of high and low bone turnover. Transiliac bone biopsies taken pre- or post- kidney transplantation, of sufficient quality for a full histomorphometric analysis were included (n = 205). The cohort was randomly split to provide separate exploration and validation subsets. Diagnostic performance was evaluated by area under the receiver operator characteristics curve (AUC). All histomorphometric parameters were significantly different across categories of low (24%), normal (60%), and high (16%) bone turnover, and all were significant predictors of both high and low bone turnover (AUC 0.71–0.84). Diagnostic performance was very good for high turnover, as a combination of static parameters resulted in negative and positive predictive values (NPV and PPV) of 80% and 96%, respectively. For low turnover, the combined model resulted in PPV of 71% and NPV of 82%. We conclude that in the absence of tetracycline labels, static histomorphometry provide an acceptable alternative for a diagnosis of bone turnover in renal osteodystrophy.

Key words

Bone histomorphometry, Chronic kidney disease – mineral and bone disorder, Chronic kidney disease, Kidney transplantation

Abbreviations

AUC=Area under the receiver operator characteristics curve, BAr=Bone area, BFR=Bone formation rate, BPm=Bone perimeter, CKD-MBD=Chronic kidney disease – mineral and bone disorder, EPm=Eroded perimeter, NPV=negative predictive value, OAr=Osteoid area, ObPm=Osteoblast perimeter, OcPm=Osteoclast perimeter, OPm=Osteoid perimeter, OWi=Osteoid width, PPV=positive predictive value, TAr=Total tissue area, TC=tetracycline

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1. Introduction

A transiliac bone biopsy with a subsequent quantitative histomorphometric analysis remains the gold standard for diagnosing renal osteodystrophy.[1] By current recommendations, an evaluation of bone turnover, mineralization, and volume is given,[2] followed by a full semi-quantitative report by the bone pathologist. The key parameter for the evaluation of bone turnover is the bone formation rate (BFR), calculated from the incorporation of tetracycline in bone. This tetracycline labeling is achieved by administering two separate courses of oral tetracycline prior to the biopsy procedure.[3] However, tetracycline labeling may fail due to patient non-compliance, gastrointestinal side effects, or very low bone turnover. In such instances, it is still paramount to gain as much clinically relevant information as possible from the extracted bone sample, through an evaluation of static histomorphometry. There is no consensus on how to utilize the static parameters of skeletal remodeling for the diagnosis of low, normal, or high bone turnover, and robust data on the relationship between static and dynamic histomorphometric parameters of bone remodeling is lacking. Previous studies reported on osteoblast and osteoclast surfaces in bone, with various cutoffs proposed,[4–6] none of which have been validated. This study aimed to investigate the diagnostic accuracy of static histomorphometric parameters for high and low bone turnover as diagnosed by a full histomorphometric analysis by an experienced bone pathologist.

2. Material and methods

2.1. Cohort

This is an interim analysis of an ongoing, prospective, observational study on the evolution of mineral- and bone-disorder after kidney transplantation (clinical trial identifier: NCT01886950). Any patient with a successfully performed bone biopsy pre- or post-transplantation was eligible for inclusion. All bone biopsies were performed at Leuven University Hospitals, Leuven, Belgium between Sept 2010 and Dec 2019. Out of 288 available bone biopsies, 37 were excluded to avoid patient duplicates, 23 due to the quality of the bone sample being too poor for histomorphometric analysis, 20 patients due to missing labels, and 3 patients due to anti-resorptive treatment, either prior to, or at the time of, the bone biopsy. The final cohort totaled 205

patients with a full bone histomorphometric analysis. Demographic variables were extracted from electronic patient files.

All clinical and research activities reported here are fully consistent with the Principles of the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. The study adhered to the principles of the Declaration of Helsinki and was approved by the Ethical Committee of KU Leuven (study identifier: S52091). All patients provided written informed consent prior to participation in this study.

