

Can we use circulating biomarkers to monitor bone turnover in CKD haemodialysis patients? Hypotheses and facts

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ABSTRACT

Assessing bone turnover is a key diagnostic tool in the global management of chronic kidney disease-mineral and bone disorder (CKD-MBD). Since bone biopsy is invasive and cannot be repeated in clinical practice and because bone histomorphometry is less available due to the lack of specialized laboratories, we will focus on potential biomarkers used to assess and monitor bone turnover. After briefly reviewing the pathophysiology of bone turnover in CKD and haemodialysis patients, we will focus on the strengths and limitations of the now recommended biomarkers, i.e. parathormone and bone-specific alkaline phosphatase. We will consider the clinical and also the biological aspects of the topic and also insist on the use of these biomarkers for the monitoring, and the follow-up of the turnover in haemodialysis subjects. Finally, we will discuss some of the most promising, but still not recommended, emerging biomarkers.

Keywords: biomarkers, bone turnover, parathormone

INTRODUCTION

Since 2006, chronic kidney disease-mineral and bone disorder (CKD-MBD) has replaced the terms 'renal osteodystrophy' (ROD) which is now used to strictly define histological lesions [1]. This change in semantic emphasized the pathophysiological link between the various systemic complications of CKD-MBD including serum biological abnormalities (hyperphosphataemia low vitamin D levels etc.), bone histological lesions [2] and the clinical features associated with

CKD, namely extra-osseous and vascular calcifications, fractures and growth retardation [1, 3]. Morbidity and mortality related to CKD-MBD is huge [4, 5]. In this context, the assessment of the bone turnover is one of the most important (but not the only one) diagnostic tools. Indeed, the therapeutic strategies for the management of CKD-MBD (for example, use of phosphate binders or calcimimetics) depend, at least in part, on the bone turnover level of the patient. The gold standard to assess bone turnover is doubtless bone histology after tetracycline double labelling [1, 3, 6–9]. However, bone biopsies are invasive and cannot be repeated. Moreover, bone histomorphometry is performed in a limited number of (hyper) specialized centres and may not be available for all clinicians. For these reasons, bone biomarkers are used for both the diagnosis and monitoring of bone turnover. In the following paragraphs and after a brief reminder of the CKD-MBD definition and pathophysiology, we will review classical and new biomarkers of interest for bone turnover assessment. Before that, we will, in this first part, briefly remind readers of the 2009 recommendations published by Kidney Disease Improving Global Outcomes (KDIGO) and, in the second part, insist on an important analytical concept when biomarkers are considered.

The KDIGO guidelines suggested that the measurements of serum parathormone (PTH) or bone-specific alkaline phosphatase (b-ALP) should be used to evaluate bone disease because markedly high or low values predict underlying bone turnover [1]. One important word in the title of this article is the verb 'monitor'. This word implies a longitudinal follow-up of patients and this concept is also underlined by the KDIGO guidelines. Indeed, KDIGO stressed that therapeutic strategies should not depend on a single PTH value but rather on the trend of several PTH values evolution. The guidelines actually

make a recommendations about the frequency of PTH and b-ALP measurements according to the CKD stage [1]. Thus, we would like to emphasize, in the present review, the implications of the use of biomarkers for longitudinal follow-up and monitoring of bone turnover which necessarily implies that the clinicians correctly apprehend the concept of 'critical difference' or 'least significant change' (LSC) [10]. The critical difference can be defined as the smallest change in consecutive results in the same patient which is not due to chance. It is dependent of the analytical coefficient of variation (CV_a) and the intra-individual (or biological) coefficient of variation (CV_i). In other words, when a biological variable is monitored in a given patient, this critical difference (in percentage) must be reached before asserting the difference between the two results is clinically relevant [11].

