Circulating biochemical markers of bone remodeling in uremic patients

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Circulating biochemical markers of bone remodeling in uremic patients. Chronic renal failure is often associated with bone disorders, including secondary hyperparathyroidism, aluminum-related low-turnover bone disease, osteomalacia, adynamic osteopathy, osteoporosis, and skeletal β2-microglobulin amyloid deposits. In spite of the enormous progress made during the last few years in the search of noninvasive methods to assess bone metabolism, the distinction between high- and low-turnover bone diseases in these patients still frequently requires invasive and/or costly procedures such as bone biopsy after double tetracycline labeling, scintigraphic-scan studies, computed tomography, and densitometry. This review is focused on the diagnostic value of several new serum markers of bone metabolism, including bone-specific alkaline phosphatase (bAP), procollagen type I carboxy-terminal extension peptide (PICP), procollagen type I cross-linked carboxy-terminal telopeptide (ICTP), pyridinoline (PYD), osteocalcin, and tartrate-resistant acid phosphatase (TRAP) in patients with chronic renal failure. Most of the observations made by several groups converge to the conclusion that serum bAP is the most sensitive and specific marker to evaluate the degree of bone remodeling in uremic patients. Nonetheless, PYD and osteocalcin, in spite of their retention and accumulation in the serum of renal insufficient patients, are also excellent markers of bone turnover. The future generalized use of these markers, individually or in combination with other methods, will undoubtedly improve the diagnosis and the treatment of the complex renal osteodystrophy.

Bone is a specialized connective tissue composed of cells and an extracellular matrix that has the unique ability to become calcified, thereby forming, in conjunction with cartilage, the skeletal system [1]. Its metabolism is characterized by two opposed processes: formation and resorption. The formation depends on osteoblasts, which are bone lining cells responsible for the production of bone matrix constituents, such as collagen and ground substances. Bone resorption depends on osteoclasts, which are giant multinucleated cells that are usually found in contact with calcified bone surfaces and within the lacuna resulting from its own resorptive activity. The combination of these two processes is defined as bone remodeling [2]. Despite the fact that they occur along the bone surface at random and that bone formation only takes place where resorption of old bone has already occurred, both processes appear to be very well coupled in normal conditions, to the point that the equilibrium between bone resorption and bone formation will determine the gain, loss, or balance of total bone mass [1, 2].

Most of the metabolic bone diseases are characterized by an alteration in the bone resorption/formation balance. In the course of chronic renal failure, before kidney graft, situations are often found in which there is a diffuse or local increase of bone resorption such as secondary hyperparathyroidism, osteoporosis, mixed bone diseases, and β2-microglobulin osteoarthropathy [3–6]. There are also situations in which there is a rather normal or decreased bone remodeling such as aluminum-related low bone turnover, osteomalacia, adynamic osteopathy, and extraskeletal calcifications [3–13]. After renal transplantation, in addition to the persistence of one or several of the previously mentioned disorders, three histologically and less characterized entities can also be observed: painful legs syndrome, avascular bone necrosis, and immunosuppression-related bone disease (Table 1) [14].

To date, the only method of making a clear-cut distinction between high, normal, and/or low bone remodeling in these patients relies on the static and dynamic histological analyses of the iliac bone biopsy after double tetracycline labeling [3, 12]. This histological diagnosis can be complemented by clinical and radiographic signs, tomodensitometry, densitometry, scintigraphy, and the results of isotopic kinetic studies using radiolabeled calcium and bisphosphonates [15–19]. Unfortunately, the prescription of bone biopsies has markedly decreased during the last decade, probably because of the difficulties in finding...
experienced teams and appropriate laboratory facilities. Other workers have become reluctant to perform bone biopsies, claiming that the procedure is particularly painful and often refused by many patients. However, it has been proved that such a procedure is safe and free from major complications, with the incidence of complications being lower than 0.7%, without death or permanent disabilities [20, 21]. Moreover, many of the studies mentioned earlier in this article are also invasive or too expensive to be performed routinely. Thus, considerable efforts have been devoted to the development of reliable noninvasive methods to assess bone metabolism in uremic patients.

Thus, for a long time, the biochemical diagnosis or the presumption of the histological type of renal osteodystrophy has been essentially based on the plasma intact parathyroid hormone (iPTH) concentration because of its relative good correlation with bone histomorphometric parameters [22–26]. However, it is now obvious that the measurement of a single parameter such as plasma iPTH does not provide sufficient information to make a final diagnosis. First, the plasma iPTH concentration basically reflects the degree of activity of parathyroid glands. Second, given that the uremic state is often associated with a resistance of bone cells to the actions of PTH, the relationship between plasma iPTH levels and bone formation rate (BFR) is not always maintained [27–29]. Since 1992, this has led to the assumption that plasma iPTH values should be maintained between 120 and 400 pg/ml in order to preserve an appropriate level of bone remodeling [30], whereas usually normal values are below 65 pg/ml. Third, plasma iPTH values obtained with current radioimmunologic methods might be overestimating the real iPTH concentration by 1.0- to 1.5-fold [31, 32].