2.2. Bone histomorphometry

A trans-iliac bone biopsy was performed in an outpatient setting under local anesthesia and light sedation. The bone core was extracted from a site 2 cm posterior and 2 cm inferior to the anterior iliac spine using an 8G trephine with an internal diameter of 4.5 mm (Biopsybell [Mirandola, Italy]). Double-tetracycline-labeling was performed prior to the procedure by administration of 500 mg oral tetracycline twice daily for 3 days, repeated after a tetracycline-free interval of 11 days; the bone biopsy was scheduled 4 days after the last intake of tetracycline. Patients without visible tetracycline labels (n = 35) were handled as follows: In cases of low bone turnover, a value 1 µm²/mm²/day was set for BFR (n = 13), provided that the patient was noted to have taken tetracycline without irregularities. In cases of diffuse, unmeasurable labels (n = 2) a value of 2500 was used for BFR, set arbitrarily as 20% above the highest measured BFR in the cohort (2071 µm²/mm²/day). The remaining 20 patients with missing TC labels were excluded, as detailed above. Bone histomorphometric analyses were performed at the Laboratory of Pathophysiology of the University of Antwerp, Belgium. Bone cores were fixed in 70% ethanol and embedded in a methylmethacrylate resin. Un-decalcified 5-µm thick sections were stained by the Goldner trichrome method to determine static bone parameters. Unstained 10-µm thick sections in 100% glycerol were examined by fluorescence microscopy for the assessment of tetracycline labels and determination of dynamic parameters. A commercially available image analysis software (AxioVision version 4.51, Zeiss Microscopy, Zeiss, Germany) was utilized, running a custom program. All bone histomorphometric parameters are reported in two dimensions, using standardized nomenclature.[7] Parameters assessed included active osteoblasts per bone and osteoid perimeters (ObPm/BPm, ObPm/OPm, %), active osteoclasts per bone and eroded perimeters (OcPm/BPm, OcPm/EPm,

%), eroded per bone perimeter (EPm/BPm), osteoid per bone area (OAr/BAr) and the presence or absence of fibrosis. Patients were diagnosed as having low, normal, or high bone turnover in a semi-quantitative assessment by an experienced bone pathologist, based primarily on the key dynamic parameter of bone formation rate on total tissue area (BFR), using a previously published normative reference range of 97 – 613 $\mu m^2/mm^2/day$.[8] Patients were categorized as high turnover if BFR > 613, *or* signs of excessive bone resorption (EPm/BPm above normal range of 0.5 – 3.4%), *or* evidence of disordered bone formation (marrow fibrosis > 5 %). Patients were categorized as low turnover if BFR < 97, *and* with limited amounts of osteoid (OAr/BAr, normal range 0.23 – 5.83 %) and without presence of fibrosis.

2.3. Statistical analysis

Descriptive statistics are given as mean±SD if normally distributed, median [IQR] if skewed, or *n* (%) if categorical. Differences in histomorphometric parameters across categories of bone turnover were tested by Kruskal-Wallis equality-of-populations rank test, followed by the Wilcoxon rank-sum test against the "Normal" category. Relationships between dynamic and static parameters were assessed using Spearman's correlation coefficients with Bonferroni-adjusted *p* –values to account for multiple comparisons. Linear and logistic regression models were performed to assess multivariable associations between BFR or turnover category as outcome, and static histomorphometric parameters as explanatory variables. Diagnostic performance was evaluated by receiver operator characteristics (ROC) curve statistics.[9] The cohort was randomly split into two subset, an exploration cohort (n = 105) and a validation cohort (n = 100), with estimation of optimal cutoffs in the exploration cohort, after which diagnostic accuracy of these cutoffs was evaluated in the validation cohort. Prevalence of disease was set as the total cohort prevalence for calculation of test predictive values. Key parameters included area under the ROC curve (AUC), sensitivity and specificity, negative and positive predictive values (NPV and PPV). AUC values were considered poor if < 0.6, fair if 0.6 – 0.7, good if 0.7 – 0.8, very good if 0.8 – 0.9, and excellent if > 0.90. Statistical significance was set at a two-sided *p* value < 0.05. All analyses were performed using STATA IC version 16.1 (StataCorp LP, College Station, TX, USA)

3. Results

3.1. Demography

Mean age was 56±13 years, 67% were men, 22% had diabetes mellitus, and 10% had undergone a parathyroidectomy. The cause of chronic kidney disease was cystic or hereditary disease (26%), glomerulonephritis or vasculitis (25%), diabetic nephropathy (7.5%), hypertension or atherosclerosis (8.5%), chronic interstitial nephritis (7.5%), other (3.5%), or unknown (22%). Eighty-five patients (41%) were biopsied pre-transplant, and 120 were biopsied post-transplant, the majority of which (85%) were performed at 12 months after kidney transplantation. Immunosuppression was achieved with tacrolimus, in combination with mycophenolate mofetil and oral corticosteroids. At the time of biopsy, 106 patients received oral corticosteroids, at a daily dose of ≤ 5 mg for all but three patients. Eight patients received calcimimetics at the time of the bone biopsy.

3.2. Histomorphometry

Bone histomorphometric parameters by category of bone turnover are given in **Table** 1.

