



Quantitative histomorphometric analysis of halved iliac crest bone biopsies yield comparable ROD diagnosis as full 7.5mm wide samples

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Full title: Quantitative Histomorphometric analysis of halved iliac crest bone biopsies yield comparable
ROD diagnosis as full 7.5mm wide samples

Running head: Small bone biopsies for the diagnosis of ROD.

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Abstract

Background and objectives: Histomorphometric analysis of a transiliac bone biopsy is the gold standard for the diagnosis of renal osteodystrophy (ROD). This procedure is costly, invasive and usually performed with a trephine with an internal diameter of 7.5mm. Our objective was to evaluate the accuracy of ROD diagnosis on halved histological bone sections to determine if they are comparable to the standard 7.5mm samples.

Design: We included 68 bone biopsies performed in CKD patients for diagnostic purposes with a 7.5mm diameter trephine. Quantitative histomorphometric analysis of the whole bone samples was performed including assessment of bone mineralization, turnover and volume. Each histological section (representing the whole 7.5mm diameter biopsy) was then divided lengthwise in two hemi-sections (representing the 3.5mm diameter biopsy). Histomorphometric analysis was repeated this time on the two hemi-biopsies for each sample, blinded from initial results. Diagnoses were classified as osteitis fibrosa, adynamic bone disease, mixed uremic bone disease, osteomalacia or other. Correlations between the whole sample and the hemi-biopsies for each parameter were studied. Concordance between the various bone parameters and final ROD diagnosis obtained from the whole section versus the two hemi sections was evaluated.

Results: Highly significant correlations were found between parameters measured on the whole section and the corresponding hemi-sections, with r coefficient of 0.98 for osteoid surface and thickness and bone formation rate, 0.97 for osteoclast surface, and 0.96 for bone volume ($p < 0.001$). Final diagnosis was in full accordance between the whole biopsy and the two corresponding hemi-biopsies in 91% of cases.

Conclusions: Accurate diagnosis of ROD type was obtained by evaluation of bone surface areas of 3 mm diameter. These data suggest that small invasive bone biopsies might provide accurate ROD diagnostics while decreasing both invasiveness and cost of the procedure.

Introduction

Chronic Kidney Disease (CKD) induces early changes in phosphate and calcium metabolism, becoming a concern for the majority of patients by the time they reach end-stage renal disease (ESRD). The clinical consequences of these perturbations in mineral metabolism include increased bone fragility with a high risk of fractures that are associated with increased mortality among CKD patients, whether requiring hemodialysis¹ or not². Moreover, CKD patients are considered at high cardiovascular risk due to extensive vascular calcifications that are a consequence of anomalies in calcium and phosphorus metabolism³ and bone turnover⁴.

The systemic disease related to bone and calcium and phosphate metabolism in kidney failure is called Chronic Kidney Disease - Mineral and Bone Disorders (CKD-MBD), as defined by the Kidney Disease Improving Global Outcome (KDIGO) consensus in 2006⁵. The term renal osteodystrophy (ROD) is now used specifically for the histological bone changes observed in CKD-MBD, including alterations of bone turnover (T), mineralization (M) and volume (V).

Assessment of bone disease is therefore critical for both the treatment of CKD-MBD and the mitigation of vascular calcification risk. To date the gold standard for the diagnosis of ROD is the histomorphometric analysis of a tetracycline double labeled anterior iliac crest biopsy⁶. Unfortunately, bone biopsy is not commonly obtained in clinical practice as it is considered an invasive procedure due to the 7.5mm internal diameter Bordier/Meunier trephine that is thought to be required to obtain adequate bone tissue sample. Moreover, it is costly, as the trephine is not disposable, and some patients may require a post-biopsy inpatient hospital stay. As a consequence, current guidelines

recommend non-invasive assessment of bone turnover with bone biomarkers such as serum parathyroid hormone (PTH) and bone specific alkaline phosphatase (BAP)⁷⁻⁸. However, the overall diagnostic performance of these surrogate markers to evaluate bone turnover at the individual level is disappointing⁷⁻⁹. Moreover, neither PTH nor BAP provide information on bone volume and mineralization status.