Fortunately, it has now become possible to estimate the degree of BFR by measuring either plasma levels of bone-specific alkaline phosphatase (bAP), osteocalcin (OC), or procollagen type I carboxy-terminal extension peptide (PICP). Similarly, the intensity of bone resorption can be evaluated by measuring serum levels of tartrate-resistant acid phosphatase (TRAP) and/or some of the multiple products resulting from the degradation of type I collagen. Other circulating molecules might also prove to be of potential interest in evaluating bone turnover, namely, bone sialoprotein, β2-microglobulin, cathepsins, nitric oxide, advanced oxidation protein products (AOPPs), advanced glycation end products (AGEs), cytokines as interleukins (IL-1, IL-6, and IL-11), soluble IL-6 receptor, tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), bone morphometric proteins (BMBPs) and their soluble receptors, growth factors such as insulin growth factor-I (IGF-I), macrophage colony-stimulating factor (MCS-F), and granulocyte-macrophage colony stimulating factor (GMCF-F; Table 2) [33–45].

In this review, we analyze several of the most recent serum markers of bone remodeling, including bAP, PICP, procollagen type I cross-linked carboxy-terminal telopeptide (ICTP), pyridinoline (PYD), deoxypyridinoline (DPD), OC, and TRAP, and evaluate their usefulness as noninvasive tests for the assessment of the degree of bone remodeling in the setting of renal insufficiency.

**Table 1. Osteo-articular complications of chronic renal failure**

| Before kidney transplantation | Osteomalacia (vitamin D deficiency) | Secondary hyperparathyroidism (HPTH-II) | Aluminum-related osteopathy | Mixed osteodystrophy (aluminum and HPTH-II) | Osteoporosis | Adynamic osteopathy | Extra-skeletal calculations | β2-microglobulin osteo-arthropathy | After kidney transplantation | Painful legs syndrome | Avascular bone necrosis | Immunosuppression-related bone disease |

**Table 2. Circulating biochemical markers of bone remodeling**

| Bone formation | Total alkaline phosphatases (tAP) | Bone-specific alkaline phosphatase (bAP) | Osteocalcin | Procollagen type-I carboxy-terminal extension peptide (PICP) | Insulin-like growth factor-I (IGF-I) | Bone resorption | Tartrate-resistant acid phosphatase (TRAP) | Pyridinoline (PYD) | Deoxypyridinoline (DPD) | Procollagen type-I cross-linked carboxy-terminal telopeptide (ICTP) | β2-microglobulin (β,m) | Bone sialoprotein | Advanced glycation end-products (AGEs) | Advanced oxidation protein products (AOPP) | Cytokines (IL-1, IL-6, IL-11, TNFα, TGFβ, IFNγ) | Growth factors (MCS-F, GMCS-F) | Other potential markers | Osteopontin | Osteonectin | Fibronectin | Bone morphometric proteins (BMBPs) and their soluble receptors | Prostaglandin E2 | Plasminogen activator factor (PAF) | Inhibitor of the plasminogen activator factor (PAF-I) | Cathepsins | Integrins | Nitric oxide |

**BONE-SPECIFIC ALKALINE PHOSPHATASE**

Six alkaline phosphatase isoenzymes have been identified so far: hepatic, intestinal, skeletal, renal, placental,
and tumoral [46–49]. Such diversity is partly due to the existence of at least four human genes, three of them on chromosome 2q34-37 and the other one on chromosome 1p36.1-34. One single gene codes for the tissue non-specific group of alkaline phosphatase (AP) that consists of liver, bone, and kidney isoforms, and these isoenzymes differ only by post-transcriptional glycosylation [50]. The bone-specific isoenzyme or bAP has a molecular weight of 80 kDa. It is neither dialyzable nor filtrable by the kidneys; therefore, its plasma concentration is not modified by variations in the renal function. Bone AP is exclusively produced by osteoblasts, first in an intracellular tetrameric form. Then it is anchored to the outer surface of the cellular membrane to inositol phosphate by a glycan ester [48]. In vitro studies indicate that bAP activity is released from the osteoblastic surface in either of two forms: an anchorless, soluble (hydrophoric) form and an anchor-intact, insoluble (hydrophobic) form [51]. Most of the bAP that appears in human serum is essentially in the anchorless or soluble form. Its plasma concentration depends on the rate of release from osteoblasts and the rate of hepatic degradation [52].

Bone AP is a robust molecule because most of its activity is still found after 72 hours at 4°C, after several years stocked at −20°C, and after several cycles of freezing and thawing. The physiological role of bAP is yet to be fully understood. However, bAP seems to be essential for the process of mineralization and bone formation. It catalyzes the hydrolysis of phosphate ester, thereby enhancing the mineralization by hydrolysis of pyrophosphate, which is an important inhibitor of mineralization, and by providing a high phosphate concentration at the osteoblastic surface [46, 53]. Moreover, molecular experiments showed that the transfection of its cDNA confers the capacity of mineralization to cells normally lacking the alkaline phosphatase gene [50]. Likewise, alkaline phosphatases may be important in the vitamin B metabolism. Transgenic mice in which the AP gene has been knocked out by homologous recombination die of sepsis and vitamin B6 deficiency [54].