Table 1 Bone histomorphometric variables of remodeling across categories of bone turnover

	Low (n = 49)	Normal (n = 123)	High (n = 33)	p
Bone formation rate, µm²/mm²/day	31 (1; 52) †	209 (137; 379)	830 (606; 1176) †	<0.001
Adjusted apposition rate, µm/day	0.1 (0.0; 0.3) †	0.3 (0.2; 0.6)	0.6 (0.4; 1.3) †	<0.001
Mineralisation lag time, days	80 (24; 139) †	27 (14; 46)	15 (9; 27)	<0.001
Mineral apposition rate, µm/day	0.6 (0.5; 0.9) †	0.8 (0.7; 1.0)	1.1 (0.9; 1.4) †	<0.001
Osteoid/Bone area, %	0.9 (0.4; 2.0) †	2.8 (1.3; 5.5)	5.4 (3.4; 10.4) †	<0.001
Osteoid/Bone perimeter, %	9.3 (4.6; 19.9) †	22.4 (12.1; 39.1)	43.4 (23.3; 56.3) †	<0.001
Osteoid Width, µm	5.5 (4.8; 6.5) †	8.3 (6.4; 10.5)	10.7 (8.0; 13.6) †	<0.001
Fibrosis, any, n(%)	1 (2.0)	12 (9.8)	25 (75.8)	<0.001
Fibrosis >5%, n (%)	0 (0.0)	3 (2.4)	15 (45.5)	<0.001
Eroded/Bone perimeter, %	1.3 (0.6; 2.6) †	3.3 (1.3; 6.0)	6.9 (4.7; 11.1) †	<0.001
Osteoblast/Bone perimeter, %	0.6 (0.0; 1.7) †	3.0 (0.5; 10.2)	12.2 (5.9; 27.3) †	<0.001
Osteoblast/Osteoid perimeter, %	7.0 (0.0; 18.1) †	16.7 (0.0; 33.4)	33.3 (23.9; 54.7) †	<0.001
Osteoclast/Bone perimeter, %	0.0 (0.0; 0.7) †	0.5 (0.0; 1.5)	2.0 (1.1; 3.0) †	<0.001
Osteoclast/Eroded perimeter, %	0.0 (0.0; 32.1)	18.3 (0.0; 30.7)	28.8 (16.9; 34.4) †	0.005

Data are median (IQR) with p by the Kruskal-Wallis equality-of-populations rank test, † marks $p < 0.05$ compared to the "Normal" category

Both dynamic and static parameters of remodeling were significantly different across categories of low,

normal, and high bone turnover. Separation was particularly good for high turnover, with a more considerable overlap between the categories of normal and low bone turnover.

3.3.Diagnostic accuracy

AUC values for the prediction of bone turnover by static parameters of bone histomorphometry are shown in **Figure** 1. All static parameters were significant predictors of both high and low bone turnover, with AUCs in the range of 0.70 – 0.85, corresponding to a "good to very good" discriminatory ability. The most informative variables for high turnover were cell counts (ObPm/BPm and OcPm/BPm), EPm/BPm, and the presence of fibrosis, while osteoid indices achieved the highest AUC values for low turnover (**Figure** 2).

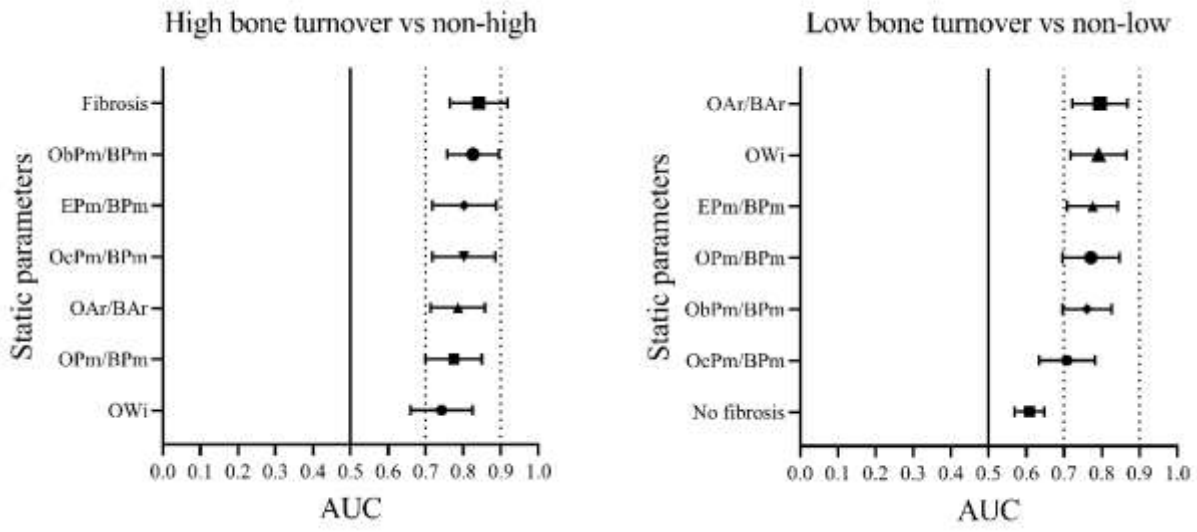

Figure 1 Area under the receiver operator characteristics curve of static histomorphometric parameters for a diagnosis of high or low bone turnover in the overall cohort (n = 205)

