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Biomarkers predicting bone turnover in the setting of CKD

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Abstract

a) **Purpose of the review:** Impaired bone quality contributes to the increased fracture risk in chronic kidney disease patients. Both low and high turnover bone disease may compromise bone quality. The question arises whether bone biomarkers may be additive or replace bone histomorphometry for diagnosing the extremes of bone turnover. .

b) **Recent findings:** Studies exploring the performance of established and emerging bone biomarkers such against histomorphometric assessment of bone turnover are limited and overall yield inconclusive results as to their diagnostic utility.

c) **Summary:** Bone biomarkers, although promising, currently fail to meet the needed diagnostic accuracy to replace bone histomorphometry, and thus are not yet ready for clinical use. Bone biomarkers have several advantages, but also important limitations such as high biological variability, retention with kidney disease, pre-analytical issues, and inter-assay variability. These important issues must be considered when developing and evaluating bone biomarkers. There is an urgent need for harmonization and standardization of available assays and additional bone biopsy studies.

Keywords

Non-invasive diagnosis, bone histomorphometry, bone biomarkers, bone turnover, bone formation, bone resorption, chronic kidney disease, renal osteodystrophy

Introduction

Patients with CKD are at increased risk of fractures. The fracture risk steadily increases along the progression of renal disease to become 4 times as high in end stage renal disease patients as compared to healthy controls¹. The risk further increases following renal transplantation, at least transiently². CKD patients with fractures have an increased risk of mortality compared to those without fractures³. Both a high fall risk and an impaired bone strength account for the increased fracture risk in CKD⁴. Bone strength is determined by bone quantity and bone quality. Several lines of evidence indicate that CKD is a state of low bone mass and accelerated bone loss⁵. Since adjustment for bone mineral density (as a proxy of bone mass) does not fully explain the association between CKD and increased fracture risk, CKD may be equally considered a state of impaired bone quality. Bone turnover is an important determinant of bone quality. Although traditionally high-turnover bone disease predominated in CKD patients, recently there appears to have been a shift towards predominance of low-bone turnover disease, especially in white populations^{6,7}. Both low and high turnover bone disease may compromise bone quality, albeit through different mechanisms⁸.

The histomorphometric analysis of the tetracycline double-labeled bone biopsy is considered the gold standard for quantifying bone turnover. A bone biopsy not only provides information on bone turnover, but also on bone volume and mineralization. Bone biopsies can only be obtained at the iliac crest, except for surgical samples taken at other sites. Given the heterogeneity of the skeleton, caution is warranted when extrapolating iliac crest bone biopsy results to other skeletal sites. Taking a bone biopsy is invasive and requires the necessary skills whilst its analysis is expensive and necessitates specific histopathological expertise which is not widely available. Therefore, a bone biopsy is not feasible in all patients all of the time⁹. Noninvasive imaging techniques (including isotope techniques)¹⁰ and bone biomarkers have been suggested as surrogate of or adjuvant to bone biopsy to assess bone turnover, to classify risk of bone loss and fractures, and to guide therapeutic decisions.

The exact role of biochemical bone biomarkers in the management of metabolic bone diseases remains a topic of controversy. Many reviews have already critically evaluated the utility of bone biomarkers for the work-up and follow-up of osteoporosis¹¹. In this review we

will present an up to date review of bone biomarkers predicting bone turnover in the setting of CKD. After discussing general aspects we will briefly discuss established and emerging bone biomarkers.

General aspects of bone biomarkers

The deliberate use of bone biomarkers requires knowledge about their strengths and limitations. Undoubtedly the *ease of sample collection* is an important asset of biochemical bone biomarker. Biochemical bone biomarkers reflect changes in bone turnover more *rapidly* than changes in other clinical tests such as bone mineral density and bone histomorphometry (e.g. the duration of bone remodeling at any given site is 3 to 6 months) Though fraught with challenges¹², monitoring bone biomarkers may be advocated to capture ‘acute’ effects of disease and therapy. The concentration of bone biomarkers reflects the turnover rate of the *skeleton as a whole*, whereas histomorphometric indices reflect its activation at a definite site. This may be relevant as heterogeneity of the skeleton (site, endosteal vs periosteal envelope) exists, both with regard to the response to aging, disease and therapy¹³.