Different methods have been employed in order to measure plasma AP isoforms, such as heat denaturation, chemical activators or inhibitors of specific AP isoenzymes, wheat germ lectin or concanavalin A precipitations, eventually followed by separation by agarose gel electrophoresis or by high-performance affinity chromatography [55–59]. However, these methods are expensive, laborious, time consuming, and of insufficient sensitivity to be easily and reliably applied in clinical practice. Fortunately, specific monoclonal antibodies against bAP have been successfully developed and used in radioimmunochemical and immunoenzymatic assays [60–64]. The values obtained using immunological assays such as Ostease®, IRMA from Hybritech (San Diego, CA, USA), which measures the mass of bAP, and Alkaphase®, enzyme-linked immunosorbent assay (ELISA) from Metra Biosystems, Inc. (Palo Alto, CA, USA), which measures the activity, correlate well with the values obtained by separation methods such as Isoval® from Beckman (Palo Alto, CA, USA) [65]. It has been suggested, however, that the electrophoretic method is more sensitive than the other methods when measuring low concentrations of bAP [65, 66].

As expected, using these new methods, recent observations have shown that plasma bAP is more sensitive than total alkaline phosphatases (tAP), OC, and osteocalcin in the evaluation of bone remodeling in chronic renal patients [27, 29, 67–71]. Furthermore, because the monoclonal antibody against bAP exhibits a low cross-reactivity with other alkaline phosphatases, it has become possible to differentiate the elevation of tAP seen in liver diseases from that caused by osteopathies [56, 65, 72–77].

During life, there are two age-dependent physiological peaks of plasma bAP, one during infancy and the other during puberty. Both peaks are disturbed in pediatric uremic patients in accordance with their altered longitudinal growth [78]. Nonetheless, in such children, plasma bAP correlated with iPTH and not with tAP and showed a better correlation with height velocity than tAP. Plasma bAP correlates also with bone trabecular density, the metabolically most active part of bone, pointing to its significance as a marker of bone formation [77].

In adult hemodialysis patients, values of plasma bAP higher than 20 ng/ml are constantly associated either with biological and histological signs of secondary hyperparathyroidism or with high bone turnover (Table 3) [27]. In addition, plasma bAP correlates well with iPTH. Such correlation is better than that of tAP with iPTH (Fig. 1). Plasma bAP also correlated better than iPTH and tAP with most bone histomorphometric parameters, including osteoclast and osteoblast surfaces [29]. Accordingly, when based on these bone parameters, the sensitivity and specificity of a bAP of more than 20 ng/ml can

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Abbreviations are: PICP, procollagen type-I carboxy-terminal extension peptide; ICTP, procollagen type-I cross-linked telopeptide; tAP, total alkaline phosphatases; bAP, bone-specific alkaline phosphatase [22].
reach 100% in the prediction of a high bone turnover disease and a positive predictive value of 84% [29]. This does not seem to be the case in the absence of bone biopsy evidence. If the diagnosis of secondary hyperparathyroidism is made on the basis of a iPTH of more than 200 pg/ml and a bAP of more than 20 ng/ml, then the sensitivity of bAP decreases to 56% and the specificity to 92%, probably because a lot of patients have iPTH values higher than 200 pg/ml without elevated bAP. Its high specificity suggests, however, that a plasma bAP higher than 20 ng/ml formally excludes the existence of a low or normal bone turnover [27,29]. Similar results have recently been reported by other workers [79].

In contrast, to give a plasma bAP value below which there would be a great probability of having an adynamic or low-turnover bone disease appears to be more difficult. The two bone biopsy-based studies reported so far employed different and noncomparable methods of bAP measurements [29,66]. On the other hand, the low number of adynamic bone disease (ABD) patients in one of the studies did not allow us to obtain a sensitive plasma bAP concentration predictive of ABD. However, from these observations, it can be proposed that the diagnosis of ABD in hemodialysis patients should be suspected when plasma iPTH levels are less than 150 pg/ml and that bAP levels are lower than 7 ng/ml (Ostase®) or 27 U/liter (Isopal®) [29,66]. A recent study in 41 hemodialysis patients who underwent a bone biopsy demonstrated that a plasma bAP concentration lower than 12.9 ng/ml had a sensitivity of 100%, a specificity of 94%, and a positive predictive value of 72% in the prediction of a low bone turnover [80].

The correlation between plasma bAP and iPTH shown in Figure 1 illustrates how difficult it is to separately interpret either of the values. Only 15 to 20% of the patients have both bAP and iPTH within normal ranges. In the zone of high values, there is a great variability. For example, for comparable plasma bAP levels of 60 to 80 ng/ml, some patients have iPTH values of 500 to 600 pg/ml and others 1000 to 1100 pg/ml, probably reflecting different degrees of skeletal response to PTH. Forty-five to 50% of them have either low or high bAP and iPTH. Twenty-five to 30% of the patients have increased plasma iPTH levels above 200 pg/ml and normal bAP. Less frequently observed, 10 to 15% of the patients have increased plasma bAP levels and normal iPTH (Fig. 1) [27].

