NEPHROLOGY

*Nephrology* **22**, Suppl. 2 (2017) 22–26

# Is there a practical role for bone biopsy in chronic kidney disease?

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**KEY WORDS:**
bone biopsy, histomorphometry, mineralization, renal osteodystrophy, turnover.

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doi: 10.1111/nep.13017

**ABSTRACT:**

**Bone biopsy is currently the only means to accurately assess renal osteodystrophy and responses to therapeutic interventions. With sedation, the technique is relatively painless, and complications are uncommon. Bone biopsy should be considered when the aetiology of symptoms or biochemical abnormalities is in question, and results may lead to changes in therapy. Although it remains prudent to use antiresorptive drugs cautiously in patients with chronic kidney disease (CKD) stages 3a–4 and low bone mineral density, bone biopsy may not be warranted before commencing therapy in these patients. Histomorphometric indices adopted for bone biopsy assessment are turnover (T), mineralisation (M) and volume (V). Often, only measurements of trabecular bone are reported; however, marked cortical changes are common in CKD and may be critical to bone structure and integrity. MicroCT of bone biopsies can rapidly assess static parameters and provides information on the cortical and trabecular compartments that may influence management. Limitations of bone biopsy include the time required for pre-biopsy tetracycline labelling and sample processing, and a paucity of facilities to process and report samples. Patients with CKD may not respond predictably to treatments, and because the biopsy sample is illustrative of activity at only one skeletal site and one point in time, assessment of real-time laboratory trends is always required. Optimally, we need a non-invasive 'virtual bone biopsy' that provides information for initiating and monitoring therapy. However, bone biopsy is the current standard by which the accuracy of investigational imaging techniques, hormonal values and biochemical turnover markers are judged.**

## INTRODUCTION

Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD) encompasses the mineral, bone, hormonal and calcific cardiovascular abnormalities that develop in patients with CKD.[1] These are influenced by age, nutrition, menopausal status, corticosteroids, vitamin D status, levels of FGF23 and other factors including changes over time in dialysis regimens and the choice of phosphate binders.[2] Fracture risk in patients with CKD-MBD is much higher than in the general population and increases across the CKD spectrum. Both bone volume and bone quality, incorporating changes in turnover, mineralization, cortical porosity and trabecular bone architecture, deteriorate through CKD stages 3 to 5. Bone mineral density (BMD) by standard dual-energy X-ray absorptiometry does not capture many of these factors, and consequently, BMD thresholds for quantitating fracture risk are higher in CKD patients.[3] Measurement of serum parathyroid hormone (PTH) and biochemical bone turnover markers are helpful but also lack sensitivity and specificity for diagnosing the bone pathology that is present. Until newer diagnostic modalities such as use of the trabecular bone score, high-resolution peripheral quantitative CT and quantitative CT with finite element analysis have been adequately trialled in the CKD cohort, bone biopsy remains the gold standard for assessing the evolving skeletal changes of renal osteodystrophy (ROD) and the only currently available tool that accurately delineates effects of therapies.

Alterations to bone morphology in CKD-MBD comprise a heterogeneous group of metabolic bone disorders that develop as CKD progresses and affect the volume of cortical and trabecular bone (osteoporosis/osteosclerosis), bone turnover (high in hyperparathyroidism, and low in adynamic bone disease) and mineralisation rates (prolonged in osteomalacia). To achieve standardization in the reporting of bone biopsies, and so that the limited number of biopsy studies might be compared, the histomorphometric indices that have been adopted are turnover (T), mineralisation (M) and bone volume (V); the TMV

classification. Often, only measurements of trabecular bone are reported; however, prominent cortical changes of increased porosity and reduced cortical thickness occur in patients with CKD (Fig. 1). These under-reported changes are critical, because the cortex contributes significantly to bone structure and integrity in patients with CKD as it does in the normal population.