Recently, an alternative approach for bone biopsy analysis has been favored by clinicians. In 2017, an analysis of the bone biopsy practice patterns across Europe showed that 40% of the procedures were performed with smaller, disposable 3mm inner diameter Jamshidi-type trephines, instead of the classical Meunier/Bordier trephines¹⁰ (Figure 1).

Figure 1: Modified Meunier/Bordier trephine (left) with an internal diameter of 7.5mm and a disposable Jamshidi trephine (right) with an internal diameter of 3mm.



Moreover, recent studies involving bone histomorphometry used data provided by such smaller needles¹¹⁻¹². The main concern however is that a smaller diameter tissue core might not provide enough trabecular tissue to achieve acceptable diagnostic accuracy.

In a move to determine the accuracy of a smaller iliac crest bone biopsy, 3mm in width, we evaluated the accuracy of ROD diagnosis on halved histological bone sections obtained from a 7.5mm wide trephine to determine if these smaller samples are comparable in their diagnosis of the standard 7.5mm samples

Materials and Methods

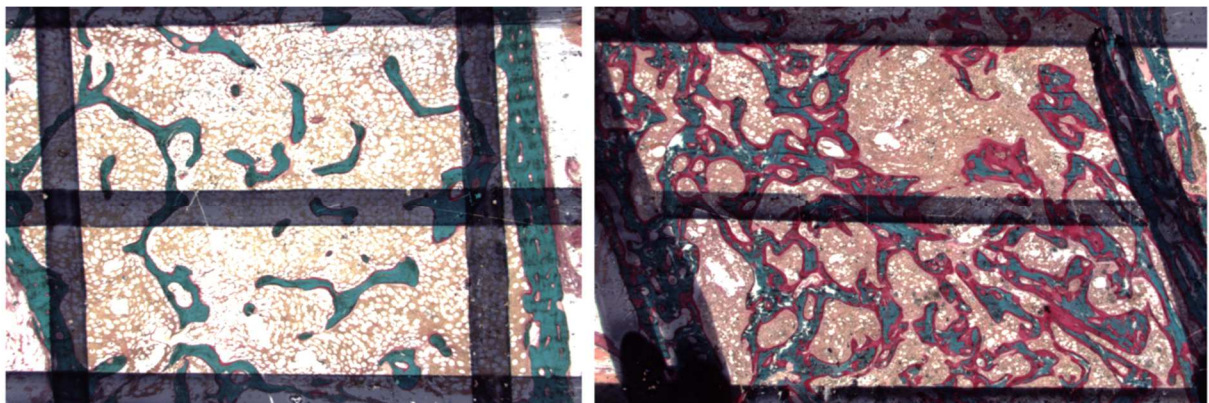
Study Population

We analyzed 68 iliac crest bone biopsies that had been performed in ESRD patients for ROD diagnostic purposes. All biopsies were performed with a Bordier/Meunier trephine with an internal diameter of 7.5mm. Bone samples were collected from the registries of Saint-Etienne or Antwerp Universities. Biopsies were not included for further analysis when the sample was either fractured or incomplete. Additional histological sections were cut from the initial MMA blocks if the available histological sections were poorly preserved.

Sample division in two 3mm-wide hemi-biopsies

Each histological mounted section was longitudinally divided with the help of a binocular magnifier in two equal portions by drawing a straight 250 μ m-wide line (in width) crossing the middle of the internal and external cortices, using an permanent felt pen. The external edges of the histological sections (250-300 μ m each) were also excluded from the region of interest for the measurements, as they usually contain dilacerated bone tissue that is not suitable for quantitative histomorphometric analysis. The histological sections were then oriented to define two hemi-biopsies arbitrarily called hemi-biopsy 1 and 2. Then, each hemi- biopsy was visually consistently identified as 1 or 2 from one section to the other, according to trabecular shape and orientation. This allowed us to non-invasively simulate tissue samples we would have obtained if the biopsy had been performed with a smaller needle with an internal diameter of 3mm. (Figures 2).