ROD: DEFINITION AND PATHOPHYSIOLOGY

Definition

CKD affects not only bone turnover but also the two other components of bone, namely, bone mineralization and bone mass. Bone turnover consists in the succession of an osteoclastic resorption phase followed by a phase of formation, which both occur in a defined time frame (~3 months in healthy humans) and at a specific location at the bone surface. The rate of turnover depends on the number of remodelling units (i.e., a team of osteoclasts + a team of osteoblasts) at work. In CKD patients, the rate of turnover may be normal, high or low. After the collagen has been deposited by osteoblasts in the lacuna made by osteoclasts, it is progressively mineralized, generally in <30 days. This process is called primary mineralization and, in CKD, it may be normal or decreased. The bone mass (or volume) is evaluated based on the bone volume/tissue volume ratio for cancellous bone and on thickness and porosity for cortical bone. Bone turnover and bone mass are physiologically linked, since the variations in bone mass are the result of changes in bone remodelling rate. However, bone mineral density measurement using dual energy X-ray absorptiometry is not a tool for assessing bone remodelling in CKD patients. In contrast, although its interpretation may be difficult in a context of ROD, it may be useful for the evaluation of fracture risk. Bone volume may be normal, high or low in CKD. Various combinations of mineralization and turnover disorders lead to four types of bone lesions: osteitis fibrosa (OF, high bone turnover), adynamic bone disease (ABD, low bone turnover), osteomalacia (mineralization defect) and uraemic mixed osteopathy (high turnover and mineralization defect). In each of these four lesions, bone mass may be increased or decreased [2, 12].

Pathophysiology of ROD

The pathophysiology of ROD is complex and evolves constantly over the course of new discoveries. Thus, the hierarchy, kinetics and weight of each of the factors described below, in ROD development, are potentially subjected to change. Phosphate retention, which seems to be one of the earliest events in CKD, is due to nephronic reduction. As a consequence, an

increase in fibroblast growth factor 23 (FGF23) production by osteocytes occurs in bone (via unknown mechanisms) and leads to an increase in phosphate excretion by the remaining nephrons and a temporary maintenance of phosphate serum levels. FGF23 signals via its receptor and a co-receptor, Klotho. High FGF23 serum levels together with nephronic reduction and high cell phosphate load contribute to the reduction in calcitriol production by the kidney [13]. Meanwhile, secondary hyperparathyroidism develops via a combination of interlinked factors. The decrease in serum calcitriol levels is responsible for decreased intestinal calcium absorption and loss of the negative feedback on PTH transcription, whereas hyperphosphataemia increases the half-life of PTH transcripts. In addition, within the parathyroid glands, the gradual decline in the expression of calcium-sensing receptors, as well as vitamin D and FGF23 receptors and Klotho, result in progressive parathyroid gland autonomization that will be achieved via parathyroid cell-cycle abnormalities.

At the bone level, high PTH serum levels increase bone turnover and lead to OF lesions with various degrees of severity. However, we have to remember that bone remodelling is under the control of a number of local and systemic factors other than PTH which may be involved in changes in CKD-induced bone turnover when PTH levels are not too high. Low bone turnover is found when PTH has been excessively suppressed by calcium salts and/or 1-alpha-hydroxylated vitamin D derivatives. It should be kept in mind, however, that a bone resistance to PTH action, of poorly understood (probably multiple) reasons, appears progressively with the progression of renal disease, so that a normal or even high serum level of PTH does not exclude low bone turnover. Other risk factors for ABD include peritoneal dialysis, age and diabetes. Interestingly, ABD was observed in CKD patients before dialysis who had not received any treatment related to calcium and phosphate metabolism demonstrating that specific factors related to uraemia may be involved in low bone turnover. Thus, ABD may be related to skeletal resistance to PTH together with accumulation of factors slowing down bone turnover [2]. Recent works on CKD population reporting increase in serum levels of sclerostin, a potent negative regulator of bone formation, are in line with this latter hypothesis [14].

PTH: INTEREST AND LIMITATIONS

In dialysis patients, high PTH serum levels are not only associated with high bone turnover, but also with increased risk of all-cause mortality [5], whereas low PTH levels are associated with low bone turnover and, at least in some studies, with early mortality [15]. Low PTH [16] as well as severe hyperparathyroidism [2] has been associated with increased fracture risk. PTH is an 84 amino acid peptide hormone secreted by the parathyroid glands when ionized calcium serum level decreases. Although its role is to increase serum calcium, it also decreases phosphataemia by decreasing phosphate reabsorption in the proximal tubule. However, as renal function deteriorates, and especially in dialysis patients, PTH increases phosphataemia because of its effect on the release of