## BONE AND MINERAL DERANGEMENTS ASSOCIATED WITH CKD

Although specific forms of ROD can only be accurately diagnosed by quantitative histomorphometry, biochemical measures and clinical features often provide valuable clues to the diagnosis. Some examples are as follows:

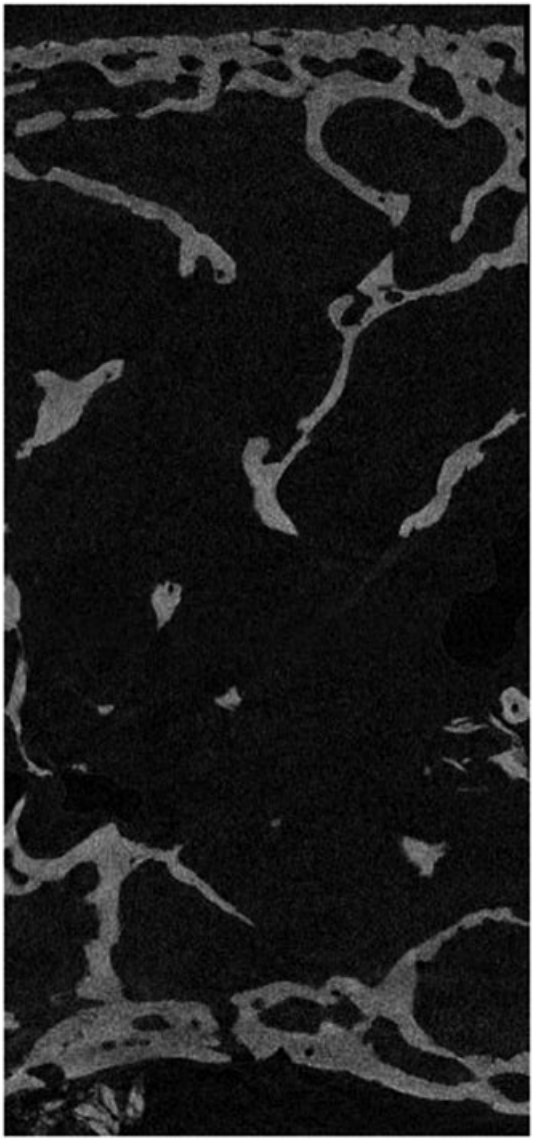

**Fig. 1** MicroCT of a transiliac bone biopsy. The patient is a 57-year-old woman with a failed transplant who returned to haemodialysis in 2012. Note the thinned and porous cortices that have become trabecularised, trabecular thinning and perforation, and increased trabecular spacing.

*Osteomalacia* may be suggested by low values of 25-hydroxyvitamin D and an elevated alkaline phosphatase (ALP), bone specific alkaline phosphatase (BSAP) and PTH. Proximal limb girdle pain and muscle weakness are common. However, identical laboratory and sometimes clinical features may occur with hyperparathyroid bone disease. Aluminium toxicity is now a rare cause of osteomalacia or low bone turnover. Bone biopsy distinguishes between these.

*Hypercalcemia* may be a feature of hyperparathyroid bone disease but can also be secondary to low bone turnover or 'adynamic' bone disease. In high turnover states, uncoupling (an imbalance of formation and resorption) may cause excess mineral release from bone and subsequent hypercalcaemia. On the other hand, hypercalcaemia may ensue if turnover is low and dietary calcium cannot be sequestered in bone or renally secreted.

*Low bone turnover* is generally associated with low or low normal PTH levels and often results from excessive use of calcium, calcitriol or its analogs or cinacalcet. However, *low* turnover may coexist with elevated levels of PTH, and *high* turnover may coexist with low PTH levels. Intact-PTH (iPTH) values generally rise with progressive renal impairment because of the accumulation of long C-terminal fragments (detected by the iPTH assay). Bone biopsy with double tetracycline labelling can differentiate between these turnover states.

*Osteoporosis* cannot be diagnosed with certainty in CKD stages 3–5 on BMD criteria established by the World Health Organization or the presence of fragility fractures, because low BMD and fracture occur with other forms of ROD.

### Treatment effects on bone disease

Patients can transition from one histological form of ROD to another, either by natural biologic means, or from induction by pharmacologic agents.[4] For example,

*Osteomalacia* may be treated with vitamin D or, in the case of hypophosphataemic osteomalacia due to long dialysis hours, by adding phosphate to the dialysate. The turnover may normalize, restoring osteoid surfaces to normal, or transform from low bone turnover and impaired mineralisation to high bone turnover and increased mineralisation.

