Biochemical markers of bone turnover in the diagnosis of renal osteodystrophy: what do we have, what do we need?

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Introduction

Chronic renal failure (CRF) is often associated with bone disorders (renal osteodystrophy) including secondary hyperparathyroidism, mixed uraemic osteodystrophy, low turnover osteomalacia, adynamic bone disease, aluminium toxicity-related osteopathy and β/2-microglobulin bone deposition. Bone histology remains the gold standard for the diagnosis of renal osteodystrophy, and the distinction between high and low bone turnover disease in these patients frequently requires invasive and costly methods such as bone histomorphometry (including static and kinetic variables after double tetracycline labelling).

The ideal biochemical marker of bone turnover should be unique to bone, reflect total skeletal activity and be well correlated with histomorphometric and radiocalcium kinetics results. We are still looking for a specific and sensitive serum biochemical test for monitoring bone turnover in uraemia. During the last years, several enzymes and matrix proteins synthesized by osteoblasts and protein fragments released after bone matrix breakdown, have been proposed as serum biochemical markers of bone formation and bone resorption. The interpretation of serum or plasma concentrations of these markers is hindered, in CRF, by different factors (circadian rhythms, diet, age, gender, menopause, liver function, clearance rates).

The development of highly precise radioimmunometric assays for intact parathyroid hormone (iPTH) in the last decade has shown that measurement of iPTH is a useful predictor of bone histology and can serve as a non-invasive tool in distinguishing between high turnover (HTBD) and normal or low turnover bone disease (N/LTBD) when large groups of patients are considered [1]. However, in an individual patient, serum iPTH alone frequently is unable to distinguish adynamic bone from hyperparathyroid bone disease [2]. For that reason, different optimal iPTH cut-off levels have been proposed to optimize the sensitivity and the specificity of this serum marker [3,4] (Figure 1).

Besides iPTH, the most commonly explored biochemical serum markers of bone remodelling are listed in Table 1.

Biochemical serum markers of bone formation

Alkaline phosphatase (AP) is a glycosylated protein produced by at least five different organs: liver, bone, kidney, intestine and placenta. In bone, AP is produced by osteoblasts and osteoblast precursors, and participates in the mineralization process. Several laborious and time-consuming techniques have been used to detect the osteoblast enzyme, bone-specific alkaline phosphatase (bAP), and to enhance the sensitivity of this marker, including: heat inactivation, wheat germ lectin or concanavalin A precipitation, inhibition by amino acids and urea, high-performance affinity chromatography and agarose gel electrophoresis. The development of monoclonal antibodies specific for bAP provided the basis for a more specific index of bone formation [5].

In a recent study, in 42 haemodialysis patients, we showed that plasma bAP (measured with a new direct immunoradiometric assay—IRMA) was better correlated with bone formation and bone resorption histomorphometric parameters than iPTH or total AP [6]. Values of bAP >20 ng/ml had a sensitivity of 100% and a specificity of 100% for the diagnosis of HTBD, and there was an excellent correlation between plasma bAP and the bone formation rate (BFR). When these limits of bAP values were associated with serum iPTH >200 pg/ml, the positive predictability value for the diagnosis of HTBD increased from 84 to 94%. These results have been confirmed by other groups not only in HTBD patients [7], but also in the diagnosis of adynamic bone disease (ABD) [8], in the latter case by using the agarose gel electrophoresis method for detection of low levels of plasma bAP.
Predictive value of serum iPTH levels for bone turnover in dialysis patients

Osteocalcin (BGP) represents one of the most abundant non-collagenous bone proteins, but is also present in dentin and calcified cartilage. It is produced by osteoblasts and has been regarded as a marker of bone formation. Serum intact osteocalcin seems to reflect the excess of produced protein not integrated in bone matrix. The new immunoradiometric assays, which use specific monoclonal antibodies to measure only intact osteocalcin [9], may be more accurate, since they will exclude BGP fragments. Nevertheless, BGP suffers from poor stability and is removed via the kidneys. After the menopause, its serum concentrations increased less than those of bAP, showing inferior sensitivity in the diagnosis of bone turnover in this osteopathy [10].

Procollagen type I carboxy-terminal propeptide (PICP) have been used as serum markers of bone formation since they are by-products of collagen synthesis, and collagen type I is the most abundant protein of bone, accounting for >90% of the proteins of bone matrix. In pre-dialysis chronic renal failure, PICP evidenced high correlation with dynamic histomorphometric parameters, but not with static histomorphometric parameters nor with other humoral markers of bone turnover [11]. We found increased levels of PICP in our haemodialysis patients, but we did not observe a significant correlation with any of the histomorphometric parameters examined [12], which is in accord with the results of Mazerferro et al. [13].

In a population of 18 patients on haemodialysis, Hamdy et al. found inappropriately increased PICP concentrations in patients with aluminium overload (38% of their patients) [14], also indicating a relatively low specificity and lack of responsiveness of PICP.