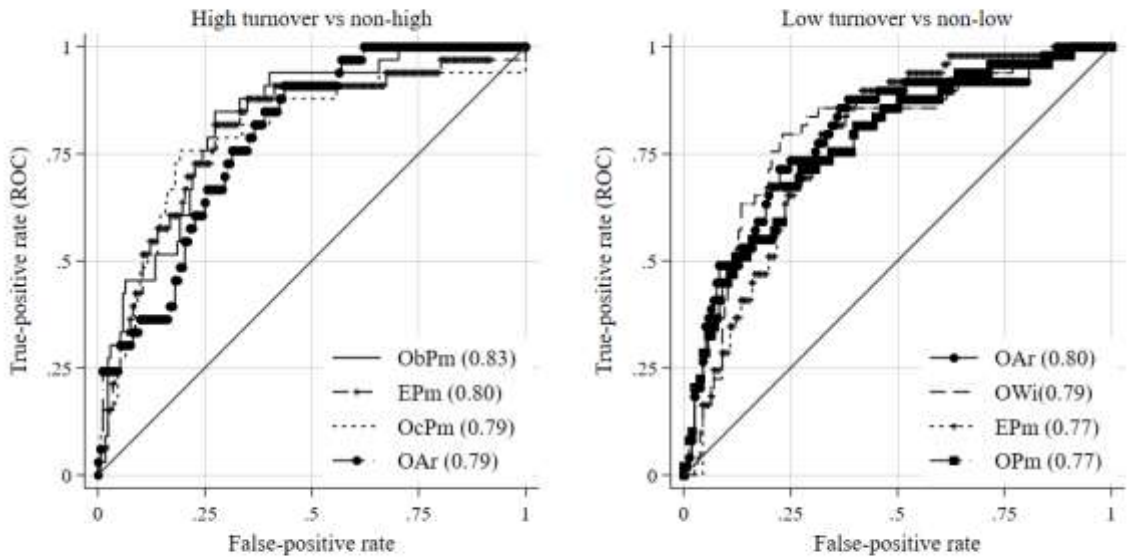

Figure 2 Receiver operator characteristics (ROC) curve for the most informative static histomorphometric parameters of high turnover and low turnover

The cohort was randomly split to provide exploration and validation subsets. Comorbidities, medications, and BFR categories were well balanced between the two groups (Suppl **Table** 1). **Table** 2 lists the calculated optimal cutoffs in the exploration cohort (n = 105), and **Table** 3 shows the diagnostic performance of these as evaluated in the validation cohort (n = 100).

Table 2 Diagnostic cutoffs of static histomorphometric parameters for a diagnosis of high and low bone turnover

	Exploration cohort (n = 105)		Validation cohort (n = 100)	
High turnover	**AUC**	**Cutoff**	**Sensitivity**	**Specificity**
Fibrosis, any	0.81 (0.70, 0.92)	Yes	92%	90%
OcPm/BPm, %	0.81 (0.69, 0.92)	>1.46	75%	76%
EPm/BPm, %	0.80 (0.70, 0.91)	>4.51	83%	69%
ObPm/BPm, %	0.79 (0.69, 0.89)	>5.37	100%	73%
OAr/BAr, %	0.77 (0.67, 0.87)	>2.44	92%	57%

OPm/BPm, %	0.77 (0.67, 0.86)	>22.33	83%	53%
OWi, µm	0.73 (0.61, 0.85)	>10.41	50%	76%
Low turnover	**AUC**	**Cutoff**	**Sensitivity**	**Specificity**
OWi, µm	0.87 (0.79, 0.95)	<5.81	45%	87%
OAr/BAr, %	0.86 (0.77, 0.95)	<1.63	59%	79%
OPm/BPm, %	0.81 (0.70, 0.92)	<8.10	31%	90%
EPm/BPm, %	0.76 (0.66, 0.86)	<2.68	83%	68%
ObPm/BPm, %	0.74 (0,63, 0,84)	<1.88	72%	72%
OcPm/BPm, %	0.63 (0.52, 0.74)	<0.89	86%	49%
Fibrosis, none	0.61 (0.56. 0.65)	No	97%	27%

Area under the receiver operator characteristics curve (AUC), with calculated cutoffs by Liu's method, and corresponding sensitivity and specificity

Combinations of the three parameters with the highest AUC values were tested, in addition to a pre-defined model of fibrosis, ObPm/BPm, OcPm/BPm, and OAr/BAr, to capture the different aspects of skeletal remodeling. Diagnostic performance was very good for high turnover bone disease, as the predefined combination provided a correct diagnosis in 94% of patients, with a NPV of 96% and PPV of 80%. Results were more modest for low turnover bone disease, with a correct diagnosis in 80% of cases by the predefined model, and a NPV of 82% and PPV of 71%.