In the cases of high plasma bAP associated with plasma iPTH levels, which are either low or within the limits considered as normal in such patients (less than 200 pg/ml) [30], several hypotheses have been presented to explain this phenomenon. First, the cut-off value of 20 ng/ml might be inadequate in the presence of a concomitant increase of tAP. As reported earlier in this article, the antibodies against bAP show a cross-reactivity of only 16% with tAP. However, it has been demonstrated that its radioimmunological measurement can still be considered reliable if the elevation of tAP does not exceed 2.6 times the upper normal limit [62]. Second, as the liver, kidney, and bone AP isoenzymes are encoded by the same gene, there could be an extraskeletal synthesis of bAP, either hepatic or renal. It is known that liver cells of dialysis patients are often abnormally activated by virus contamination [49, 81, 82]. Third, the production of skeletal bAP could be independent of the PTH-stimulated osteoblastic activity. Several cytokines and growth factors have been shown to exert a PTH-like action on bone cells. Among them, IL-1, IL-6, and TNF are usually found increased in the plasma of hemodialysis patients [37,83]. Fourth, the serum concentration
of certain electrolytes could influence the concentration of bAP. It has been demonstrated that bAP activity is proportional to the concentration of phosphate in the culture milieu [84]. The release of bAP activity into the circulation can be negatively regulated by calcium [51]. Likewise, an inverse relationship between plasma urea concentration and bAP activity has been observed in the rat [85]. Finally, the association of high bAP with low iPTH levels might surprisingly indicate the sign of a certain degree of skeletal aluminum overload [86].

The finding of low plasma bAP concentrations with iPTH levels higher than 200 pg/ml in 15 to 20% of hemodialysis patients may have several explanations. First, this strongly suggests that an increased plasma iPTH is not always indicative of high bone turnover in these patients. Second, a low plasma bAP level probably reflects reduced bAP synthesis by uremic osteoblasts. It has been shown that cultured osteoblasts from hemodialysis patients respond less well to several stimuli, including PTH, than those from normal individuals [87]. Third, the poor response of osteoblasts to PTH and the low plasma bAP may result from down-regulation of PTH receptors in these uremic cells [28]. Fourth, the presence of a genetic alkaline hypophosphatasemia could be responsible of the low plasma bAP levels as well [82, 88]. Finally, there is the possibility that no direct correlation exists between plasma bAP and iPTH on the ground that immunoassays overestimate the concentration of the active form of iPTH. It has recently been demonstrated that a non-(1-84) molecule of PTH that accumulates in the serum of uremic patients can interfere with the measurement of iPTH by currently used methods [31, 32, 89].

We cannot exclude that the dissociation between bAP and iPTH could also reflect an uncoupling between bone formation and bone resorption. In this regard, on the basis of bone histomorphometry, some patients have no tetracycline double-labeled bone formation surfaces and maintained bone resorption (unpublished personal results).

After surgical parathyroidectomy (PTX), the plasma bAP concentration behaves like iPTH, decreasing from an approximate mean value of 40 ng/ml before PTX to 10 ng/ml three months after PTX. The coexistence of another high turnover bone disease should be suspected if plasma bAP levels persist elevated after successful PTX, as evidenced by the decrease in iPTH [90]. After successful kidney transplantation, hypocalcemia, hyperphosphatemia, and calcitriol deficiency are often corrected, and plasma iPTH returns to normal values in 70 to 90% of patients. Plasma OC levels decrease with the normalization of renal function. Paradoxically, plasma bAP levels show a tendency to increase, probably because of the augmentation of bone turnover induced by most of the immunosuppressive drugs [14, 71]. However, bone resorption appears to predominate over bone formation, as evidenced by the bone loss observed during the first year following a kidney graft. Unfortunately, our understanding of bone metabolism during kidney transplantation is limited, as few studies with bone histomorphometrical data have been performed so far.

In most cases, the response to medical treatment in uremic patients with secondary hyperparathyroidism such as oral or intravenous calcitriol has been based on the diminution of plasma iPTH levels alone. However, it has been noted that calcitriol can have a direct effect on bone turnover independent of its effect on PTH synthesis [68]. Calcitriol decreases the expression of PTH receptors on osteoblasts and, thereby, the action of PTH on bone remodeling. Such an effect could lead to the initiation of a low bone turnover bone disease even in the presence of relatively high plasma iPTH levels [9, 68, 91]. We have observed that plasma bAP levels can help in making a decision to lower the dose or to stop calcitriol altogether [68].

In summary, all of the observations described earlier in this review suggest that in the absence of bone histology, plasma bAP alone provides useful information about the rate of bone remodeling in hemodialysis patients. Its combination with plasma iPTH improves the sensitivity, the specificity, and the predictive value in the diagnosis of the type of bone turnover. When there is a dissociation between both parameters, the sensitivity of bAP is higher than that of iPTH, and it is, therefore, a better indicator of bone remodeling. Nevertheless, thus far, bone histomorphometry appears to be still indispensable in making the distinction between patients with normal bone and patients with adynamic bone disease and in situations in which there is a discordance between plasma bAP and iPTH values. Again, it should be stressed that an independent augmentation of either plasma iPTH or bAP may not always correspond to an increased bone turnover.

The idea that in hemodialysis patients one must try to maintain plasma iPTH levels three to four times higher than the normal values should not be taken as a general rule, with each patient requiring an individual evaluation. As described before, paradoxically, histomorphometric signs of high bone remodeling can be seen in patients with normal or low plasma iPTH and vice versa.