phosphate from bone. Since 1987 [17], the so-called 'intact' PTH assays, also called second-generation PTH assays, are available. These assays measure 1–84 PTH, which contains the first three amino acids of the N-terminal portion of the PTH molecule necessary for its hypercalcaemic–hyperphosphaturic effect. However, these assays also measure, with various cross-reactivities, 7–84 PTH, a PTH fragment accumulating in CKD and having some opposite biological properties [18, 19]. More recently, third-generation assays that measure 1–84 PTH but not 7–84 PTH became available [20]. The clinical information provided by these two different assay-generations seems similar, but it must be underlined that the measured concentration is lower with the third-generation assays (which measure one molecular form only) than with the second-generation assays (which measure two molecular forms). Some years ago, a study comparing bone biopsy data with the PTH concentrations measured with a second- and a third-generation assay in dialysis patients found that the 1–84 PTH/7–84 PTH ratio was promising in that it was significantly better correlated with the histomorphometric values than any single PTH value [8]. However, three other studies published during the following years did not find any improvement in the prediction of the bone turnover level with this ratio compared with a single PTH value of either assay-generation [21–23]. It must be stressed that the patients included in these various studies differed in terms of past or current vitamin D therapy, and that this may represent a possible explanation for the discrepancies between these studies. Another aspect which should be taken into account in such studies is the ethnic origin of the patients. Indeed, second-generation PTH levels correlated with the bone turnover index in Caucasians, but not in African-American dialysis patients [24, 25]. Furthermore, for a given level of ALP, the PTH level is usually higher in African-American than in Caucasians, suggesting that optimal bone turnover may be associated with different levels of PTH in these two ethnic groups. Finally, beside the evaluation of altered bone turnover, other outcomes may be considered. For example, it was shown in one study that a third-generation PTH assay (but not a second-generation assay, or the 1–84/7–84 ratio) was predictive of all-cause mortality in a cohort of incident dialysis patients [26].

According to these data, we share the opinion of the KDIGO experts that both generations of PTH assays seem similarly informative, and that there are currently not enough evidence to ask the medical laboratory to switch from a second-generation assay to a third-generation assay. Concerning the 1–84/7–84 ratio, we acknowledge that further studies, especially bone biopsy studies, are mandatory to propose this ratio as a routine tool in dialysis patients all the more than that two PTH assays, one second- and one third-generation assay, are required to calculate this ratio, thus considerably increasing the cost of this evaluation.

To maintain a similar level of bone turnover to that of patients with normal renal function, CKD and dialysis patients require higher PTH levels. The Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines proposed to maintain the serum PTH level within the 150–300 pg/mL range in dialysis patients as, in several already ancient studies, this range of

values was associated with a (grossly) normal bone turnover as diagnosed on bone biopsies [27]. However, while this target range was based on studies that used the (no longer available) Allegro intact PTH assay, it was shown that the many available PTH assays presented a very high inter-method variability, with a factor of almost 4 between the concentrations obtained by the kits that produced the lowest and the highest levels [19]. Since the standardization of PTH assay methods is highly difficult to achieve due to the lack of an international standard made of recombinant 1–84 PTH, and also to the variable cross-reactivity of PTH fragments from one assay to another, the KDIGO work group proposed to use a target range for serum PTH based on multiples of the upper limit of the normal values rather than on absolute concentrations [1]. After the publication of the KDOQI guidelines, Baretto *et al.* performed a study on a dialysed population and assessed bone turnover on bone biopsies. In line with previous work, ABD was more likely to be found with PTH values <150 pg/mL and OF with PTH >300 pg/mL. However, they demonstrated that any type of bone lesion could be found for PTH values between 150 and 300 pg/mL and that only 3% of the patients had normal bone turnover [28]. Furthermore, other studies reported that for dialysis patients, PTH concentrations are associated with mortality only for the highest concentrations (>400–600 pg/mL), albeit generally without mention of the nature of the PTH assay [5, 15]. For these reasons, the KDOQI PTH range for dialysis patients (150–300 pg/mL) [27] has been expanded in the KDIGO guidelines to two to nine times the upper normal limit (corresponding, for example, to 130–585 pg/mL with the Allegro PTH assay when the manufacturer's upper limit of normal of 65 pg/mL is considered) [1]. This is a pragmatic approach which may reduce the inter-method discrepancies in the interpretation of PTH concentrations in dialysis patients. However, we have some concerns about the way PTH reference values have been established. PTH reference values are obtained by measuring PTH in a reference population of apparently healthy subjects. Exclusion criteria are very important when recruiting this population (i.e. any subject with a potential cause of altered PTH secretion). Two very frequent causes of potentially elevated PTH levels, vitamin D deficiency/insufficiency and decreased GFR have generally not been taken into account when recruiting a reference population to establish the PTH normal values of the many available PTH kits. In a recent study published in this journal, we have compared the normal values of 10 PTH kits as provided by the manufacturers, with the values obtained with these 10 kits in 240 healthy subjects with a 25 hydroxy-vitamin D (25OHD) level >30 ng/mL, and an eGFR >60 mL/min/1.73 m². We found that the upper limit of normal in our population was always lower than the one proposed by the manufacturers. The difference was sometimes marginal (Abbott Architect kit) and sometimes huge (Beckman-Coulter Access). With most kits, our upper normal limit was 25–35% lower than the one provided by the manufacturers with a significant impact on the KDIGO target range (see Table 1) [29]. Owing to the inter-method variability in PTH results, KDIGO recommend that the clinical laboratories inform the nephrologists of any change in the PTH assay used. We believe that they should also inform the clinician