Table 3 Diagnostic accuracy of combined static variables of histomorphometry for the diagnosis of high or low bone turnover

	Validation cohort (n = 100)			
High turnover	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Fibrosis, OcPm/BPm	67%	94%	62%	95%
Fibrosis, OcPm/BPm, Epm/BPm	67%	94%	62%	95%
Fibrosis, ObPm/BPm, OcPm/BPm, OAr/BAr	67%	98%	80%	96%
Low turnover	**Sensitivity**	**Specificity**	**PPV**	**NPV**
OWi, OAr/BAr	34%	94%	71%	78%
OWi, OAr/BAr, Opm/BPm	24%	97%	78%	76%
Fibrosis, ObPm/BPm, OcPm/BPm, OAr/BAr	52%	92%	71%	82%

Sensitivity, specificity, positive and negative predictive values (PPV, NPV), and overall accuracy using diagnostic cutoffs as shown in Table 2

Abbr.: BAr=Bone area, BPm=Bone perimeter, EPm=Eroded perimeter, OAr=Osteoid area, ObPm=Osteoblast perimeter, OcPm=Osteoclast perimeter, OPm=Osteoid perimeter, OWi=Osteoid width

Further, we investigated the prediction of bone turnover by static histormorphometry through multivariable logistic regression. We performed a stepwise selection of variables for high and low bone turnover in the exploration cohort, and investigated the performance of these models in the validation cohort. The presence of fibrosis and ObPm/BPm were selected as predictors of high turnover, while OWi and ObPm/BPm were selected for low turnover. The logistic prediction model improved sensitivity and accuracy for low turnover, while no added benefit was seen for the diagnosis of high turnover (Supplementary **Table** 1).

The overall contribution of static histomorphometric parameters to the diagnosis of high and low bone turnover was investigated through an all-in multivariable logistic regression model. By including the full set of static parameters, a pseudo R^2 of 48% was achieved for the prediction of high turnover, and a pseudo R^2 of 41% for low turnover.

3.4.Bone formation rate

As a secondary analysis, we examined whether the static parameters could predict bone turnover as categorized by BFR alone, using cutoffs based on a normal range of 97 – 613 μm²/mm²/day. BFR was below, within, and above the normal range in 66 (32%), 110 (54%), and 29 (14%) patients, respectively. The agreement between the two diagnostic approaches (full histomorphometric analysis *vs.* BFR alone) was 83% (kappa 0.705, $p < 0.001$), corresponding to substantial agreement. The main disagreement for high turnover was re-classification of patients with marrow fibrosis ($n = 5$) or high bone resorption ($n = 4$) as high turnover despite a BFR < 613. For low turnover, disagreement was seen for 19 patients with a BFR < 97, which were categorized as normal by the bone pathologist based on normal static parameters of osteoid and resorption.

The relationship between static histomorphometry and BFR was examined using univariate correlation analyses. Spearman's *rho* with *p*-values (adjusted for multiple comparisons) were: ObPm/BPm (*rho* = 0.555, $p < 0.001$), ObPm/OPm (*rho* = 0.422, $p < 0.001$), OcPm/BPm (*rho* = 0.382, $p < 0.001$), OcPm/EPm (*rho* = 0.202, $p = 0.13$), EPm/BPm (*rho* = 0.413, $p < 0.001$), OAr/BAr (*rho* = 0.567, $p < 0.001$), OPm/BPm (*rho* = 0.557, $p < 0.001$), and OWi (*rho* = 0.434, $p < 0.001$) (**Figure** 3).