Bone biomarkers also have limitations. Bone biomarkers originating from the degradation of type I collagen *lack tissue specificity*. Indeed, type I collagen is not only present in bone, but also in other connective tissues such as the skin and tendons. More importantly, most bone biomarkers are characterized by a high *variability*. Sources of variability can be pre-analytical (related to patient characteristics and sampling conditions), analytical (related to the assay)¹¹ and post-analytical (including biological variability). Many of circulating bone biomarkers exhibit a circadian rhythm. The circadian rhythm is more pronounced for markers of bone resorption than those of bone formation and partly related to food intake. Some biomarkers are retained with progressive kidney failure, hampering their interpretation in the setting of CKD. Other pre-analytical confounding factors include age, gender, menopausal status, ethnicity, geographical location and therapy. Glucocorticoids, for example, decrease levels of bone formation markers dose-dependently within 2 days of onset of therapy¹¹. Sample type (serum or EDTA plasma), sampling location (fistula or dialysis catheter) and analyte stability should be accounted for as well. Bone biomarkers are most commonly determined by immunoassays. Automated methods are not uniformly available and inter-method or inter-manufacturer variability is high. The latter is related to problems of antibody specificity and a lack of standardization. Establishment of reliable reference ranges for bone biomarkers is also challenging. Indeed, the reference population should be free of any condition that might lead to secondary hyperparathyroidism including kidney disease, hypophosphatemia, hypocalcemia,

vitamin D deficiency or low bone turnover due to older age and diabetes even without CKD. Unfortunately, most manufacturers do not take these confounding factors into consideration, which may lead to normal reference ranges which are wide with both too high upper and lower limit. Another post-analytical issue that should be accounted for is the biological variability (CVi)^{14;15}. The CVi is the random natural variation around an individual homeostatic set point. The CVi determines how much the concentration of an analyte must vary between two results before the change should be considered as clinically significant. This change is commonly referred to as the critical difference or least significant change (LSC) and corresponds to 3 times the CVi. In hemodialysis patients, the LSC for bone alkaline phosphatase is 36%, whilst it is 72% for PTH. These figures are several fold higher than those observed for other common biochemical parameters such as creatinine¹⁴.

These characteristics of bone biomarkers may explain why the correlation between histomorphometric parameters obtained from the iliac bone and the integrated mean of the overall skeletal turnover represented by serum bone biomarker concentration is at best only modest¹⁶ (*Table 1*)

Bone biomarkers in the setting of CKD

Bone biomarkers may roughly be classified as circulating factors that affect bone turnover (e.g. parathyroid hormone [PTH], sclerostin) and factors that reflect bone cell number and/or activity (figure 1). The latter are generally subdivided into two categories: markers of bone formation and markers of bone resorption. Bone formation markers derive from the osteoblastic activity and include bone specific alkaline phosphatase (BsAP), osteocalcin, N-terminal propeptide (PINP), and C-terminal propeptide of type-I procollagen (PICP). The markers of bone resorption include degradation products of the type-I collagen such as the intermolecular crosslinks pyridinoline (PYD) and deoxypyridinoline (DPD), the C-terminal telopeptide (CTX), the N-terminal telopeptide (NTX) and matrix-metalloproteases (MMP)-generated (CTX-MMP or ICTP) type I collagen fragments and osteoclasts enzymes, such as type 5b tartrate-resistant acid phosphatase (TRAP-5b) and cathepsin K. The International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine recommend that PINP and CTX-1 are used as reference analytes in clinical osteoporosis studies, because of their robust nature and dynamic response to treatment. It should be emphasized however, that in the setting of osteoporosis, bone biomarkers are neither used

for diagnostic means, nor to inform treatment decisions. The 2009 Kidney Disease Improving Global Outcomes (KDIGO) guidelines suggested that “measurements of serum PTH or BsAP can be used to evaluate bone disease because markedly high or low values predict underlying bone turnover” (paragraph 3.2.3; grade 2B). PTH monitoring in advanced CKD is recommended every 3 months and alkaline phosphatase activity yearly, or more frequently if levels of PTH are elevated (paragraph 3.1.2; not graded)¹⁷.