Table 1. Comparison, for 10 different PTH assays, of the reference range (in ng/L) and KDIGO target range for dialysis patients proposed by the kit manufacturers, and reference values and KDIGO target range established in our laboratories from the same population of 240 healthy subjects (120 men and 120 women) with a serum 25 hydroxy-vitamin D level >30 ng/mL and an eDFG (MDRD) > 60 mL/min/1.73 m².

Assay (name of the manufacturer)	Manufacturer's reference range	KDIGO target (x2–x9 upper normal) derived from manufacturer's range	Reference range obtained in our laboratories	KDIGO target (x2–x9 upper normal) derived from manufacturer's range
Second-generation assays				
Architect (Abbott)	15–68	136–612	16–65	130–585
Immulin (Siemens)	12–65	130–585	0.5–50	100–450
Vitros (Ortho-clinical)	7.5–53	106–477	11–48	96–432
Liaison N-tact (DiaSorin)	17.3–73	146–657	21–68	136–612
TiPTH (Scantibodies)	14–66	132–594	8–50	100–450
Elecsys (Roche Diagnostics)	15–65	130–585	14–50	100–450
DiaSorin IRMA (DiaSorin)	13–54	108–486	7–36	72–324
Access 2 (Beckman-Coulter)	12–88	176–792	10–47	94–423
Third-generation assays				
CA-PTH (Scantibodies)	5–39	78–351	7–31	62–279
Liaison (DiaSorin)	5.5–38	76–342	5–26	52–234

With some assays, the difference between the manufacturer's KDIGO range and the one derived from our own reference values established in vitamin D sufficient patients with an eDFG >60 mL/min/1.73 m² is huge.

on the way the reference values have been established. Finally, it must be underlined that several experts consider the KDIGO target range for serum PTH too broad and argue for a preferable range of approximately two to four times the upper normal value [30, 31].

As already stated, the concept of LSC is fundamental when considering the monitoring of PTH. To our knowledge, two studies have recently reported the LSC for PTH levels in haemodialysis patients. Gardham *et al.* reported that the LSC was 72 and 86% for EDTA plasma PTH with a second-, and a third-generation assay, respectively [32]. More recently, two of us (P.D. and E.C.) reported a lower (but still high) LSC of 39 and 43% for serum PTH with a second- and a third-generation PTH assay, respectively [10]. In other words, even when considering the more favourable results from our group, a PTH concentration of (say) 300 pg/mL cannot be considered different of 200 or 400 pg/mL.

Although PTH has important effects on bone turnover, serum PTH concentrations cannot be considered as a surrogate marker of bone turnover. It must be remembered that bone turnover is quite a slow process, which can take a few weeks in cases of high bone turnover, to a few months, or even years, in cases of ABD, whereas secretion of PTH is a very quick process (minutes) in response to variations in ionized calcium (Figure 1). Thus, an isolated measurement of serum PTH is unlikely to provide a valid representation of bone turnover, except in cases with extremely low or high values. This is not to say that PTH measurement is useless but other bone biomarkers must probably be used in complement.

b-ALP: A NEW GOLD STANDARD?

