Review Article

Bone markers and chronic kidney diseases

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Abstract: Patients suffering from chronic kidney diseases are at higher fracture risk due to impaired bone quality. Bone quality is mediated by bone turnover, together with bone microarchitecture, mineralization, microfractures and bone matrix and composition. Since bone histomorphometry can only be performed in specialized centers, is invasive, costly and cannot be used for the follow-up of patients, bone turnover markers (BTMs) are thus generally presented as an alternative for the evaluation of bone turnover. In this critical review, we will present the advantages and limitations of BTMs. Notably, knowledge of the limitations is very important for a good interpretation of the results. Among them, we will insist on the lack of standardization and on the high variability that can come from the preanalytical, the analytical and the postanalytical phases. More studies comparing bone biopsies results and BTMS are also definitely needed.

Keywords: Bone alkaline phosphatase; parathyroid hormone (PTH); Kidney Disease Improving Global Outcomes (KDIGO); metabolic bone disease of chronic kidney disease (CKD-MBD); bone turnover

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Introduction

Patients suffering from chronic kidney disease (CKD) are at increased risk of fractures whether they are dialyzed (1) or not (2) because they suffer from increased or decreased bone turnover, linked to over- or under-secretion of parathyroid hormone (PTH). The gold standard to evaluate bone turnover is bone biopsy. Unfortunately, bone biopsies are invasive and need specialized centers for correct interpretation of the results (3). Also, repeating bone biopsies for monitoring the evolution of bone turnover is impossible. Hence, bone turnover markers (BTM) determination is essential in clinical practice to evaluate bone turnover. KDIGO (Kidney Disease: Improving Global Outcomes) guidelines thus recommend measurement of PTH and bone specific alkaline phosphatase (BALP) in the assessment of metabolic bone disease of CKD (CKD-MBD) (4,5).

General aspects of BTMs

BTM are important biomarkers that reflect changes in bone turnover more rapidly than changes in bone mineral density and bone histomorphometry. Also, their concentrations reflect the whole turnover of the skeleton, which is not the case of histomorphometric indexes, focusing on definite sites. However, BTMs also do have limitations, the most important one being their high variability. This variability can come from the pre-analytical phase (related to the characteristics of the patient, the sampling process and the handling of the samples), the analytical phase (related to the assays) and post-analytical phase (mainly related to the reference ranges).

Regarding the pre-analytical phase, some BTMs can exhibit a circadian rhythm, can be influenced by food intake and can accumulate with GFR loss. This is particularly the case of the C-terminal peptide of type I collagen (CTX), which is a marker of bone resorption. Other pre-analytical confounding factors include age, gender, menopausal status, ethnicity, and geographical location. Sample type (serum or EDTA plasma) and sampling location (fistula or dialysis cathether) can also impact the results of BTMs results: for CTX, EDTA plasma is recommended, but serum

will be mandatory for the determination of bone alkaline phosphatase. Finally, other diseases like liver failure and medication like glucocorticoids may affect circulating levels of BTMs.

Regarding the analytical phase, BTMs are generally determined by immunoassays. Unfortunately, inter-method variability is quite high, mainly linked to problems of antibody specificity and lack of standardization. Finally, from the post-analytical phase perspectives, the establishment of good reference ranges for BTMs is also challenging. Indeed, the reference population should be free of any condition that might lead to secondary hyperparathyroidism, among which CKD and vitamin D deficiency. Unfortunately, most of manufacturers have not taken these parameters into consideration, which leads to falsely elevated upper reference range for BTMs, and notably PTH (6). Another post-analytical issue that should be taken into account for is the biological variability (CVi) (7,8). The CVi is the random natural variation around an individual homeostatic set point and determines how much an analyte's concentration must vary between two results before the change is considered as clinically significant. This change, commonly referred to as the critical difference or least significant change (LSC), corresponds to about 3 times the CVi. For most BTMs, the LSC can range (in hemodialyzed patients) from 36% (for bone alkaline phosphatase) to 72% (for PTH), which is several fold higher than what is observed for other common biochemical parameters such as creatinine.

Abovementioned characteristics of BTMs may explain why the correlation between histomorphometric parameters obtained from the transiliac bone biopsy and the integrated mean of the overall skeletal turnover represented by serum BTM concentration is quite modest, ranging from 0.54 to 0.65 (9).