Table 1. Biochemical serum markers of bone turnover in renal osteodystrophy

<table>
<thead>
<tr>
<th>Markers of bone formation</th>
<th>Markers of bone resorption</th>
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<tr>
<td>Alkaline phosphatase (AP)</td>
<td>Tartate-resistant acid phosphatase (TRAP)</td>
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<tr>
<td>Bone-specific alkaline phosphatase (bAP)</td>
<td>Type I collagen cross-linked telopeptide (ICTP)</td>
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<tr>
<td>Osteocalcin (OC, BGP)</td>
<td>Pyridinoline (Pyr, free and total)</td>
</tr>
<tr>
<td>Procollagen type I carboxy-terminal propeptide (PICP)</td>
<td>Deoxypyridinoline (Dpy, free and total)</td>
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Biochemical serum markers of bone resorption

Tartrate-resistant acid phosphatase represents the fraction of acid phosphatase that shows resistance, in its mobility on acrylamide gel, to inhibition by tartrate. This is a feature of the bone acid phosphatase, but the degree of specificity to osteoclasts is not well defined, and a similar fraction enzyme is produced by other cell types [15] (as we can see, for instance, in hairy cell leukaemia and Gaucher’s disease). The recent development of immunoassays for this enzyme molecule should offer increased specificity, but clinical data is still insufficient [16,17].

Assays for type I collagen cross-linked telopeptide (ICTP) using a polyclonal antibody have been developed [18] but with disappointing clinical results [10]. Its elimination rate depends on the glomerular filtration rate, and its serum levels respond poorly to hormone replacement therapy after menopause [19].
Biochemical markers of bone turnover in the diagnosis of renal osteodystrophy

In our dialysed patients, we did not observe a significant correlation between serum ICTP and any static or dynamic histomorphometric parameter, suggesting that ICTP is not a sensitive marker of bone metabolism in uraemia [12]. On the contrary, Mazzaferrro et al. found ICTP serum levels correlated to serum AP, bAP, iPTH and to some histomorphometric indices of bone turnover, and pointed out ICTP as a useful humoral marker of bone turnover in dialysis renal osteodystrophy [13].

Pyridinoline cross-links of collagen exist under two chemical forms: hydroxypyridinoline (or pyridinoline—Pyr) and lysylpyridinoline (or deoxypyridinoline—Dpy). These molecules are markers of type I and II collagen breakdown, and their urinary excretion has already been validated as an excellent marker of bone resorption in several metabolic bone diseases. We have demonstrated recently, for the first time, that serum Pyr concentrations can be measured reliably with an accurate competitive enzyme immunoassay [20] in dialysis patients, and that these patients had markedly increased serum Pyr compared with normal individuals. Among our 37 haemodialysis patients, those with the highest rate of bone resorption showed the highest values of serum Pyr. We observed a good correlation between serum Pyr, the number of osteoclasts/mm² and the percentage of bone covered by osteoclasts [12].

Besides the biochemical serum markers of bone turnover listed above, other mediators involved in the process of bone remodelling (including local activators, such as cytokines and their inhibitors) might have a role in the non-invasive diagnosis of renal osteodystrophy. Recently, we observed that patients with histological HTBD had greater serum β2-microglobulin (β2M) than patients with N/LTBD [21], and that serum β2M correlated with two serum markers of bone formation rate, namely osteocalcin and bAP, and with a specific serum marker of bone resorption, serum free pyridinoline, but not with intact PTH. The association of high serum β2M with higher bone cell number and with serum markers of bone turnover suggests that β2M could be either a direct or an indirect activator of bone cells or another marker of bone cell activity.

The involvement of cytokines and their inhibitors in the process of bone remodelling has been demonstrated by different groups, and there has been some speculation by others about its hypothetical role in renal osteodystrophy [22]. Interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α) are known to be direct stimulators of osteoblastic proliferation and secretion [23], and IL-6 is produced by osteoblast cells and possesses a bone-resorbing activity, probably by increasing the formation of mature osteoclasts from hematopoietic progenitors [24]. The balance between these cytokines and their specific inhibitors is impaired in haemodialysis patients [25,26]. Recently, we had the opportunity to measure circulating cytokines and cytokine inhibitors, by specific enzyme-linked immunoabsorbent assay (ELISA), in the sera of 16 chronic haemodialysis patients, sampled at the time of transplant.

In conclusion, the evaluation of bone turnover should include a combination of different markers, so that the balance between bone formation and bone resorption can be evaluated adequately. The immunoassays of human bAP for bone formation and of Pyr for bone resorption seem to be currently the most sensitive and specific serum markers of bone turnover in renal osteodystrophy. These markers should be combined with serum iPTH, with serum β2M and with serum aluminium. Finally, in the presence of previous aluminium overload, a bone biopsy frequently is needed to determine the exact type of bone lesion, and to quantify the aluminium bone deposits.

References