Bone Turnover Biomarkers

PTH

Because PTH is a key regulator of bone remodeling and because of the wide availability of rapid and automated PTH immunoassays, regular monitoring of PTH levels has become the *lingua franca* of renal bone disease management. Much has been inferred from PTH values, both in terms of bone turnover, and fracture risk. The correlation between circulating PTH levels and bone turnover is rather weak¹⁷, unless at the extremes of the PTH concentration range. The area under the receiver operating characteristic (ROC) curve (AUC) for PTH in predicting either low or high bone turnover averages a ‘moderate’ 0.70. Results are similar for the intact (2nd generation; iPTH) and 3rd generation or biointact (1-84PTH; biPTH) assay. In addition to the abovementioned sources of variability, the responsiveness of bone to PTH is an additional confounder of the association between PTH and bone turnover. Competing downstream signals, inhibitory local factors and PTH receptor downregulation, suppress the skeletal response to PTH to a variable extent in CKD. Factors contributing to PTH hyporesponsiveness (formerly referred to as PTH resistance) in CKD include oxidative stress, uremic toxins, and C-terminal PTH fragments¹⁸. It was hypothesized that the simultaneous quantification of 1-84PTH and 7-84PTH fragments and calculation of their ratio would reveal superior predictive power regarding bone turnover. Unfortunately, this hypothesis could not be uniformly confirmed in bone biopsy studies¹⁹.

Sclerostin

Sclerostin, a protein produced by osteocytes, is a negative regulator of bone formation; it decreases bone formation through inhibition of the (canonical) Wnt– β -catenin pathway²⁰. Clinical data show increasing circulating sclerostin levels with progression of CKD to levels that are 2-4-fold higher in dialysis patients compared to healthy controls²¹. Circulating

sclerostin levels correlate negatively with histomorphometric parameters of bone turnover in dialysis patients^{22,23}. A low circulating sclerostin was superior to a high iPTH for the negative prediction of high bone turnover; a high sclerostin level is a better predictor of non-high turnover bone than a low PTH level. The exact value of sclerostin as a biomarker of bone turnover remains yet to be established in particular in CKD patients not yet in dialysis. Unfortunately, an automated method for sclerostin measurement is currently lacking and commercially available ELISAs provide incongruent results^{24;25}.

Bone formation biomarkers

Type I collagen and other bone matrix components are produced by osteoblasts, which also produce enzymes essential for the mineralization of osteoid. Bone formation markers reflect different stages of bone formation.

Propeptides of type I procollagen

In the early phase of bone formation unmineralized osteoid is formed. Around 90% of osteoid consists of type I collagen. The formation of type I collagen requires cleavage of the propeptides of type I procollagen, C-terminal (PICP) and N-terminal (PINP), which are released into the circulation. In human serum, PINP is present in two major forms, an intact trimeric form and a monomeric one; the latter accumulates in patients with renal failure. PINP is cleared by the liver. The serum concentration of PINP shows little diurnal or seasonal variation and does not differ between men and women. Trimeric PINP is a promising bone biomarker in CKD patients even if the literature on its use in such patients is scarce. PINP determination is easy and can be performed either with automated or manual methods. However, there is between assay discordance, as the antibodies used do not recognize to the same extent the monomeric form of the peptide²⁶. Data from a recent KDIGO supported large cross-sectional bone biopsy study in 492 hemodialysis patients showed an AUC for PINP (Roche Elecsys) of 0.65 and 0.74 in predicting low (<498.9 ng/mL) and high (>621.1 ng/mL) bone turnover as assessed by bone histomorphometry, respectively⁷.