**OSTEOCALCIN OR GLA PROTEIN**

Osteocalcin or GLA protein (protein containing γ-carboxyglutamic acid) is the most abundant noncollagenic protein of the bone matrix [92–94]. γ-Carboxylation occurs principally at glutamine residues 17, 21, and 24 and depends on the presence of the cofactor vitamin K. Carboxylation of the glutamic acid at position 17 seems to be essential for the structural and spatial conformation
of the molecule allowing the interaction with hydroxyapatite crystals [95, 96]. When there is a deficiency in vitamin Ks (K1 or phylloquinone and K2 or menaquinones: MK-4, MK-7, and MK-8), the carboxylate fraction of OC tends to decrease and can be associated with a reduced bone mass and a high risk of fractures (abstract; Nakazawa et al, Nephrol Dial Transplant 13:A43, 1998) [97–100]. Indeed, intestinal absorption of vitamin K is in part regulated by apolipoprotein E (apoE). Recently, an association has been demonstrated between the level of plasma vitamin K and bone density, and the risk of fractures in hemodialysis patients and in postmenopausal women having the polymorphism E4 in the apoE gene [96, 100, 101]. In addition, another recent study has suggested the possibility that a deficiency in the menaquinone MK-7 could be one of the risk factors predisposing to adynamic bone disease in hemodialysis patients (abstract; ibid). The physiological explanation of this is probably that the incorporation of vitamin K-charged lipoproteins into intestinal, hepatic, and bone cells depends exclusively on the attachment of apoE to its specific receptor. In the presence of the apoE4 phenotype, the incorporation of vitamin K seems to be reduced [100].

Human osteocalcin possesses 49 amino acids and is produced by only osteoblasts and odontoblasts under the control of 1,25-OH2D3. Incidentally, it was on the ity in the dosage of OC with most of the current assays the incorporation of vitamin K seems to be reduced could be one or more of these fragments measurable [95–100]. The synthesis of osteocalcin by mature osteoblastic cells is probably one of the signals used by PTH to slow osteoblastic activity and thereby bone formation [105–108].

Numerous studies have shown the degree of interest in the use of plasma osteocalcin measurements in several metabolic bone diseases [61, 109–112]. It has always been considered to be a useful marker of the rate of bone formation. However, its use in the context of renal osteodystrophy is still hampered by several problems. First, many osteocalcin fragments of yet unknown function are retained in the plasma of uremic patients. Second, at least three forms of intact osteocalcin can be measured in the plasma: total, carboxylated, and decarboxylated. Third, the intact molecule is rapidly degraded at room temperature. Thus, the concentration measured depends on the characteristics of the antibodies used in the assay. Many of these antibodies recognize the intact molecule but also some of the fragments. As already mentioned, osteocalcin fragments are also liberated during bone matrix degradation. It is possible that in the future there could be one or more of these fragments measurable in the plasma that is specific for bone resorption [98, 113–115]. Finally, perhaps because of the limitations previously described, there is a great intra-analysis variability in the dosage of OC with most of the current assays [15, 92, 19–115].

In patients with chronic renal failure, circulating intact osteocalcin represents 26% of total osteocalcin. The remaining 74% comprises mainly four fragments: N-terminal, midregion, midregion C-terminal, and C-terminal [116, 117]. A recent study compared the measurement of osteocalcin using six different assays: ELSA-OST-NAT IRMA (Cis Bio Int.), ELSA-OSTEO IRMA (Cis Bio Int.), Osteocalcin IRMA (Nichols Institute, San Juan Capistrano, CA, USA), OSTK-PR RIA (Cis Bio Int.), OSCA Test Osteocalcin RIA (Henning), and Osteocalcin RIA (Nichols). The authors concluded that there was a good correlation of Osteocalcin values obtained with these kits when they examined normal individuals, premenopausal, and osteoporotic women. In contrast, there was no correlation when uremic patients and patients with Paget’s disease were studied [118].

Using the ELSA-OSTEO IRMA kit (Cis Bio Int.), which uses two antibodies, one recognizing the end N-terminal fragment and the other recognizing a midregion amino acid sequence, we and others have shown osteocalcin values four to six Z-scores higher in hemodialysis patients than in normal individuals [15, 61, 119, 120]. In spite of this accumulation, the plasma osteocalcin concentration demonstrated good sensitivity in making the distinction between patients with hyperparathyroidism and those with normal or low bone turnover. The values were 555 versus 198 ng/ml, respectively (Table 3).
[119]. As with other biochemical markers, diagnostic sensitivity was low when the aim was to differentiate patients with adynamic bone disease from those with normal bone turnover [15, 61]. Although weaker than bAP and iPTH, the correlations of plasma osteocalcin with bone histomorphometric parameters in hemodialysis patients were quite good [27, 29, 119].

The results of these studies demonstrate the limitation of the use of plasma osteocalcin as a biochemical marker of bone remodeling in patients with impaired renal function and in patients under hemodialysis treatment. Obviously, a clear understanding of the physiological role of osteocalcin and its fragments is still lacking. The development of new assays will certainly increase its sensitivity in the evaluation of renal osteodystrophy [121, 122].