Figure 2: Representative histological sections stained with Goldner's trichrome of two 7.5mm transiliac bone biopsies, divided in two hemi biopsies 1 and 2 (Mild Osteitis Fibrosa, left; Osteomalacia, right, 125 X)



Bone histomorphometry

The parameters of bone structure were measured with an automatic image analyzer (Bone; Explora Nova, La Rochelle, France). The static as well as the dynamic parameters reflecting bone turnover and mineralization status were measured using a semiautomatic image analyzer (Tablet' Measure; Explora Nova). Osteoid parameters (OS/BS, O.Th and OV/BV) were measured on sections stained with Goldner Trichrome. Osteoclast parameters were measured on TRAP stained sections with Aniline blue counter staining. Histodynamic parameters were measured on unstained sections by analyzing fluorescent tetracycline labels under UV light.

Three sets of parameters were thus measured for each bone sample; i.e. on the whole specimen and on the two hemi-biopsies. All histomorphometric analyses are reported according to the 2013 standard international nomenclature¹³. These successive analyses were carried out by an operator single-blinded for the results obtained from one set of measurements to another.

Diagnosis of Renal Osteodystrophy

For each bone biopsy, the diagnosis of renal osteodystrophy was assessed thrice using the Turnover Mineralization Volume (TMV) classification¹³: on the whole specimen, and the arbitrarily defined hemi-biopsies 1 and 2.

Diagnoses were classified as previously reported¹⁴: *Osteitis Fibrosa* (OF), *Adynamic Bone Disease* (ABD), *Osteomalacia* (OM), and *Mixed Uremic Osteodystrophy* (MUO). Bone Surface referent Bone Formation Rate (BFR/BS, $\mu\text{m}^3/\mu\text{m}^2/\text{day}$) was calculated as

[Mineralizing Surface per Bone Surface, MS/BS, %] x Mineral Apposition Rate (MAR, $\mu\text{m}/\text{d}$). Mineralization Lag Time (MLT, days) was calculated as Osteoid Thickness, (O.Th, μm) divided by Adjusted Apposition Rate ($\text{Aj.AR} = \text{MAR} \times \text{MS/OS}$, $\mu\text{m}/\text{day}$), where OS represents Osteoid Surface. The diagnosis criteria for Osteitis Fibrosa were: $\text{BFR/BS} > 0.083 \mu\text{m}^3/\mu\text{m}^2/\text{day}$, Osteoclastic Surface, $\text{Oc.S/BS} > 1\%$, presence of woven bone and no signs of mineralization defect; for Adynamic Bone Disease: $\text{BFR/BS} < 0.031 \mu\text{m}^3/\mu\text{m}^2/\text{day}$, Osteoid thickness $< 11 \mu\text{m}$; for Osteomalacia: $\text{MLT} > 100$ days, $\text{O.Th} > 11 \mu\text{m}$, $\text{OV/BV} > 10\%$ and no signs of increased resorption or woven bone¹⁵; and for Mixed Uremic Osteodystrophy: $\text{BFR/BS} < 0.031 \mu\text{m}^3/\mu\text{m}^2/\text{day}$, $\text{O.Th} > 10 \mu\text{m}$, $\text{MLT} > 50$ days and $\text{Oc.S/BS} > 1\%$. The reference values are those of Garcia-Carrasco *et al*¹⁶ for the bone volume and static bone formation parameters, Palle *et al* for resorption and osteoclast parameters¹⁷ and those of Vedi *et al.* for the dynamic parameters¹⁸. The diagnosis was classified as “*Other*” when the measurements did not fit within the criteria for any of the diagnoses mentioned.

Statistical Analysis

Spearman rank correlation tests were performed between the measurements on the whole sample and each hemi-biopsy, as well as between the two adjacent hemi-biopsies.

Diagnosis concordance between whole samples, and the corresponding hemi-biopsies 1 and 2 was checked. We considered diagnoses to be fully concordant only when in full accordance on the whole sample, on hemi-biopsy 1 and hemi-biopsy 2.