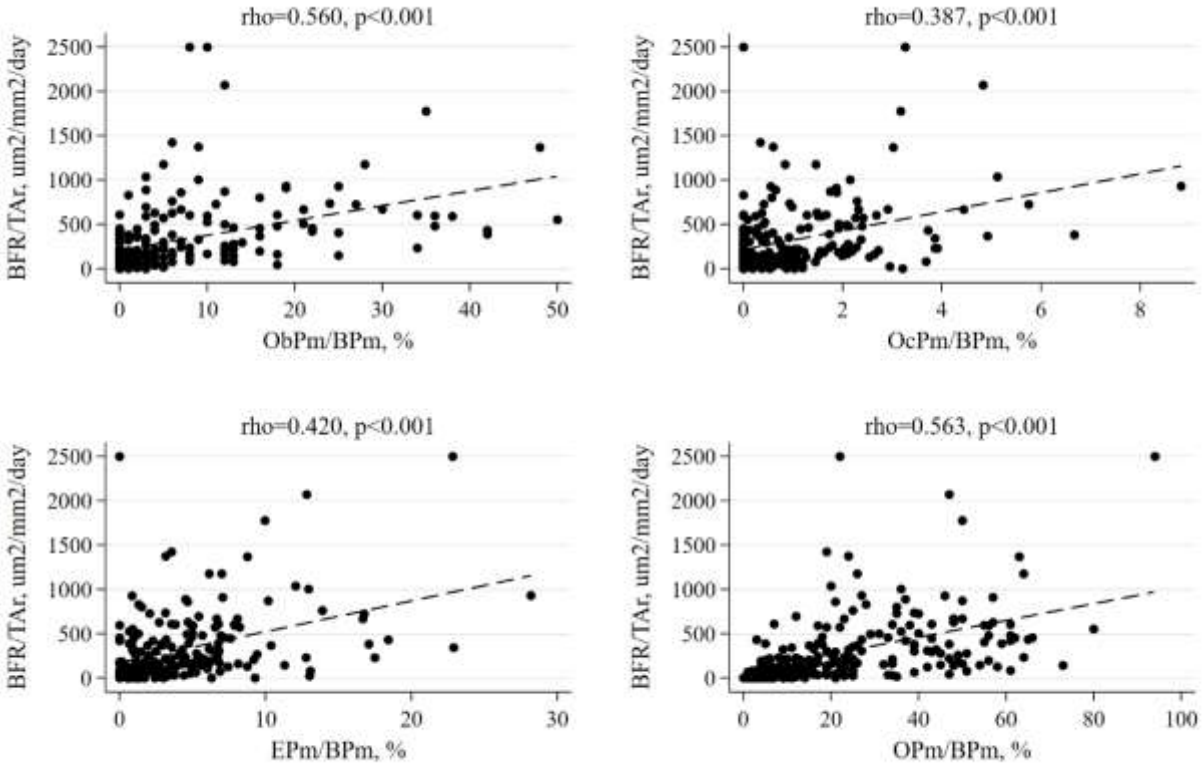

Figure 3 Scatterplots between bone formation rate (BFR/TAr) and static histomorphometric parameters; osteoblasts on bone perimeter (ObPm/BPm), osteoclasts on bone perimeter (OcPm/BPm), eroded per bone perimeter (EPm/BPm), and osteoid per bone perimeter (OPm/BPm)

The diagnostic performance of static histomorphometric parameters for predicting BFR categories are shown in **Table** 4. Overall, static parameters were somewhat less accurate in predicting categories of BFR compared to bone turnover by the full histomorphometric analysis. AUC values were in the range of 0.70 – 0.76 for a high BFR, and 0.70 – 0.83 for low BFR.

Table 4 Diagnostic accuracy of static variables of histomorphometry for the diagnosis of high or low bone formation rate

	Exploration cohort (*n* = 105*)*		Validation cohort (*n* = 100)	
BFR/TAr>613	AUC	Cutoff	Sensitivity	Specificity
OcPm/BPm, %	0.76 (0.63. 0.89)	>1.46	58%	74%
ObPm/BPm, %	0.75 (0.65, 0.85)	>2.97	100%	60%
OWi, µm	0.75 (0.63, 0.87)	>10.41	50%	76%
EPm/BPm, %	0.74 (0.61, 0.88)	>3.14	75%	52%
OAr/BAr, %	0.73 (0.63, 0.83)	>2.44	92%	57%

OPm/BPm, %	0.72 (0.62, 0.83)	>22.33	92%	55%
Fibrosis	0.71 (0.59, 0.84)	Yes	75%	88%
BFR/TAr<97	AUC	Cutoff	Sensitivity	Specificity
OAr/BAr, %	0.83 (0.74, 0.92)	<1.69	56%	80%
OPm/BPm, %	0.82 (0.72, 0.91)	<16.23	62%	73%
ObPm/BPm, %	0.75 (0.65, 0.84)	<2.37	76%	68%
EPm/BPm, %	0.75 (0.66. 0.85)	<3.07	79%	67%
OWi, µm	0.73 (0.63, 0.84)	<6.46	56%	77%
OcPm/BPm, %	0.70 (0.60, 0.80)	<0.46	68%	62%
Fibrosis	0.62 (0.57, 0.67)	No	91%	30%

Data are area under the receiver operator characteristics curve (AUC) with optimal cutoffs by Liu's method, and corresponding sensitivity and specificity

Abbr.: BAr=Bone area, BFR=bone formation rate, BPm=Bone perimeter, EPm=Eroded perimeter, OAr=Osteoid area, ObPm=Osteoblast perimeter, OcPm=Osteoclast perimeter, OPm=Osteoid perimeter, OWi=Osteoid width, TAr=Total tissue area

As in our primary analyses, we next determined the diagnostic performance of combinations of parameters for the prediction of high or low BFR. Results were comparable to the prediction of a full histomorphometric analysis, but with lower positive predictive values, particularly for high turnover (Table 5).