BTMs and CKD

BTMs are generally classified as circulating factors that affect bone turnover, like PTH, and factors that reflect bone cell number and/or activity, which are generally subdivided into two categories: markers of bone formation and markers of bone resorption. Bone formation markers derive from the osteoblastic activity. BALP and the N-terminal propeptide of type I procollagen (PINP) are the most frequently used markers of bone formation. The markers of bone resorption include degradation products of the type-I collagen such as the C-terminal cross-linking telopeptide of type I collagen (CTX) and osteoclasts enzymes, such as type 5b tartrate-resistant acid phosphatase (TRAP-5b). The International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine recommend that PINP and CTX are used as reference biomarkers in clinical osteoporosis studies, because of their robust nature and dynamic response to treatment (10). The 2017 Kidney Disease Improving Global Outcomes (KDIGO) guidelines suggested that "*In patients with CKD G3a to G5D, we suggest that measurements of serum PTH or bone-specific alkaline phosphatase 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 3–6 months and alkaline phosphatase activity yearly, or more frequently if levels of PTH are elevated (paragraph 3.1.2; not graded) (5). Finally, recent markers like sclerostin have emerged to better understand the bone physiopathology. Sclerostin is now considered as the protein responsible for the relation between mechanical stress and bone formation and also acts as an inhibitor of bone formation by inhibiting the canonical Wnt pathway (11,12).

Factors that affect bone metabolism

PTH

PTH is a key regulator of bone remodeling and its monitoring has been recommended in nephrological guidelines for years. KDIGO recommend now that the PTH levels of HD patients should be maintained between 2 to 9 times the upper reference limit of normality. However, PTH is not a "true" bone marker since PTH levels are primarily influenced by serum calcium concentration. Like other BTMs, PTH determination is not free from issues in the preanalytical, analytical and postanalytical phases. Briefly, PTH stability is a matter of an intense debate, which could be summarized by saying that EDTA plasma is preferred if samples cannot be processed immediately. From the analytical point of view, PTH assays are traditionally referred as "second" or "third" generation PTH if they measure or not the large N-truncated PTH fragments that accumulate in CKD patients. The magnitude of difference between these assays can be very important and PTH assays are not yet standardized. Finally, reference ranges proposed by the manufacturers for PTH assays are not generally accurate since the subjects of the selected reference population were not necessarily free from secondary hyperparathyroidism. Finally, the intense

oxidative stress observed in dialyzed patients can lead to the oxidation of the molecule. If it is know that oxidized PTH is biologically inactive, the different assays present on the market do not make the distinction between oxidized and non-oxidized moieties.

Despite these limitations, PTH has a high positive predictive value for high bone turnover (9,13) and a recent study in HD patients demonstrated that PTH was a good marker of bone turnover, able to discriminate high from non-high and low from non-low turnover based on KDIGO targets for PTH. Addition of BALP marginally improved the situation and addition of other bone biomarkers did not add any value (14).

Sclerostin

Sclerostin, a protein produced by osteocytes, is a negative regulator of bone formation; it decreases bone formation through inhibition of (canonical) Wnt-β-catenin pathway (15). Unfortunately, sclerostin determination is, to date, a great challenge since various ELISA present on the market exhibit large differences, potentially leading to opposite clinical findings (16,17). Clinical data show increasing circulating sclerostin levels along the progression of CKD, to reach levels that are 2–4-fold higher in dialysis patients as compared to healthy controls (18) but it is not confirmed yet if these high levels are due to sclerostin itself or to accumulating fragments.

There are very few papers dealing with sclerostin and bone biopsies. In a study in 60 stage 5 CKD patients, sclerostin was shown to be superior to PTH for the positive prediction of high activation frequency (Ac.f.), bone formation rate/bone surface ratio (BFR/BS), and osteoblasts numbers (NOb/BPm) (19). However, the ability of sclerostin to make the distinction between low and high bone turnover was actually very low.

Bone formation markers

Propeptides of type I procollagen

The first step of bone formation consists in the formation of osteoid, mainly constituted by type I collagen. During the formation of type I collagen, a cleavage of propeptides, C-terminal (PICP) and N-terminal (PINP) occurs and these propeptides are released in the circulation. PINP is present in serum in two major forms, a trimeric form and a monomeric one, this latter tending to accumulate in CKD patients. The liver clears trimeric form of PINP. PINP is not affected by diurnal or seasonal variation and is not different in men and women. PINP is a promising BTM 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, these kits are not equivalent, as they do not recognize to the same extent the monomeric form of the peptide (20). Intact PINP (and BALP) have been shown to better discriminate CKD stage 4–5 patients with low bone turnover than PTH in CKD patients (9)

Bone alkaline phosphatase

BALP is an ectoenzyme produced by the osteoblasts which is essential to bone mineralization. It acts by binding to bone matrix proteins and inducing bone mineralization through stimulation of pyrophosphate hydrolysis. The two major isoforms of alkaline phosphatase are the bone and liver forms, each one accounting for approximately 50% of the total activity. Together with PTH, BALP has been selected by the KDIGO for monitoring CKD-MBD because serum concentrations are not affected by kidney function due to its molecular weight of 80 kDa. Also, BALP possesses a relatively long half-life of 1.5–2.3 days, is very stable in serum and has a relatively low intra-individual variability (7,8). However, BALP determination does suffer from some analytical and clinical issues (21). Elevated liver alkaline phosphatase might confound the measurement of BALP, as there can be up to 20% cross reactivity between bone and liver isoforms. Three BALP isoforms (B/I, B1 and B2) have been identified in healthy individuals. B/I and B2 isoforms are specially increased in CKD. Moreover, a fourth isoform (B1x) has been discovered in serum from CKD stage 4 and 5 and not in normal subjects (22). BALP is mostly measured by automated immunoassays even if a manual radioimmunoassay and an ELISA remain also available. Some assays measure the "Ostase" i.e., the mass of BALP present in the serum whereas some others measure the activity of the BALP enzyme. The first ones will provide results in µg/L whereas the others will provide results in U/L which brings confusion since the results are not interchangeable.