PYRIDINOLINES

Eighty-five to 90 percent of bone matrix consists of type I collagen (Fig. 2) [123–125]. It is this molecule that mostly provides the mechanical force of bone tissue because of its particular intramolecular and intermolecular cross-links, which are responsible for the tensile strength of collagen fibers [125]. Mutations in the gene of type I collagen α1 chain have been associated with low bone density and osteoporosis [126]. The mechanisms leading to the formation of these cross-links are still poorly understood, although several biochemical processes have been described. First, lysine residues located at specific regions in the type I collagen α1-chain (amino acids 103 and 1043) are hydroxylated (Fig. 3) [124–128]. Then, in presence of copper, the enzyme lysyloxidase deaminates hydroxylated lysine residues giving rise to aldehyde groups. It is thought that PYD [hydroxylsylylpyridinium (HP)] is formed from the condensation, ring closure, and oxidation of one hydroxysine and two hydroxysine-derived aldehyde molecules, and DPD [lysylpyridinium (LP)] is formed from one lysine and two hydroxysine-derived aldehydes (Fig. 3) [124, 129, 130]. To date, only these two main collagen intermolecular cross-link molecules have been thoroughly studied; however, another kind of intramolecular cross-link, which is formed from the condensation of two aldehyde groups and serves to joint the α1 chains of the same collagen molecule, has also been described [123–132].

Both PYD and DPD are present in bone and cartilage, although most of the pyridinium cross-links found in the cartilage are in the form of PYD, in contrast with DPD, which is predominantly in bone [123, 124, 133]. Metabolically, these molecules are nonreducible and cannot be reused during new collagen synthesis (abstract; Robins et al, J Bone Miner Res 6:C642, 1991) [134]. Furthermore, diet-derived cross-link molecules are not absorbed from the intestine [134]. Following bone resorption and bone collagen degradation by specific osteoclast-related enzymes, PYD and DPD are released into the circulation, and because of their low molecular weight (429 to 591 Da), they are normally excreted into the urine, as much as 40% in their free forms [124, 135–138]. Usually, serum levels of PYD and DPD are extremely low and undetectable in normal individuals; therefore, their measurement is commonly performed in urines. The renal clearance is different for free and conjugated forms. The fractional clearance of free forms is higher than one, suggesting tubular secretion, whereas the fractional clearance of the conjugated forms is less than one [139]. Based on these properties, it has been suggested that quantitative analysis of PYD and DPD in the serum as well as in the urine could provide valuable information about bone resorption rate.
In the last two decades, intense research has focused on the development of methods capable of accurately assessing urinary and plasma concentrations of PYD and DPD, as well those found in a diversity of tissues including bone, cartilage, tendon, dentin, skin, cornea, and vessels. The first methods were based on conventional chromatography after borohydride reduction of collagen cross-links, high-performance liquid chromatography (HPLC) using aminopropyl-silica column and radioactivity monitoring, followed by HPLC and fluorescence detection techniques. Last, reproducible results have been obtained using reversed-phase, ion-pair quantitative HPLC (abstract; Robins et al, ibid) [124, 136–141]. Certainly, HPLC-based techniques are highly quantitative and reliable; however, they are too time consuming and cumbersome to become a routine clinical application.

Fortunately, specific polyclonal and monoclonal antibodies have recently been successfully developed for the direct measurement of PYD and DPD (abstract; Robins et al, ibid) [142, 143]. The concentrations of PYD and DPD measured by ELISA methods are usually 4 to 10 times lower than the value obtained by HPLC [137, 142]. Nevertheless, the values obtained with either method are excellently correlated ($r = 0.85$ to $0.90$). In addition, some authors have even reported that the measurement of DPD by ELISA is more reliable than by HPLC [136, 137, 142, 143]. Moreover, measurement of PYD and DPD, released after hydrolysis of the urine sample, either by HPLC or ELISA (Table 4), has been shown to serve as an excellent marker of bone resorption rate in malnourished children, osteoporotic and postmenopausal women, primary and secondary hyperparathyroidism, rheumatoid arthritis and osteoarthritis, Paget’s
Table 4. Measurement of pyridinolines in metabolic bone disease

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Abbreviations and symbols are: DPD, deoxypyridinoline; PYD, pyridinoline; HPLC, high performance liquid chromatography; ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay; =, non-modified; ↑, increased; ↓, decreased.