Table 5 Diagnostic accuracy of combined static variables of histomorphometry for the diagnosis of high or low bone formation rate

	Validation cohort (n = 100)			
High turnover, BFR/TAr>613	**Sensitivity**	**Specificity**	**PPV**	**NPV**
OcPm/BPm, ObPm/BPm	58%	82%	30%	94%
OcPm/BPm, ObPm/BPm, OWi	33%	97%	57%	91%
Fibrosis, ObPm/BPm, OcPm/BPm, OAr/BAr	50%	95%	60%	93%
Low turnover, BFR/TAr <97	**Sensitivity**	**Specificity**	**PPV**	**NPV**
OAr/BAr, OPm/BPm	50%	80%	57%	76%
OAr/BAr, OPm/BPm, ObPm/BPm	47%	85%	62%	76%
Fibrosis, ObPm/BPm, OcPm/BPm, OAr/BAr	44%	91%	71%	76%

Sensitivity, specificity, positive and negative predictive values (PPV, NPV), using diagnostic cutoffs as shown in Table 2

Abbr.: BAr=Bone area, BFR=bone formation rate, BPm=Bone perimeter, EPm=Eroded perimeter, OAr=Osteoid area, ObPm=Osteoblast perimeter, OcPm=Osteoclast perimeter, OPm=Osteoid perimeter, OWi=Osteoid width, TAr=Total tissue area

Finally, to assess how much of the information presented by the BFR could be captured by static histomorphometry, we performed a stepwise multivariable linear regression model for the prediction of BFR,

with a backwards selection of static parameters as explanatory variables. ObPm/BPm (β 13.61, $p < 0.001$), EPm/BPm (β 27.99, $p < 0.001$), and OWi (β 22.91, $p < 0.001$) were selected as independent predictors, and the model achieved an adjusted R^2 of 47% ($p < 0.001$).

4. Discussion

4.1.Main findings

The main finding of this study is that static histomorphometric parameters of bone remodeling have acceptable diagnostic accuracy for high and low bone turnover. In the absence of successful tetracycline labeling, static histomorphometry provide a suitable alternative for the categorization of bone turnover in renal osteodystrophy.

All static histomorphometric parameters were significantly different across categories of low, normal, and high bone turnover, although overlap was noticeable between categories of low and normal turnover. AUC values of static parameters were in the range of 0.70 – 0.85 for both high and low bone turnover. The diagnostic accuracy was very good for high bone turnover, as a combination of variables achieved a PPV of 80% and a NPV of 96%. Results were more moderate for low turnover, with PPV and NPV both in the range of 70 to 80% using a combination of static histomorphometric variables.

A recently published study similarly investigated the role of static bone histomorphometry for the diagnosis of renal osteodystrophy. Salam *et al*[10] reported on the diagnostic performance of osteoblast, osteoclast, and eroded surfaces, and concluded that these variables were not useful in predicting bone turnover. While the limited number of bone biopsies included ($n = 43$) may have reduced their statistical power, methodological differences between our two studies should also be considered. Salam *et al* categorized patients by BFR alone, while in we used a full histomorphometric analysis as the diagnostic standard in our primary analyses. We chose this approach to mimic the real-life situation of having a bone biopsy without tetracycline labels, and still wanting to arrive at a diagnosis of high or low turnover. Thus, it was important for us to keep the diagnostic standard used by our center as the outcome of our analyses. However, as the semi-quantitative evaluation of bone histomorphometry include consideration of the static parameters, this approach would be expected to positively inflate the diagnostic performance. We therefore added, as a secondary analysis, the

diagnostic accuracy for categories of BFR, using the normative range utilized by our lab.[8] This analysis resulted in lower diagnostic performance, which were more in line with what was reported by Salam *et al*. However, with NPV and PPV in the range of 70 to 80%, the diagnostic performance could still be considered acceptable. Secondly, while Salam *et al*[10] and others[5,6,11] limited their analyses to two or three static histomorphometric parameters, our results indicate that combinations reflecting different aspects of skeletal remodeling may yield better diagnostic performance, whether the diagnosis of bone turnover is determined by the full histomorphometric analysis or BFR alone. Finally, differences in diagnostic cutoffs between laboratories performing histomorphometry should be mentioned. Several normative references are currently in use, and the most suitable reference range for the evaluation of renal osteodystrophy has not yet been established.