We agree with the guideline about the use of b-ALP as a confirmatory and complementary test to assess bone turnover.

ALPs are glycoproteins produced in different organs, like liver, placenta, kidney, leucocytes and intestine [33]. ALPs are enzymes that remove phosphate from proteins and nucleotides at alkaline pH. Specific b-ALP is released by osteoblasts and plays a major role in bone mineralization. Its serum concentration seems independent from glomerular filtration rate. Previous works showed that b-ALP could be of interest to assess bone turnover in CKD patients. In transversal studies, several authors demonstrated a significant correlation between b-ALP and PTH in CKD patients [6, 7, 10, 34–42]. In studies with bone histology available as a reference, most of the authors showed a slightly higher correlation between bone formation rate (BFR) and b-ALP than total ALP [9, 34, 39, 40, 42] and, for some authors, between BFR and PTH [6, 7, 9]. In the same vein, similar or better sensitivity-specificity was shown for b-ALP to diagnose low versus high bone turnover in comparison with PTH [6, 7, 9, 34, 36, 43]. Combination of both biomarkers was the best diagnostic tool for some authors [9, 43]. For example, Urena *et al.* showed that b-ALP concentrations >20 ng/mL formally excluded low bone turnover in haemodialysis patients. Couttenye *et al.* also proposed a b-ALP cut-off value with the best performance to detect low bone turnover [43]. Superiority of b-ALP was, however, less evident in other studies using bone histology [8, 40, 44]. Of interest, some authors reported an association between total ALP and risk of fractures in dialysis patients [45]. In the same view, recent observational studies showed a positive linear and independent association between total ALP and mortality risk in haemodialysis [46–48] and pre-dialysis CKD patients [49]. This association was still present even after adjustment for other liver enzymes. Compared with association between PTH and risk of mortality, the association of mortality risk with total ALP seems more linear and incremental [47, 49]. Such an association with mortality has also been recently suggested with b-ALP [50, 51].

Kinetics of variations of PTH and Bone Parameters

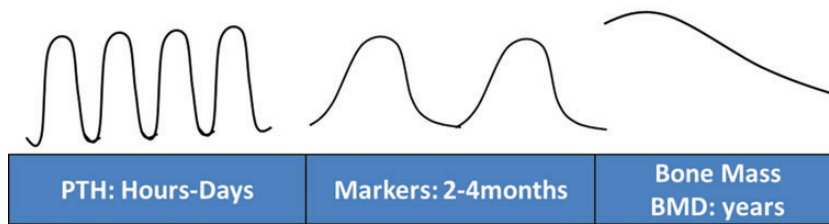


FIGURE 1: Kinetics of variations of PTH and bone parameters. PTH, parathormone; BMD, Bone Mineral Density.

The main advantages of b-ALP over PTH could also be analytical. Indeed, b-ALP can be determined with either automated or manual immunoassays that determine the mass or the activity of the enzyme. As Beckman-Coulter has the property of the epitope, this company licenses and currently supplies the antibody named 'Ostase BAP' to other selected companies, harmonization of the various assays is easier. On the other hand, b-ALP is more stable than PTH and it is not influenced by fasting status and kidney functions [52, 53]. Regarding the monitoring of bone turnover, the superiority of b-ALP could also be an argument. Indeed, LSC has been shown to be lower with b-ALP than with PTH. Sardiwal *et al.* showed an LSC of 36 and 72% for b-ALP and PTH, respectively [54]. We (P.D. and E.C.) confirmed the better LSC of 23% for b-ALP compared with 39% for PTH [10, 54].

As we previously stated, transversal studies showed a direct correlation between b-ALP and PTH serum concentrations. Consequently, the KDIGO guidelines presented b-ALP and PTH measurements as complementary [1]. However, as clinicians and considering the longitudinal follow-up of patients, we frequently observe that these two biomarkers frequently change in opposite directions (PTH increasing and b-ALP decreasing or *vice versa*). We (P.D. and E.C.) recently illustrated this fact in a descriptive study [35]. We did not observe any significant correlation between PTH (Δ PTH) and b-ALP variations (Δ b-ALP) over a short (6 weeks) or a long (52 weeks) period of time. PTH serum concentrations rapidly follow any acute modifications of calcium serum concentrations, whereas b-ALP bone turnover-related changes take longer because its serum levels will depend on bone remodelling, which is a process slower than PTH variations (Figure 1). In the same view, the half-life of b-ALP in serum is from 1 to 2 days, whereas the half-life of PTH is only few minutes [55]. Differences between the kinetics of the 'minute to minute' calcium regulation by PTH, which has a short half-life, and the time needed for bone to be altered, which is sometimes over 1 year, was illustrated by the way in peritoneal dialysis patients moving to low calcium dialysate [56] and in haemodialysed patients treated with cinacalcet [57]. Even if correlation between Δ PTH and Δ b-ALP might become significant on a longer period of time, discrepancies between biomarkers in specific patients remain and need to be further studied [37].