The large bone biopsy study by Sprague *et al.* showed an AUC for BALP (Quidel) in predicting low (<33.1 U/L) and high (>42.1) bone turnover of 0.757 and 0.711, respectively. BALP (IDS iSYS) at a value of ≤21 µg/L also presented the higher AUC (0.824) to identify patients

with low bone turnover, with a PPV and a NPV of 53 and 96%, respectively. To identify patients at high risk of bone turnover, BALP value >31 µg/L had an AUC of 0.750, with a PPV and NPV of 69% and 74%. For that purpose, it was supersede by intact PINP, CTX and PTH.

Bone resorption markers

CTX

Peptide fragments of collagen are released into the circulation when bone is resorbed. Amino- (NTX) and Carboxy-terminal (CTX) telopeptides of type I collagen are non-helical fragments of type I collagen. CTX can be measured on automated analyzers (IDS iSYS and Roche Cobas) or by manual Elisas (IDS). CTX is cleared by the kidneys and its concentration increase in patients suffering from CKD. In non-hemodialyzed patients, they show an important circadian rhythm and their levels are decreased by food intake (23), probably by amylin secretion (24). The International Osteoporosis Foundation has recommended that the serum CTX level be used as the reference marker for bone resorption. There are however very few data available in the literature regarding CTX in CKD patients. Jean *et al.* have reported that serum CTX (and BALP) evolutions correlated well with one another, but correlated poorly with PTH changes over a 18-month period in prevalent HD patients (25). Our team has also reported that, compared to BALP, only CTX correlated well with PTH variation in a short-term period of 6 weeks (26). Jean *et al.* have recently shown that patients entering hemodialysis with cut-off value of >1,200 ng/L for CTX were at increased risk of developing severe secondary hyperparathyroidism (27). Even more limited are the studies evaluating CTX compared with bone biopsy. The accuracy of CTX for identifying patients with low bone turnover is quite disappointing, but concentrations >2,390 ng/L are quite specific (96%) and present an interesting positive predictive value in identifying patients with high bone turnover (9).

Tartrate resistant acid phosphatase isoform 5b

TRAP-5b is an enzyme produced by osteoclasts during bone resorption and serum concentration of TRAP-5b correlates with the osteoclasts number (28). TRAP-5b is not influenced by kidney function (29) and could thus be considered as the only useful biomarker that reflects bone resorption in patients with CKD (30). An automated immunoassay for determination of TRAP-5b has recently been developed by IDS on the iSYS platform. The intra-individual variation coefficient of variation of TRAP-5b in HD patients is low and the LSC is about 24% (7). However, histomorphometry data are limited. In a bone biopsy study in 14 HD patients, serum TRAP-5b levels were observed to be highly correlated with dynamic and static parameters of bone turnover. The study also showed that TRAP-5b activity is a more sensitive and specific marker of osteoclast activity (31). TRAP5b concentrations ≤4.6 U/L were shown to have a 96% negative predicting value to identify patients with low bone turnover, whereas concentrations higher than this cut-off had 82% negative predicting value to identify patients with high bone turnover (9).

Conclusions and future perspectives

Undoubtedly, the "ideal" bone marker does not exist. Indeed, this biomarker should undergo little degradation, show minimal variability diurnally and longitudinally, not accumulate with GFR loss, nor be cleared by dialysis. It should also be analyzed by a high-throughput, accurate and affordable methodology and provide clear clinical added information (32). Finally, this biomarker should be superior to PTH in predicting bone turnover in CKD.

Basically, we can consider that bone biomarkers are not useful for diagnosis of low or high bone turnover, unless they present extremely low or high values. Hence, they are more useful for the follow-up of over time. These guidelines recommend using a bone formations marker (BALP) to monitor the patients. Unfortunately, BALP has many limitations, mainly from the analytical point of view. PINP could be an interesting bone formation candidate, but more studies, with bone histomorphometry are need. Regarding bone resorption markers, the literature is also very scarce. However, CTX, and probably more certainly TRAP-5b could be of interest. But for these markers also, more data are needed.

Bone strength is a combination of bone volume (quantified by cortical and trabecular bone density) and bone quality, which is mediated by bone turnover, together with bone microarchitecture, mineralization, microfractures and bone matrix and composition (33). BTMs determination allows thus a partial view of a complex phenomenon and that should be taken into consideration when interpreting results.

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