*Secondary hyperparathyroidism* treated with calcitriol or its analogs, calcium supplements, high dialysate calcium or cinacalcet may correct from high to normal turnover or, if PTH suppression is excessive, low bone turnover.[5]

*Osteoporosis.* Antiresorptive therapies may be effective for patients with uncomplicated osteoporosis and CKD stages 3 to 4.[6,7] However, bisphosphonates and denosumab are relatively contraindicated in patients with glomerular filtration rates $<30$ mL/min/1.73m$^2$, who may have low bone turnover and impaired mineralisation (adynamic bone disease and osteomalacia), because these therapies may cause additional suppression of bone turnover and potentially increase fracture risk. Histomorphometry may identify these high-risk patients.

*Aluminium bone disease* is now uncommon. When treated with desferrioxamine chelation, bone histomorphometry can change

from low bone turnover to high bone turnover as aluminium is removed from osteoid surfaces allowing more responsiveness to PTH.

Forms of ROD, as defined by some commonly used histomorphometric indices, together with supportive laboratory measures, are illustrated in Table 1.

## KDIGO GUIDELINES

The 2009 KDIGO (Kidney Disease Improving Global Outcomes) guidelines suggested a bone biopsy be considered in patients with CKD stages 3–5D in various settings, including unexplained fractures, persistent bone pain, unexplained hypercalcemia, unexplained hypophosphatemia, possible aluminium toxicity and when considering therapy with antiresorptive agents.[16] An example of the 2009 rationale would be a patient with a low BMD due to hyperparathyroidism, for whom lowering the PTH is a safer and more appropriate therapy than an antiresorptive therapy. There was also concern that bisphosphonates would induce low-turnover bone disease if used with insufficient discrimination. The latest draft guidelines relating to bone biopsy, add 'if knowledge of the type of CKD–MBD will impact treatment decisions' (not graded), and that the magnitude of the abnormality, its reversibility and CKD progression should also be considered (evidence level 2D) (http://www.kdigo.org/clinical_practice_guidelines/CKD-MBD%20Update/KDIGO%20CKD-

**Table 1** The spectrum of renal osteodystrophy[8–13]

| Bone histomorphometry | Structural parameters | Static histomorphometry | Dynamic histomorphometry | Laboratory indices |
|---|---|---|---|---|
| Hyperparathyroidism | Reduced cortical width. Increased cortical porosity. | Increased: ES/BS (%), active resorption surface, osteoclast number. O.Ar/B.Ar ≤ 12%. Mild: Fibrosis 0–5% Severe: Fibrosis >5%. | Mild: High normal/increased BFR/T.Ar. Severe: Increased BFR/T.Ar >613 $\mu m^2/mm^2$/day. | Variable iPTH: Supportive for dialysis patients when >9-fold the assay normal upper range. Elevated BSAP. Elevated TRAcP-5b. Elevated P1NP/DPD/CTX. |
| Osteomalacia | — | O.Ar/B.Ar >12 %. Increased O.Pm/B.Pm Increased O.Wi. Fibrosis 0%. | BFR/T.Ar <97 $\mu m^2/mm^2$/day. | Low: 25(OH)D/1,25$(OH)_2$D. Elevated BSAP. Chronic metabolic acidosis. Hypophosphataemia. Elevated aluminium after desferrioxamine (now rare). |
| Mixed uraemic osteodystrophy | Reduced cortical width. Increased cortical porosity. | O.Ar/B.Ar ≥12% With or without fibrosis. Generally increased osteoclast numbers | BFR/T.Ar may be increased in woven bone/decreased in lamellar bone | Mixed abnormalities. |
| Low turnover/ adynamic | — | O.Ar/B.Ar ≤12%. Fibrosis 0%. | BFR/T.Ar <97 $\mu m^2/mm^2$/day. | Variable iPTH: supportive for dialysis patients when <2-fold the assay normal upper range. Normal/low BSAP and TRAcP-5b. Normal/low P1NP/DPD/CTX. Elevated aluminium after desferrioxamine (now rare). |
| Osteoporosis | Reduced cortical width. Increased cortical porosity. | Reduced BV/TV. Reduced Tb.Th, Tb.N. Increased Tb.Sp. O.Ar/B.Ar ≤12%. Fibrosis 0 %. | Normal/high normal BFR/T.Ar. | BMD T-score<−2.5. Normal or elevated BSAP/P1NP/DPD/CTX. Possible deficient/insufficient 25(OH)D. |
| Dialysis-related Amyloid | — | — | — | Abnormal imaging. Amyloid on biopsy. |