Antibodies against crosslink type-I collagen telopeptide

disease, acromegaly, hyperthyroidism, in patients with tumor-associated hypercalcemia, and in uremic patients (Table 4; abstract; Robins et al, ibid) [93, 119, 134–137, 140–150]. In addition, an Italian group has recently demonstrated the increase in urinary excretion of PYD and DPD in normocalcemic patients with kidney transplantation was more sensitive than plasma measurement of iPTH, bAP, and tAP for the diagnosis of secondary hyperparathyroidism [150]. In another study dealing with patients treated by peritoneal dialysis, PYD and DPD concentrations in serum, urine, and dialysis fluid were well correlated with plasma iPTH and bAP levels. Moreover, the authors observed that circulating PYD and DPD levels progressively increased with the duration of renal failure and with the time spent on peritoneal dialysis [149]. Few studies have been performed concerning the metabolism of PYD and DPD in uremic patients with or without bone diseases. Two recent observations have suggested that urinary cross-link excretion was unaffected by renal dysfunction [142, 151]. However, none of the patients included in these two studies had advanced renal failure (creatinine clearance below 10 ml/min) or were undergoing dialysis therapy. As expected, serum levels of PYD and DPD increased in patients with severe renal failure. In a study dealing with patients treated by hemodialysis and peritoneal dialysis, the authors observed that circulating PYD and DPD levels were 50 to 100 times higher than in controls, decreased postdialysis, and could be measured in the dialysate [149]. Recently, using a competitive enzyme immunoassay for the measurement of PYD in the serum (Metra Biosystems), it has been found that serum levels of PYD were significantly increased in osteoporotic women, patients with hyperthyroidism, and patients with primary hyperparathyroidism [152]. With this ELISA, we assessed serum-free PYD levels in a group of 37 uremic patients undergoing hemodialysis. We found that these patients had markedly increased (10- to 30-fold) serum PYD levels compared with normal individuals (Table 3) [119]. High serum PYD levels were generally associated with high turnover bone disease and were positively correlated with bone resorption and bone formation parameters. These correlations were comparable to those found between serum bAP and bone histomorphometric
parameters. However, serum PYD was correlated significantly better with bone histology than either serum iPTH or osteocalcin [119]. No difference was observed in serum PYD levels between patients dialyzed on high-flux or standard cellulose dialysis membrane, which may indicate that the type of dialysis membrane would not have a major influence on serum PYD levels. In a recent study, using a commercial radioimmunoassay from Nichols, it has been observed that serum DPD levels higher than 21 nmol/liter had a sensitivity of 88% and a specificity of 93% in the diagnosis of a high remodeling disease [81].

From these studies, it can be argued that serum PYD and DPD may be useful biochemical markers of bone resorption in chronic renal patients. It seems that the sensitivity and specificity of these markers are greater in cases of increased bone remodeling. Hopefully, more sensible measurements of collagen cross-linked molecules will be developed and will become common tests in clinical practice.

**TARTRATE-RESISTANT ACID PHOSPHATASES**

Acid phosphatases are lysosomal enzymes produced by osteoclasts, prostate, uterus, pancreas, spleen, blood red and white cells, and platelets. Analogous to tAPs, the different acid phosphatases can be identified and measured by enzymatic, electrophoretic, or chromatographic methods [152–155]. Thus, the bone-specific acid phosphatase (TRAP), or the purple acid phosphatase because of its iron content, has been proposed as a potential marker of bone resorption. TRAP has a molecular weight of 30 kDa but circulates in the serum as a 250 kDa complex, which also contains calcium [152]. Serum TRAP had previously been determined by virtue of its resistance to tartric acid. However, many questions have been raised about its osteoclast specificity. Moreover, even though TRAP appears to be essential to the process of bone resorption, its physiological role on the skeleton is yet ill understood. It is known that TRAP dephosphorylates a variety of bone matrix proteins such as osteopontin and sialoprotein [156]. In addition, transgenic mice with targeted disruption of the TRAP gene develop an osteoporotic phenotype with altered osteoclastic function [157]. In spite of these transgenic studies, little is known about the TRAP gene [158, 159]. It belongs to a family of genes that codes for iron transport proteins in the placenta. Accordingly, the TRAP gene seems to be regulated at the transcriptional level by extracellular iron concentrations. It possesses an iron-sensitive DNA sequence (iron-response element) on its 5’ untranslated region [160].

In some studies, the amount and the activity of TRAP seems to correlate with bone resorption rate (abstract; Lam et al, Clin Chem 30:457, 1984) [152, 154, 161–163]. However, few studies have been performed in uremic patients. A decade ago, a correlation between serum TRAP and bAP levels was demonstrated using electroraphic methods [155]. Another group, using an enzymatic method, has recently shown a correlation between serum TRAP activity and the number of osteoclasts and the percentage of eroded bone surface (abstract; Maluche et al, J Am Soc Nephrol 2:337, 1991). Similarly, serum TRAP appears to correlate with plasma tAP and iPTH [164–167].

Specific antibodies against TRAP have been successfully developed and applied in an ELISA [154, 168]. With this method, elevated levels of TRAP have been found in the serum of patients with primary hyperparathyroidism. Surprisingly, however, it was found that normal subjects had measurable amounts of serum TRAP [154]. It is likely, therefore, that the specificity of this ELISA assay may not be perfect, in that it might be recognizing other acid phosphatases or the same TRAP produced by other cells. Significant TRAP activity has recently been detected in osteoblasts and osteocytes [154].

To date, the value of serum TRAP measurement in uremic hemodialysis patients still remains to be established. The sensitivity of enzymatic methods and the few available immunological assays are still low. Certainly, progress in understanding its physiological role as well as its biochemistry and immunology should lead to the development of more sensitive assays [168].

**PROCOLLAGEN TYPE I CARBOXY-TERMINAL EXTENSION PEPTIDE**

Type I procollagen carboxy-terminal extension peptide (PICP) results from the extracellular cleavage of a molecule of type I procollagen at the moment of its incorporation into the bone matrix (Fig. 2) [169, 170]. PICP has a molecular weight of approximately 100,000 Da. Its plasma concentration is not altered by renal failure because its degradation takes place in the liver through the mannose-6-phosphate receptor. Specific antibodies against PICP have been developed and are actually employed to measure its plasma concentration [119, 169–176]. Because PICP is produced by osteoblasts during the process of bone formation, plasma PICP concentration has been proposed as a biochemical marker of bone formation.