A p value <0.05 was considered statistically significant. The statistical software *GraphPad Prism* version 5.0.3 was used for statistical analyses.

Results

Study population

Sixty-eight bone samples were included in the study, 53 from Saint Etienne and 15 from the University of Antwerp registries. Static parameters of active bone resorption (Oc.S/BS) were measured for all but two samples, for which no TRAP-stained sections were available. The distribution of the various ROD diagnoses of our population using the complete samples as reference are shown in Table 1.

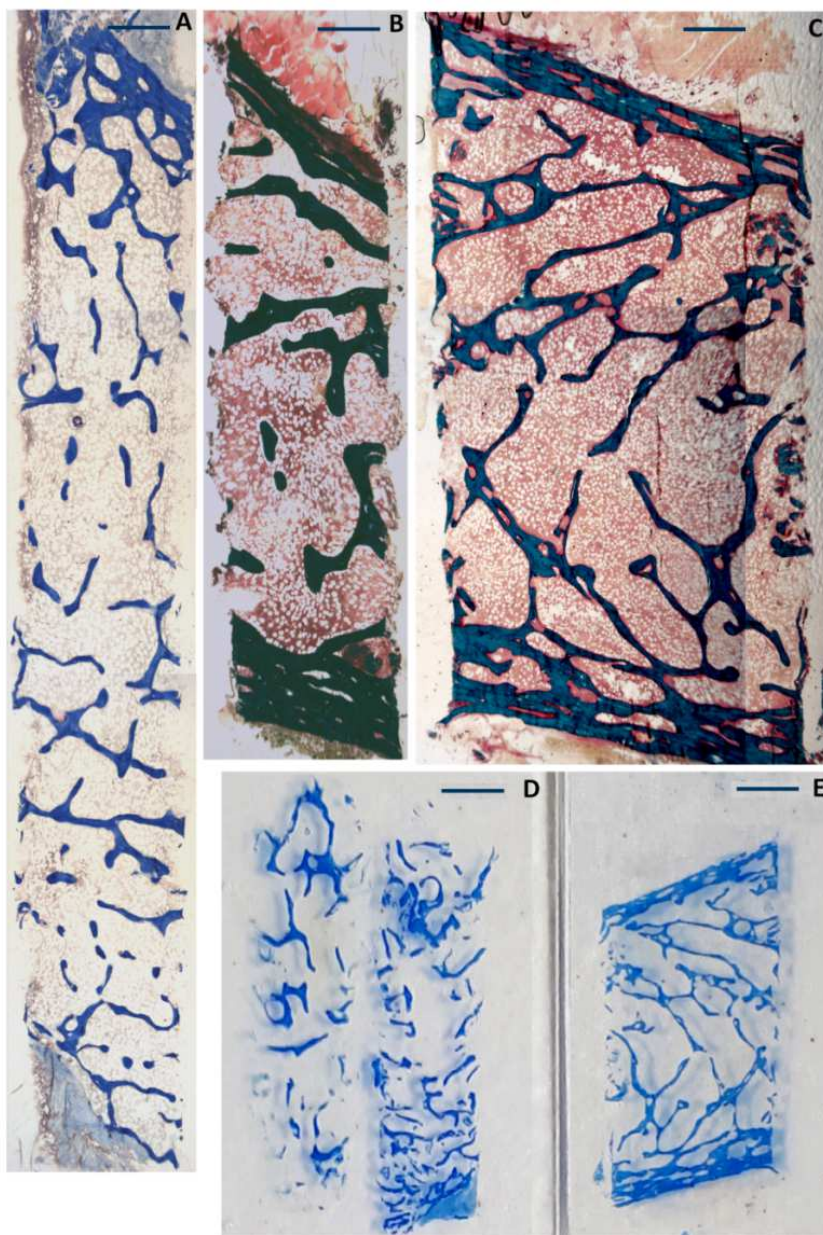
Table 1: Repartition of the initial ROD diagnoses, using the complete 7.5mm wide sample as reference.