Bone Specific Alkaline phosphatase

Approximately 2 weeks after the formation of osteoid, mineralization of the matrix occurs. Essential to this process is bone specific alkaline phosphatase (BsAP) activity, an ectoenzyme of the osteoblast which hydrolyzes pyrophosphate to inorganic phosphate. The bone isoform of alkaline phosphatase accounts for approximately half of the circulating total

alkaline phosphatase activity, the remaining representing mainly the liver isoform. Elevated liver alkaline phosphatase might confound the measurement of BsAP, in particular in patients with low bone turnover, as with immunoassays there can be up to 20% cross reactivity between bone and liver isoforms. Up to four BsAP isoforms have been identified. BsAP has features consistent with a promising biomarker: liver clearance (thus concentrations not influenced by decreased GFR), relative high half-life in serum, storage stability and relatively low intra-individual (biological) variability^{14;15;27}. In recent years, rapid, robust, and reproducible immunoassays have been developed for the quantification of circulating BsAP, often on fully automated platforms. Some assays measure the “Ostase”, i.e. the mass of BsAP present in the serum (expressed as µg/L), whereas other assays measure the activity of the BsAP enzyme (expressed as U/L). Results obtained by both types of assays, unfortunately, are not interchangeable²⁸. The role of BsAP as a biomarker of renal osteodystrophy has been investigated in patients with ESRD in several bone biopsy studies. Depending on the laboratory methods used, applied cut-off levels, and the cohorts investigated, low BsAP levels showed positive predictive values for low turnover bone disease between 89% to 100%²⁹⁻³¹. Youden indices for the diagnosis of renal osteodystrophy vary between 0.49 and 0.93^{29;31;32}. The KDIGO large bone biopsy. showed an AUC for BsAP (Quidel) in predicting low and high bone turnover of 0.76 and 0.71, respectively⁷.

Osteocalcin

The non-collagenous protein osteocalcin is a secretion product of the osteoblast.□□Post-translational modification of osteocalcin by □-carboxylation, which is vitamin K dependent, is essential for the structural and spatial confirmation of the molecule allowing its interaction with hydroxyapatite. Osteocalcin is released in the circulation during bone resorption as well as bone formation. As such osteocalcin may also qualify as a bone turnover biomarker. Although weaker than BsAP and PTH, significant correlations of circulating osteocalcin with bone histomorphometric parameters in hemodialysis patients have been reported³³. Conversely, in a bone biopsy study in 84 patients with advanced CKD not yet in dialysis, osteocalcin showed a sensitivity, specificity and AUC of 83%, 67% and 0.80 respectively in detecting adynamic bone disease, and was superior to iPTH³¹. A high (pre)analytical variability due to heterogeneity of circulating osteocalcin (fragments, carboxylated versus uncarboxylated osteocalcin) and uremic retention, however, hamper the widespread clinical implementation of serum osteocalcin measurements, especially in the setting of CKD.

Bone resorption markers

Pyridinoline and deoxypyridinoline

Collagen fibrils are strengthened by amino acids forming crosslinks between the fibrils. Pyridinium crosslinks—pyridinoline (PYD) and deoxypyridinoline (DPD) are released into the circulation as degradation products of mature collagen. The ratio of deoxypyridinoline to pyridinoline is higher in bone compared with other tissues, so it is probable that bone is the major contributing source of deoxypyridinoline in the circulation. There is very little bone biopsy data in CKD patients, but small studies indicate that serum PYD and DPD may be useful^{34;35}. Unfortunately these cross links are renally cleared and thus retention in kidney disease compromises the utility of pyridinolines.

NTX-I and CTX-I

Peptide fragments of collagen are released into the circulation when bone is resorbed. Amino-terminal (NTX-I) and carboxy-terminal (CTX-I) telopeptides of type I collagen are non-helical fragments of type I collagen. Both NTX-I and CTX-I assays have been adapted for measurement on automated analyzers. Telopeptides are retained in renal failure and show an important circadian rhythm, which, at least partly, is mediated by food intake³⁶. The International Osteoporosis Foundation has recommended that the serum CTX-I level be used as the reference marker for bone resorption. Bone biopsy studies in CKD patients exploring the utility of CTX-I are limited and overall yielded disappointing results^{33;37}.