In static histomorphometry, ES/BS means eroded surface/bone surface, O.Ar/B.Ar means osteoid area/bone area, O.Pm/B.Pm means osteoid perimeter/bone perimeter, O.Wi means osteoid width, BV/TV means (trabecular) bone volume/total volume, Tb.Th means trabecular thickness, Tb.N means trabecular number, and Tb.Sp means trabecular spacing. In dynamic histomorphometry, BFR/T.Ar means bone formation rate/total area. BFR is calculated as the mineralising surface/bone surface × mineral apposition rate (MS/BS × MAR). MS is calculated as the length of the double tetracycline label, plus ½ the length of the single label. MAR is calculated as the distance between tetracycline labels divided by the interval in days between tetracycline doses prior to the bone biopsy (mm/day). In laboratory indices, iPTH means intact parathyroid hormone, BSAP means bone specific alkaline phosphatase, TRAcP-5b means tartrate resistant acid phosphatase 5b, and BMD means bone mineral density. Markers for which values rise as renal function falls include P1NP (procollagen 1N-propeptide), DPD (deoxypyridinoline), and CTX (C-terminal telopeptide cross-links of type 1 collagen).

MBD%20Update_Public%20Review_Final.pdf: last accessed 22 January 2017).

Recent publications, which inform current suggestions for bone biopsy, include the study of Malluche *et al.*[12] that assessed 630 patients on maintenance haemodialysis and reported a high prevalence of low bone turnover, particularly amongst Caucasian patients, with no differences in turnover by gender or presence of diabetes. Abnormal cortical parameters were also reported, and no biopsies showed stainable aluminium or iron. Bakkaloglu and colleagues assessed relationships between histomorphometry using the TMV classification and PTH, ALP and calcium values in paediatric patients on peritoneal dialysis[17] Approximately half of the children had increased bone turnover and/or abnormal mineralisation. PTH values <400 pg/ml (43 pmol/L) plus alkaline phosphatase (ALP) <400 IU/L provided the highest prediction of normal bone turnover and mineralisation.

The very recent study of Sprague *et al.*[13] supported use of the TMV system to evaluate ROD, but reported that no biochemical markers achieved acceptable minimal criteria (receiver operator characteristic area under the curve; ROC AUC >0.7) to discriminate low from normal or high from normal bone formation rates. The ability to discriminate low from non-low bone formation was best achieved with iPTH <103 pg/ml (11 pmol/L) and with BSAP <33.1 U/L (AUC curve >0.7) and high from non-high with a PTH >323 pg/ml (34.4 pmol/L) and BSAP >42.1 U/L. The authors concluded that the identification of new biomarkers with higher discriminatory ability may help to improve diagnosis. Bone biopsy has also been used in the assessment of therapies, such as the BONEFIDE study, which reported improvements in bone histology in most dialysis patients with elevated PTH and BSAP values, who were treated with cinacalcet.[5] Importantly, a recent publication by Araujo *et al.*[18] highlights the potential risks of treating low bone turnover by altering therapies so as to increase levels of PTH. This intervention may reduce cortical thickness and increase cortical porosity, with potential to *exacerbate* fracture risk.