Diagnosis	Number of samples (%)
Osteitis Fibrosa	31 (45%)
Adynamic Bone Disease	8 (12%)
Osteomalacia	7 (10%)
Mixed Uremic Osteodystrophy	14 (21%)
Other	8 (12%)

Evaluation of the size of the samples

By dividing the gold standard anterior iliac crest 7.5 mm-wide bone sections in two equal parts, we obtained two smaller samples, with the objective to mimic two sections the size of which could be obtained with a smaller trephine such as the Jamshidi needle. After excluding the dilacerated edges of the bone samples, the mean exploitable width of our halved bone samples was $2326 \pm 305\mu\text{m}$ (n=136). The region of interest that is actually suitable for histomorphometric analysis was checked in order to verify that it accurately mimicked a real bone sample provided by a smaller needle. In order to do this, we compared the number of fields that were available for the histomorphometric analysis between our halved samples, and available mini bone biopsies at our registry that were actually performed with a 3 mm diameter needle. The mean number of fields analyzable under a 10x magnification for the hemi biopsies was 5. Comparatively, the mean number of fields at a 10X magnification, suitable for analysis provided by biopsies performed with a real 4 mm-wide Jamshidi needle in our institutions was 5 to 8, when the procedure was performed using transiliac and vertical approaches of the iliac crest, respectively (Figure 3).

Figure 3: A, B & C : Example of histological sections of anterior iliac bone biopsies obtained in different patients with a Jamshidi's trephine (A, B) and a Meunier / Bordier Trephine (C), X 125, Bar= 1.3mm A: Bone sample performed with a vertical approach of the anterior iliac crest, with the external cortex only (Aniline Blue staining). B & C: Transiliac bone samples, with both cortices (Goldner staining). D,E: Photographs of slides with bone sections stained with aniline blue, Bar= 2.5 mm, D: two sections sampled with a Jamshidi's trephine, D with a Meunier / Bordier Trephine.



Correlation of histomorphometric parameters between the regions of interest

Spearman correlations for measures of dynamic and static histomorphometry between differing regions of interest are provided in Tables 2 and 3.

Table 2: Correlation of histomorphometric parameters between whole sample and one of the corresponding hemi-biopsy.

Parameter	Number of pairs	Spearman r	P value
OS/BS	67	0.98	<0.0001
OV/BV	67	0.99	<0.0001
Oc.S/BS	66	0.97	<0.0001
MS/BS	61	0.98	<0.0001
BFR/BS	59	0.98	<0.0001
MLT	58	0.98	<0.0001
O.Th	67	0.98	<0.0001
BV/TV	68	0.96	<0.0001
Tb.th	68	0.89	<0.0001

OS: Osteoid Surface, BS: Bone Surface; OV: Osteoid Volume, Oc.S: Osteoclast Surface, MS: Mineralizing Surface, BFR: Bone Formation Rate, MLT: Mineralizing Lag Time, O.th: Osteoid thickness, BV: Bone Volume, Th.th: trabecular thickness.