Nondialyzed patients with chronic renal failure have significantly increased plasma PICP levels [171]. However, this increase does not correlate with other humoral markers of bone turnover or with bone histomorphometrical parameters. In the same study, it was observed that patients receiving 1,25OH₂D₃ had higher plasma PICP levels than patients without treatment. In patients already treated by hemodialysis, the results are contradic-
Some workers have found that plasma PICP concentration provides useful information regarding the degree of bone formation. They also observed that PICP levels correlate quite well with plasma bAP, osteocalcin, iPTH, and bone formation rate (BFR) [169]. By analogy with plasma bAP, PICP levels increased significantly during the first two weeks after surgical PTX, probably as a consequence of diminished osteoclast activity following the reduction of iPTH levels and an increase in mineral deposition [119, 173, 177]. However, our own observations have not demonstrated any great value of plasma PICP in the diagnosis of the type of bone remodeling in hemodialysis patients [29, 119, 170, 177]. Additional studies remain to be performed in order to define its value in renal osteodystrophy.

**PROCOLLAGEN TYPE I CROSS-LINKED CARBOXY-TERMINAL TEOPEPTIDE**

Telopeptides are small amino acid sequences originating from the nonhelical ends of collagen molecules. For instance, procollagen type I cross-linked carboxy-terminal telopeptide (ICTP) is a part of type I collagen released during bone resorption and containing cross-linking molecules (Fig. 2). One molecule of ICTP is composed of two C-terminal fragments from the α1 chain of type I collagen, one helical segment from the α1 or α2 chain of collagen, and a cross-linker (PYD or DPD). Its molecular weight is approximately 9,000 Da [178]. ICTP is released into the circulation after the degradation of several mature collagen molecules of the bone matrix [125, 169, 170]. Therefore, plasma ICTP levels should reflect the degree of bone resorption.

These assays of plasma ICTP use polyclonal antibodies against a small group of cross-linked peptides of the human type I collagen [179] or against a group of eight amino acids (EKHDGGGR) that correspond to the C-telopeptide domain of the α1 chain of type I collagen [180]. Several observations have demonstrated a good correlation between plasma ICTP and histomorphometric parameters of bone resorption in patients with a diversity of metabolic bone diseases [178, 181]. However, other clinical studies have not supported this method as a specific indicator of bone resorption rate [145]. In the case of patients with chronic renal failure, ICTP tends to accumulate in the serum with the decline in renal function and even further in hemodialysis patients. The few studies performed in hemodialysis patients have not supported its use as a humoral marker of bone remodeling [119, 145, 182].

**β2-MICROGLOBULIN, ADVANCED END-GLYCATION PRODUCTS, AND BONE SIALOPROTEIN**

β-2 Microglobulin (β2m) accumulates in the serum of chronic renal patients and can lead to β2m-related amyloid deposits in osteoarticular tissues [183]. In addition to being an indicator of an inflammatory state, it has been suggested that β2m could serve as a bone remodeling marker as well [184]. Serum β2m concentrations have been found to correlate with serum TRAP in postmenopausal women [185, 186]. Moreover, in a recent study, we have observed that patients with severe secondary hyperparathyroidism showed significantly higher serum β2m levels than patients with normal bone turnover. In addition, serum β2m correlated with serum bAP, PYD, and osteocalcin levels [183].

Regarding AGEs, these also have a tendency to accumulate in the serum of uremic patients. Recent studies have shown that AGEs were able to stimulate bone resorption probably through the stimulation of IL-6 production [35, 187]. Because IL-6 is one important mediator of the action of PTH on bone cells [188], it is possible that AGEs could influence bone metabolism in hemodialysis patients. Finally, bone sialoprotein, a glycoprotein of the bone matrix with a molecular weight of 70 to 80 kDa, has been shown to be a bone indicator of bone resorption in cancer patients and in menopausal women [189].

**CONCLUSIONS**

In the absence of bone biopsy information, it seems that there is no ideal marker of bone remodeling in uremic patients. The time has come to regard the plasma iPTH concentration as the marker of parathyroid activity and not as the indicator of bone turnover. As mentioned before, increased plasma iPTH levels are not always synonymous with high turnover bone disease and vice versa. Among the biochemical markers of bone remodeling revised herein, plasma bAP concentration appears to be more sensitive than iPTH, osteocalcin, TRAP, PYD, PICP, and ICTP in distinguishing the type of bone disease and, in particular, estimating the BFR. It is not yet clear whether it can separate patients with adynamic bone disease from those with normal bone remodeling. Renal osteodystrophy has been considered for a long time as a disease in which coupling between resorption and formation was thought tight. It now becomes clear that other factors besides PTH can influence bone turnover and induce a decoupling between resorption and formation. A specific marker of bone resorption would therefore be suitable. Serum DPD appears to fill such a requirement. However, further developments are necessary to improve its sensitivity and accuracy. The future generalized use of these markers, individually or in combination with other methods, will undoubtedly improve the diagnosis and the treatment of renal osteodystrophy. Further studies, based on bone histomorphometry, are clearly needed to better understand the value of these
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