**Table 2** Therapeutic options for treatment of renal osteodystrophy[4,6,7,14,15]

| | |
|---|---|
| Hyperparathyroidism | Cholecalciferol (CKD stages 1–4)<br>Calcitriol or its analogs<br>Non-calcium-based phosphate binders<br>Calcium if hypocalcaemic; note: avoid long-term positive calcium balance<br>Hormone replacement therapy<br>Cinacalcet<br>Bisphosphonates, denosumab; note: risk of acute hypocalcaemia<br>Parathyroidectomy |
| Osteomalacia | Bicarbonate dialysis<br>Bicarbonate supplements<br>Phosphate added to dialysate if hypophosphataemic<br>Cholecalciferol, calcitriol/analogs |
| Mixed uraemic osteodystrophy (MUO) | Varied; cholecalciferol, calcitriol/analogs, phosphate if hypophosphataemic.<br>note: Parathyroidectomy in patients with MUO with bone hyper-remodeling and mineralisation defect with inappropriately thick osteoid seam may induce fracturing low turnover. |
| Low turnover/adynamic | Mainly related to iatrogenic over-suppression of PTH:<br>Reduce calcitriol or its analogs/calcium supplements/high dialysate calcium.<br>Cease cinacalcet, avoid antiresorptives.<br>Remove aluminium. |
| Osteoporosis | Management with standard therapies may be possible*. |
| Dialysis-related Amyloid | Longer dialysis/high flux dialysis/transplantation. |

*Patients with CKD stages 5 and 5D have not been included in studies of drugs used for management of osteoporosis, and studies have generally excluded participants with elevated levels of PTH and ALP.

## DISCUSSION

Bone biopsy is the gold standard for the assessment of ROD, and currently the only means to accurately assess responses to therapeutic interventions. With sedation, concerns about the pain of the technique are largely misplaced, and complications are uncommon. The main risk is of haematoma in patients treated with aspirin, clopidogrel, warfarin, heparin anticoagulation on dialysis or with reduced platelet function.

Bone biopsy should be considered if knowing the aetiology of clinical symptoms or biochemical abnormalities is in question, and when biopsy results may lead to changes in therapy (Table 2). Although it remains prudent that the use of antiresorptive drugs be individualized and cautious in patients with CKD stages 3a–4 and low BMD, bone biopsy is generally unnecessary prior to antiresorptive therapy. Fracture rates are markedly increased in these patients, and although definitive trials are lacking, post hoc analysis suggests efficacy and safety of oral bisphosphonates over 3 to 4 years, including in very elderly women.[6] Retrospective analysis of the Freedom Study has also shown that denosumab may be effective in CKD stages 3a–4.[7] Prospective randomized controlled trials in the CKD cohort, and in patients with CKD stages 5 and 5D specifically are lacking, and these patients are particularly prone to acute hypocalcaemia following antiresorptive therapy. Nevertheless, there are no data to confirm that these patients develop adynamic bone disease. Following transplantation, changes in BMD and bone histomorphometry have been assessed in relation to a variety of therapies, and concerns have been voiced that bisphosphonates may cause adynamic bone disease.[19–24] However, studies are needed to determine the efficacy of protocols that use combinations of prevalent fracture, BMD, values of PTH and biochemical turnover markers to stratify therapy,[25] and no randomized controlled trials have assessed fracture outcomes.

Bone biopsy has a number of well-recognized limitations, including its lack of ready availability and, because samples need to be undecalcified, prolonged processing time. In addition, the only

way to determine therapeutic efficacy is to repeat the biopsy. Confounding results include multiple pathologies in the one sample that may make therapeutic decisions difficult. MicroCT of the biopsy sample provides more rapid assessment of static indices, but not of turnover or mineralisation, and we need new techniques for processing samples rapidly. Histomorphometry on frozen sections, soft beam microCT or electron microscopy with energy-dispersive X-ray spectroscopy may overcome some limitations of assessing mineralisation and turnover.

Optimally, we need techniques that are non-invasive and provide information similar to bone biopsy for initiating and monitoring therapy. A 'virtual bone biopsy' might combine accurate laboratory biomarkers with newer imaging techniques to provide the necessary information on turnover, mineralisation and cortical and trabecular bone volume to direct therapies including calcimimetics, calcitriol or vitamin D analogs, antiresorptive agents, hormone replacement therapy and possibly anabolic agents such as teriparatide and sclerostin antagonists. For the present, bone biopsy represents an important adjunct to the clinical and biochemical evaluation of patients with CKD, particularly when patients present with bone pain or unexplained fractures. However, even with the availability of bone biopsy data, we may find that patients do not respond predictably to treatments and that the biopsy sample is illustrative of activity at only one skeletal site and a single point in time. Thus regular analysis of biochemical indices and attention to their trends will also be required.

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