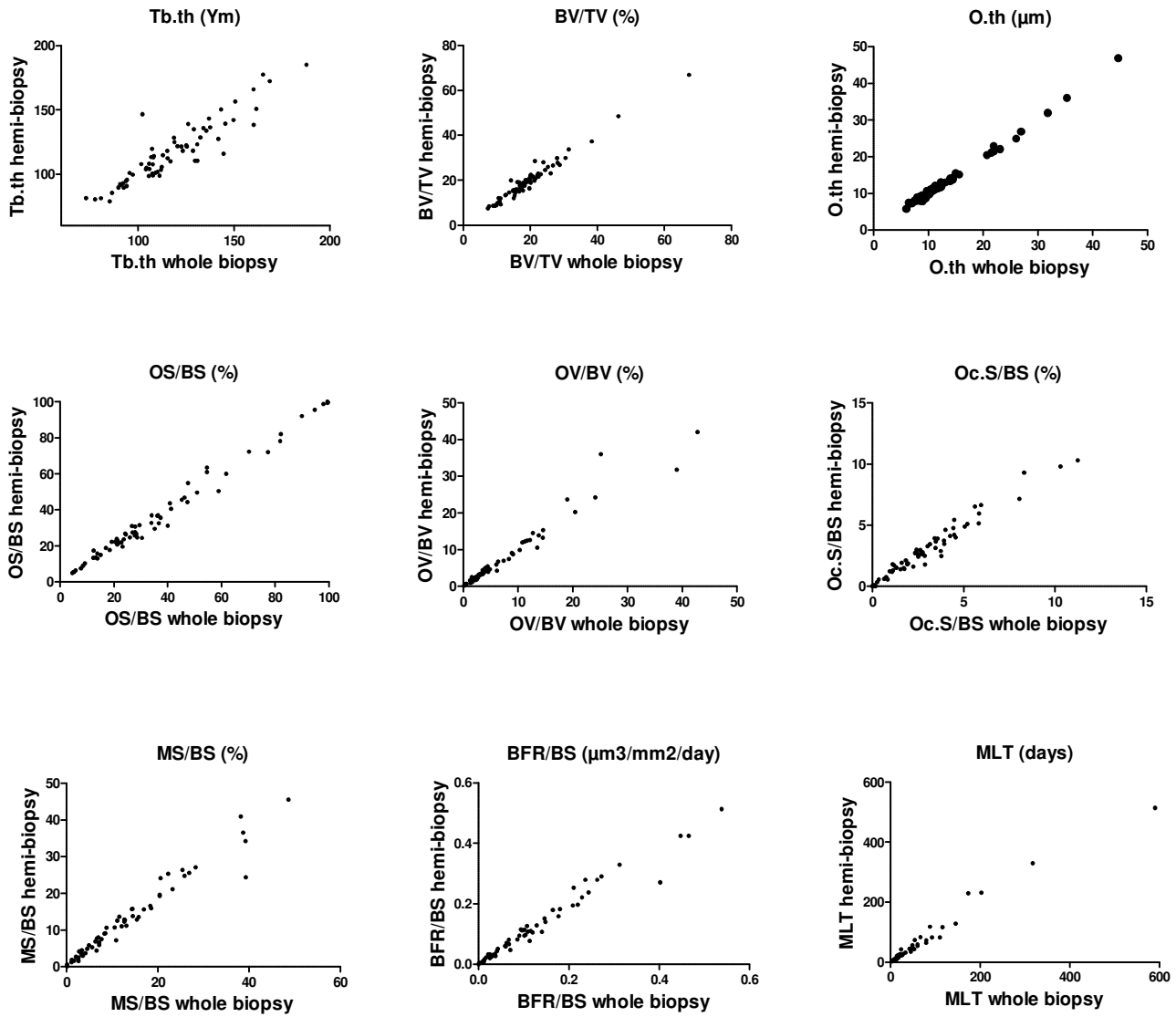
Table 3: Correlations of histomorphometric parameters between the adjacent hemi-biopsies 1 and 2.

Measured parameter	Number of pairs	Spearman r	P value
OS/BS	67	0.95	<0.0001
OV/BV	67	0.95	<0.0001
Oc.S/BS	66	0.92	<0.0001
MS/BS	61	0.94	<0.0001
BFR/BS	59	0.96	<0.0001
MLT	58	0.95	<0.0001
Oth	67	0.97	<0.0001
BV/TV	68	0.93	<0.0001
Tb.th	68	0.92	<0.0001

OS: Osteoid Surface, BS: Bone Surface; OV: Osteoid Volume, Oc.S: Osteoclast Surface, MS: Mineralizing Surface, BFR: Bone Formation Rate, MLT: Mineralizing Lag Time, O.th: Osteoid thickness, BV: Bone Volume, Th.th: trabecular thickness.

All correlations for bone biopsy parameters between regions of interests were high and significant. Scatterplots of the main histomorphometric parameters measured on the whole samples and one of the corresponding hemi-biopsy are shown in Figure 4.