However, one needs to be cautious with the interpretation of b-ALP. Its serum concentration is obviously influenced by other local or systemic bone processes such as metastases,

recent fractures and growth. Furthermore, from an analytical point of view, recent data (J.C.S. and G.J.) demonstrated that the specificity of b-ALP measurement is not perfect in patients with liver diseases [38]. The cost of the assay might also be discussed and no cost-effectiveness analysis, in comparison with PTH assays, is actually available.

'NEW' BONE TURNOVER BIOMARKERS IN DIALYSIS PATIENTS?

Recently, the International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine recommended that a marker of bone formation (serum procollagen type 1 N-propeptide, s-PINP) and a marker of bone resorption (serum C-terminal telopeptide of type I collagen, s-CTX) should be used as reference analytes for bone turnover markers in clinical studies in non-CKD populations [58]. These markers have not really thoroughly been investigated in the field of bone turnover monitoring in CKD patients yet. Another bone resorption marker, the tartrate-resistant acid phosphatase 5B (TRAP-5B) may be interesting for that purpose. We will shortly describe these markers.

TRAP-5B is secreted in the circulation by osteoclasts during bone resorption and is thus now considered as a marker of bone resorption [59, 60]. In our opinion, this marker presents different very interesting features: its serum concentrations are not influenced by kidney function, or the fasting status of the patient or circadian rhythm [61] and it is a 'true' non-collagen bone resorption marker as it correlates significantly with histological indices of osteoclast number, BFR and mineral apposition rate in uraemic patients [62]. The intra-individual coefficient of variation of TRAP-5B in haemodialysed patients is quite low (8.3%), leading to an LSC of 24% [10]. Unfortunately, no automated method is yet available for its determination, which renders it quite cumbersome by Elisa. The literature on the use of TRAP-5B in the patient's follow-up is scarce, but it was shown to correlate with PTH and other bone markers [41, 59] and with most of the pertinent histomorphometric and histodynamic parameters [7].

The amino-terminal propeptide of type 1 procollagen (PINP) consists in three subunit chains of type 1 procollagen (two pro- α 1 chains and one pro- α 2 chain), which are non-covalently linked to each other and is produced in equimolar amounts with the collagen deposited in the tissue [63]. Once

in the circulation, PINP is rapidly bound and internalized by the endothelial cells of the liver through their scavenger receptors [64]. However, in human serum, PINP is present in two major forms, an intact trimeric form and a monomeric one. Some assays recognize both forms ('Total PINP', Roche Elecsys) while other assays recognize the trimeric form only ('Intact PINP', Orion Diagnostica and IDS iSYS). The proportion of the monomeric form is elevated in patients with CKD, whereas the apparent concentration of PINP is unaffected by glomerular filtration rate in kidney disease patients when an intact assay for PINP is used [65]. PINP monomers are not cleared by conventional dialysis sessions and the LSC is of 32% for the intact assay [10]. The serum concentration of PINP shows little diurnal or seasonal variation, does not differ between men and women, but is much higher in children and adolescents due to growth. All these characteristics seem very promising, but one must admit that the literature on its use in CKD patients is scarce. One study showed, however, that serum PINP values correlated significantly more strongly than serum b-ALP values with all bone resorption markers and that PINP was significantly negatively correlated with annual changes in bone mineral density in the distal third of the radius [65].

Beta-CrossLaps (CTX) are fragments formed as a result of the degradation of type I collagen that are released during osteoclastic resorption. CTX is used as a marker for monitoring the effectiveness of bisphosphonates in patients with osteoporosis; moreover, its decrease correlates with an increase in the bone mass during osteoporosis therapy [66]. In a study involving a prospective cohort of elderly French women, higher urinary CTX levels were associated with an increased risk of hip fracture independent of the bone mass [67]. In the normal population, CTX levels reportedly display a circadian rhythm, with a higher value in the early morning [68]. This diurnal rhythm is reduced by fasting.