Tartrate resistant acid phosphatase isoform 5b

Tartrate resistant acid phosphatase isoform 5b (TRAP-5b) is produced by osteoclasts during bone resorption and is detectable in the circulation. Similar to BsAP, circulating TRAP-5b levels are not influenced by kidney or liver function and do not show a circadian variation³⁸. The immunoassay for the measurement of TRAP-5b has been optimized over years to increase specificity and to decrease analytical variability³⁹. Very recently, an automated method for determination of TRAP-5b was developed, which will facilitate its use in clinical practice. The intra-individual coefficient of variation of TRAP-5b in hemodialysis patients is low, translating in a reasonable least significant change of 24%¹⁴. Data on its correlation with bone histomorphometry in patients with CKD are limited^{34;40}. In a bone biopsy study in 14

hemodialysis patients, serum TRAP-5b levels were observed to be highly correlated with dynamic and static parameters of bone turnover. The correlation coefficient was higher for osteoclast number than for indices of erosion. This should not come to a surprise, since scalloped bone surface and erosion depth may still be evident after the osteoclast has completed resorption and undergone apoptosis. This supports the hypothesis that TRAP-5b activity is a sensitive and specific marker of ongoing osteoclast activity and resorption at the time of sampling⁴⁰, but more data is needed.

Conclusions and future directions

At present, none of the bone biomarkers fulfills all of the criteria of an ‘ideal’ biomarker (*table 2*); an ideal biomarker (a) undergoes little degradation, shows minimal variability diurnally and longitudinally, and does neither accumulate with GFR loss, nor is it cleared by dialysis; (b) can be analyzed by a high-throughput methodology that at the same time is accurate, reproducible and affordable; and (c) provides information that adds to, or improves upon existing tests, aids risk assessment or enhances patient management. Of note, no biomarker so far clearly proved to be superior to iPTH in predicting bone turnover in CKD⁷.

Inter-method variation of most, if not all bone biomarkers is high. This compromises widespread clinical implementation and calls for further standardization and harmonization. External reference material should be considered for quality control and quality assurance.

Bone biomarkers do have several advantages, but also important limitations. Additional large cross-sectional and longitudinal studies that compare bone biomarkers with the gold standard, bone histomorphometry are required. Besides established bone biomarkers, emerging bone biomarkers or the combination of bone biomarkers (which will increase the specificity – measure of true negatives – at the expense of sensitivity – true positives -) should be evaluated as well. Additional biomarkers such as sclerostin, DKK-1, cathepsin K, and RANKL should also be studied as specific treatments targeting these molecules are in development or currently used in the general population. Moreover consensus should exist on reporting parameters of diagnostic performance not only in terms of sensitivity, specificity and AUC, but also in terms of positive and negative predictive values following the Bayes’ theorem which takes into account the prevalence of a particular type of renal osteodystrophy or high or low bone turnover as assessed in a particular study population. The ultimate goal is to demonstrate utility of the bone biomarkers in predicting bone loss and fractures, likely with other radiologic tests.

Although promising clinical data have been reported for some of the bone biomarkers^{38;41-43}, evidence is not consistent^{1;44;45}. Finally, bone biomarkers may prove useful to monitor CKD-MBD therapy⁴⁶. It is likely that a panel of bone biomarkers will always prove superior than a single bone biomarker and . In addition, assessing trends over time are more informative than single time point measurements . It is hoped that with continued validation and research, that panels of bone biomarkers will become part of routine clinical evaluation of bone health in CKD patients.

Compliance with Ethical Guidelines

Conflict of Interest

Patrick D'Haese and Etienne Cavalier declare no conflicts of interest.

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Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Figure Legend

Figure 1: Bone Biomarkers