Figure 4: Scatterplots of the main static and dynamic histomorphometric parameters on whole sample and corresponding hemi-biopsy.



Diagnosis concordance between whole sample and corresponding hemi biopsies

ROD diagnosis fully matched between the whole sample and both corresponding hemi-biopsies in 91% of cases (n=62). Six mismatches between the diagnosis made on one of the hemi-biopsies and the reference diagnosis made on the whole sample were found. The parameters responsible for each mismatch are reported in Table 4.

Table 4: Description of the mismatches between ROD diagnoses made on whole sample and hemi-biopsies. Mismatched parameters are shown in bold.

Case	Mis-matched parameter	Unit	Diagnostic threshold	Value on whole sample (reference)	Value on hemi-biopsy 1	Value on hemi-biopsy 2	Consequence on final diagnosis of ROD
#1	BFR/BS	$\mu\text{m}^3/\mu\text{m}^2/\text{d}$	<0.031	0.0216	0.0327	0.0162	Normal turnover instead of ABD
#2	BFR/BS	$\mu\text{m}^3/\mu\text{m}^2/\text{d}$	<0.031	0.025	0.0327	0.020	Normal turnover instead of ABD
#3	BFR/BS	$\mu\text{m}^3/\mu\text{m}^2/\text{d}$	>0.083	0.0905	0.094	0.078	Normal turnover instead of Mild HPT
#4	MLT	Days	50	54	73	44	Normal instead of mild mineralization defect
#5	Oc.S/BS	%	1.00	0.95	1.89	0.53	MUO instead of OM
#6	Oc.S/BS	%	1.00	0.83	0.56	1.10	MUO instead of other.

BS: Bone Surface, Oc.S: Osteoclast Surface, BFR: Bone Formation Rate, MLT: Mineralizing Lag

Time

Discussion

The histomorphometric analysis of an anterior iliac crest bone biopsy is the gold standard for the diagnosis and classification of ROD. However, this procedure is both costly and invasive, explaining why nephrologists are currently reluctant to perform it, despite recognizing its added clinical value¹⁰. Recently, smaller and disposable Jamshidi type trephines with an internal diameter of 3 to 4mm have been favored instead. This method, even if already widely practiced by nephrologists in Europe¹⁰, was never validated in regard to its accuracy for quantitative histomorphometric analysis and diagnosis of renal osteodystrophy lesions. One of the main concerns is evidently that the sample provided by such a procedure is not sufficient to adequately represent the state of bone turnover, mineralization and volume at the level of the skeleton, as sample variations may occur¹⁹. First we found that, the bone area that was available for quantitative histomorphometric analysis on our halved bone samples was equivalent or slightly inferior as what is provided by an actual smaller needle with an internal diameter of 3mm. This is reassuring because it shows that our method does not overestimate the bone surface that an actual smaller needle would provide. Depending on the anatomical approach for the procedure, it might, on the other hand, slightly underestimate it, which does not impact our results.

Our results are encouraging by showing that in 91% of the cases, the halved biopsies allowed for a correct evaluation of ROD, matching the reference diagnosis made on the complete sample. Moreover, we showed that there is an excellent correlation between whole sample and hemi-biopsies for every measured parameter.

It is noteworthy that mismatched diagnoses of ROD between complete and halved bones samples occurred in six cases (9% of the samples). In each case, the mismatch occurred because the smaller region of interest yielded a result that fell only slightly out of range of the diagnostic threshold that was established for a specific turnover or mineralization parameter (Table 4). In the present study, the use of these thresholds was a prerequisite to our methodological approach. Indeed, it would have been impossible to check the concordance of the final ROD diagnosis between whole and halved samples without strict criteria that were defined *a priori*. In routine practice, in rare borderline cases such as these, qualitative observations such as areas of woven bone or peritrabecular fibrosis, osteoid in osteocyte lacunae, resorption state in cortices or at the sub-periosteal surface, co-examination of turnover parameters other than those strictly included in the diagnosis criteria, frequently helps to provide a correct final evaluation of bone turnover and mineralization status.