The KDIGO guidelines recommended that the bone-derived markers of collagen synthesis and breakdown, including CTX, should not be routinely measured in patients with chronic kidney disease stages 3–5D. The primary rationale for this recommendation was that the levels of such markers did not appear to be more effective at predicting clinical outcomes or bone histology than serum PTH or b-ALP [1].

The intra-assay coefficient of variation for the Elecsys® CTX serum assay is <2.6%; moreover, the serum CTX levels in patients undergoing haemodialysis are found to be five times that of the normal population due to the accumulation of CTX and also frequent secondary hyperparathyroidism [69, 70].

Residual diuresis, dialysis efficiency and the change during the morning and afternoon shift did not appear to influence the CTX serum levels. In patients undergoing haemodialysis, the correlation between CTX and other bone markers—including osteocalcin, TRAP-5B, b-ALP and PTH—is good. Other studies indicated similar correlations in patients undergoing peritoneal dialysis [71, 72]. However, the serum CTX levels are significantly reduced following haemodialysis, and serum samples should be obtained prior to dialysis for examination [73].

The major advantages of the use of serum CTX levels in patients undergoing dialysis are that they can be easily measured

using routine automated analysers, and they are not influenced by liver disease.

In conclusion, TRAP-5B, PINP and CTX seem to be promising markers to evaluate bone turnover and should be included in larger studies analysing their ability to predict bone turnover assessed by histomorphometry.

CONCLUSION: FROM BONE BIOPSY TO PRAGMATISM, FROM TRANSVERSAL TO LONGITUDINAL STUDIES

Nobody can question the interest of bone histology in the validation of biomarkers to assess bone turnover. Biomarkers are, and still remain, surrogate markers for bone turnover. Moreover, no marker is able to predict accurately mineralization impairment as found in mixed uraemic bone disease or in osteomalacia. These two bone lesions are admittedly less prevalent than OF or ABD [12]. Thus, while the need for a bone biopsy aiming at the diagnosis of bone turnover has become rare due to the use of bone remodelling markers, a bone biopsy remains often necessary after a fracture in CKD patients especially when treatment with a bisphosphonate is considered. The need for this gold standard to validate biomarkers is, however, also the main reason for the uncertainties remaining on the usefulness of most of them. Indeed, large collaborative multicentric studies of patients (under CKD-MBD current therapies or not) including bone histology and biomarkers measurements are still urgently needed for validating their ability to predict both bone turnover and fracture. Until now, we have to be pragmatic and, in clinical practice, we have to try to interpret at best the proposed bone biomarkers. Among these biomarkers, PTH is still the most used, including in clinical trials studying therapies of CKD-MBD. By far, PTH is *sensu stricto* not the best bone biomarker both from a physiological point of view (PTH is acting in calcium metabolism and only indirectly on bone) and a biological point of view (low stability, high LSC). In this context, we can only agree with the KDIGO recommendation to measure phosphatase alkaline and especially b-ALP which can be considered as a true 'bone biomarker'. The biological profile of b-ALP is also probably better than PTH, even if not unquestionable. We have also discussed the potential interest of other bone biomarkers. Their place in the diagnosis and monitoring of bone turnover still needs to be further studied. One risk is to multiply the tests with higher costs and potential higher discrepant results for both transversal and longitudinal evaluations. Indeed, and it is another important 'call for studies', we think it may be time to move from 'transversal' and strictly 'diagnostic' studies to 'longitudinal' and 'monitoring' studies. Again, as underlined by the KDIGO guidelines, important therapeutic decisions based on bone biomarkers assessment must take into account their longitudinal variations rather than one isolated biological result. As frequently in nephrology, this point also underlines the necessary collaboration between nephrology and clinical chemistry departments.

CONFLICT OF INTEREST STATEMENT

P.D. received honoraria from IDS. G.J. received honoraria from Fresenius, Genzyme and Amgen. E.C. received honoraria from IDS and Diasorin.

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Received for publication: 8.5.2013; Accepted in revised form: 15.5.2013