Cutoffs of ±1 SD from a normal mean has been suggested as suitable reference range for bone histomorphometry.[12,13] Using recently published normative data from healthy US Caucasian men,[14] this definition would result in the following normal ranges for static histomorphometry: ObPm/BPm 0.86 – 5.66%, OcPm/BPm 0.10 – 0.58%, EPm/BPm 0.53 – 1.79%, OPm/BPm 5.65 – 22.35%, and OAr/BAr 0.49 – 2.83%. We note that our calculated cutoffs for high turnover of ObPm/BPm (5.37%), OPm/BPm (22.33%), and OAr/BAr (2.44%) are very close to the upper normal value by this definition. For the resorptive parameters, OcPm/BPm (1.46%) and EPm/BPm (4.51%), our cutoffs for high turnover were noticeably higher than the proposed range. Calculated cutoffs for low turnover were uniformly above the lower normal values, and in the case of the resorptive parameters, even above the normal range (OcPm/BPm <1.88% and EPm/BPm <2.68%). It is presently unclear whether these discrepancies may be due to differences in age, gender, and ethnic distributions between cohorts, or inherent properties of renal osteodystrophy.

We found moderate, highly significant correlations (*rho* ~0.40 – 0.50) between BFR and all static histomorphometric parameters, with the exception of OcPm/EPm. The full set of static histomorphometric variables explained about 50% of the variation in BFR as estimated by the all-in multivariable linear regression model. These analyses demonstrate that dynamic histomorphometry does provide information that is not readily captured by static parameters. Dynamic and static histomorphometry express different aspects

of skeletal remodeling, and as each have their inherent limitations, they should be considered complimentary. While the use of tetracycline labeling for the calculation of dynamic parameters may be considered optimal for the evaluation of renal osteodystrophy, our analysis indicates that in the absence of labels, static histomorphometry may provide clinically relevant information on the state of skeletal remodeling.

4.2. Strengths and limitations

The main strength of this study is the large number of bone biopsies available for analyses, allowing a split of the cohort with internal validation of the diagnostic performance in a separate subset of patients. However, optimism bias may still be present, as no external validation was performed. Our choice of diagnostic standard may be challenged. We primarily considered the full histomorphometric analysis by the bone pathologist, while others use BFR alone for the evaluation of bone turnover. Both approaches have inherent limitations; as already detailed, static parameters form a part of the full histomorphometric analysis. The BFR, on the other hand, is an expression of bone formation, and not necessarily bone resorption, which poses a challenge in cases of excessive resorption over formation.[2] Further, careful adherence to the tetracycline regime prior to performance of the bone biopsy is necessary for a reliable result. Another limitation in our diagnostic approach is the lack of a second, independent assessment of the histomorphometry. The presence of a mineralization defect, defined as slow bone mineralization in combination with the accumulation of osteoid,[15] would change the relationship between osteoid parameters and bone turnover. We did not exclude patients based on delayed mineralization from our analyses, as only a single patient had borderline osteomalacia in this cohort, in line with current reports of a very low prevalence of severe mineralization defects in late-stage CKD.[15] Lastly, as already discussed, substantial differences exist between labs performing bone histomorphometry with regards to methodology applied and normative reference range utilized, which hampers between-study comparisons; a consensus on these issues is urgently needed in order to synthesize published data and move the field forward.[16]

4.3. Clinical application

A full histomorphometric analysis of a bone biopsy with prior tetracycline labeling remains the gold standard for the evaluation of renal osteodystrophy. Considering the invasive nature of this procedure, it is vital to achieve as much clinically relevant information as possible once the biopsy has been performed. In the

absence of successful tetracycline labeling, we propose that the diagnostic cutoffs of static histomorphometry given in this study provide an acceptable alternative for the evaluation of bone turnover. High and low bone turnover can be ruled out with high certainty by static histomorphometry, which can be used to aid clinical decisions such as whether or not to intensify medical treatment for high bone turnover, or initiate anti-resorptive treatment without fear of exacerbating a low bone turnover state.

5. Conflicts of interest

LV received speaker fee from Amgen. BB reports grants from Otsuka Pharmaceutical, other from Otsuka, other from Baxter. BM reports personal fees from Baxter, grants and personal fees from Nipro, personal fees from Astrazeneca. PE reports personal fees from Amgen and Vifor-FMC. MN and BS are senior clinical investigators of The Research Foundation Flanders (1844019N and 1842919N, respectively). Remaining authors report no conflicts of interest.

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