It is our opinion that the fact that smaller bone samples for the diagnosis of renal osteodystrophy could offer many advantages compared to the classical, 7.5mm wide bone samples. First, we hypothesize that smaller samples could allow for a significant reduction of the invasiveness of the procedure. This is debatable, as it has been demonstrated that bone biopsies performed with 7.5 mm wide trephines were associated with minimal morbidity²⁰. Still, in a study evaluating patient acceptance of such a procedure, it has been noted that severe pain occurred in 24% of cases²¹, whereas pain induced by bone marrow biopsies performed with Jamshidi trephines was considered severe in 3.7% of cases²². The biopsy site is however different (i.e. posterior).

Moreover, as advocated elsewhere²³, a longitudinal approach of the iliac crest with a smaller needle allows for a deeper access in the trabecular bone (Figure 4), and creates a single hole that can be compressed easily, should bleeding occur. On the contrary, Bordier/Meunier trephines can only be used to perform transiliac bone biopsies which create two perforations of the iliac crest and provide a limited amount of trabecular bone, while allowing for the analysis of both internal and external cortices (which is not of critical importance in the diagnosis of ROD).

However, we acknowledge that the aforementioned advantages of using smaller needles are hypothetical as our work did not include any samples provided directly by such needles (such as the Jamshidi trephine) and that no direct comparison between classical 7.5mm bone samples and 3mm bone samples were made. Since 2010, more than 200 bone biopsies have been performed at the University Hospitals Leuven in an outpatient setting using a Jamshidi-type needle with an internal diameter of 4.5 mm (Osteobell [Mirandola, Italy], Biopsybell [Mirandola, Italy]) and under light sedation (midazolam). Tolerability was excellent and complications occurred in only two cases (<1%) (1 respiratory depression caused by midazolam requiring antidote and prolonged monitoring and 1 hematoma requiring transfusion) (P. Evenepoel, personal communication).

This work has several limitations. An ideal methodological approach would have been to directly compare bone biopsies performed with both Bordier/Meunier trephines and a smaller trephine needle at the same time in CKD patients. Even if the area of bone provided by our hemi biopsies was comparable to the one provided by an actual needle with a 3mm inner diameter, our method does not equate to the comparison of two independent bone samples provided by two different trephines. We acknowledge that this pilot project is only a first step to explore the use of smaller bone samples for the diagnosis and management of renal osteodystrophy and that further prospective studies are required to directly compare the two techniques and formally validate this new approach. However, smaller bone trephines being already widely used in clinical practice; our opinion is that this preliminary work was indispensable to prompt future studies on this matter. As disposable trephine are usually relatively inexpensive and widely available, future studies should also collect data for direct cost comparisons between the different techniques of bone biopsies, which could not be performed in our work.

Conclusion

The clinical consequences of CKD-MBD are severe, with increased risk of fractures and cardiovascular events. Methods to noninvasively monitor the level of bone turnover, mineralization, and volume in CKD patients are currently lacking. As a consequence, bone histomorphometry remains the mandatory gold standard for the correct evaluation of ROD in difficult cases where serum biomarkers are inaccurate. This preliminary work shows that the histomorphometric analysis of a halved bone samples yield comparable results as full 7.5mm wide bone biopsies, and prompts future studies to directly demonstrate that smaller bones samples, provided by trephines with an internal diameter of 3mm seem sufficient to correctly diagnose the type of ROD in CKD patients. The generalization of bone biopsies performed with disposable small needles might allow for a reduction in both cost and invasiveness of this procedure.

Author contributions

M.H.L.P and T.N. designed the study; G.B. provided material assistance; E.N.C and M.H.L.P. conducted the histomorphometric measurements; E.N.C., M.H.L.P., J.P.R. and R.C. analyzed the data. E.N.C. drafted the paper. M.H.L.P., S.P., D.F., R.C., G.B., P.D'H., P.E. and T.N. revised the paper.

Disclosure of financial interests

The authors declare no competing interest for this work.

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