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Clinica Chimica Acta 371 (2006) 107–111
www.elsevier.com/locate/clinchim

Evaluation of different bone markers in hemodialyzed patients

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Received 17 January 2006; received in revised form 22 February 2006; accepted 22 February 2006
Available online 2 March 2006

Abstract

Background: Routinely, nephrologists rely on different biochemical markers like intact PTH (iPTH), bone-specific alkaline phosphatase (BALP), plasmatic calcium and phosphate. The aim of the present study was to evaluate different other bone markers like N-terminal propeptide of type 1 procollagen (P1NP), active isoform 5b of the tartrate-resistant acid phosphatase (TRAP 5b) and β-crossLaps® (CTXS) as well as full-length PTH (wPTH), presumed non-(1–84) PTH, and their ratio in the diagnosis of renal osteodystrophy with high and low turnover. We also determined 25 hydroxyvitamin D (25VTD), 1–25 dihydroxyvitamin D and homocystein (HCY).
Methods: We performed those parameters on 73 patients with end-stage renal disease according to the manufacturers' instructions.
Results: There were very strong correlations between the bone markers concentrations, particularly between BALP and P1NP ($r=0.953$). We did not observe any correlation between the ratio whole PTH/non-(1–84) PTH and any of the usual bone markers. This ratio was significantly ($p<0.05$) higher in low and high bone turnover patients than in normal patients according to the K/DOQI. We found a correlation between low levels of 25VTD and high levels of HCY.
Conclusions: BALP offers the best clinical and analytical profile as the easier marker of choice in hemodialyzed patients for the diagnosis of bone disease.

Keywords: PTH; Bone alkaline phosphatase; Homocystein; Hemodialyze; 25-OH vitamin D

1. Introduction

Patients undergoing long-term hemodialysis develop bone complications known as renal osteodystrophy (ROD). Such a terminology includes high turnover bone disease associated with secondary hyperparathyroidism, low turnover bone disease including adynamic bone disease and osteomalacia (defect of calcification) and mixed forms. The gold standard for the diagnosis of ROD is the histomorphometric and histochemical examination of a bone biopsy specimen [1]. However, this invasive procedure is only performed in specialized centres and is not easily accepted by the patients. Bone mineral densitometry [2] has been evaluated in the diagnosis and/or monitoring of renal bone disease, but data are clearly insufficient. Routinely, nephrologists rely on measurements of different serum biochemical markers to diagnose these renal bone diseases, make the follow-up of the patients and initiate appropriate treatment. Among those markers, the most frequently used are the intact PTH (iPTH) and the bone-specific alkaline phosphatase (BALP) as well as plasmatic calcium (pCa) and phosphate (P). The aim of the present transversal study was to evaluate different other bone markers in the diagnosis of renal osteodystrophy with high and low turnover: these markers were the N-terminal propeptide of type 1 procollagen (P1NP), the active isoform 5b of the tartrate-resistant acid phosphatase (TRAP 5b) and the degradation products of C-terminal telopeptides of type 1 collagen or β-crossLaps® (CTXS). In addition, we measured the full-length PTH (wPTH), the presumed non-(1–84) PTH given by the subtraction of the wPTH from the iPTH and their ratio. Determinaton of 25 hydroxyvitamin D (25VTD), 1–25 dihydroxyvitamin D (1,25VTD), albumin, folic acid and homocystein, often associated with a risk of osteoporotic fracture [3] were also performed.

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doi:10.1016/j.cca.2006.02.029

2. Material and methods

The study was performed in spring (May 2005). All blood samples were drawn from the arteriovenous fistula immediately before the start of a hemodialysis session. As pre-analytical conditions become more and more important [4], great care was taken for the specimens' handling: after collection, samples were directly brought to the laboratory, then centrifuged at 4000 rpm for 10 min at 4 °C. Sera were prepared and stored at −20 °C; they were rapidly thawed and centrifuged before assaying. For each marker, all patient serum samples were measured in the same assay run.

2.1. Population

Seventy three patients (28 women, 45 men) with end-stage renal disease from the hemodialysis unit of the University Hospital of Liège were included in the study. The median age was 71 years, ranging from 22 to 92 (mean ± SD: 65.2 ± 16.5 years). All patients underwent chronic hemodialysis treatment three times a week and the median vintage on hemodialysis was 38 months (88 ± 71 months). The main underlying renal diseases were vascular nephritis (14), chronic glomerulonephritis (12), diabetic nephropathy (10) and polycystic kidney disease (9). Twenty-one patients were receiving active vitamin D metabolites. None of them were under treatment with native vitamin D3 or calcimimetics. Eleven patients (15%) were supplemented with folic acid; calcium was the main phosphate binder, present in the treatment of 64 patients (88%), alone (72%) or in association with sevelamer, which was present alone in the treatment of 3 patients. None of the patients were receiving aluminium salts as phosphate ligand. The concentration of calcium in the dialysate bath was 1.5 mmol/l (45 patients) or 1.25 mmol/l (28 patients).

2.2. Biological markers

Intact and whole PTH were measured using the *Scantibodies duo-PTH immunoassay* (Scantibodies Laboratories Inc., Santee, CA,USA). The iPTH immunoassay uses two polyclonal human antibodies: the first raised against PTH(39–84) and immobilized onto plastic beads, the second one, directed against PTH(7–34) and labelled with ^{125}I. To determine the wPTH, the polyclonal antibody recognizing PTH(39–84) is the same as in the intact assay, but the ^{125}I-labelled polyclonal antibody is directed against the first four amino acids of the N-terminal sequence of the PTH. This assay does not cross-react with PTH(7–84). The non-(1–84)PTH was estimated by subtracting the wPTH concentration from the iPTH concentration. The inter-assay CV for both determinations is <8%. iPTH was also measured using the *Liaison® N-tact PTH assay* (DiaSorin, Saluggia, Italy). This chemiluminescence immunoassay (CLIA) uses a first antibody directed against the N-terminal region of the molecule and a second antibody directed against the C-terminal region immobilised on magnetic particles. The inter-assay CV is <10%.

25VTD was determined using the *Liaison® 25 OH vitamin D* (DiaSorin, Saluggia, Italy). The specificity is supposed to be 100% for both D2 and D3 hydroxyvitamin D. The inter-assay CV is <15%.

1,25VTD was determined with *Gamma-B 1,25-Dihydroxy Vitamin D RIA* (Immunodiagnostic Systems, Boldon, UK). The inter-assay CV is <15%.

TRAP 5b was determined with *BoneTRAP® Assay* (Medac Diagnostika GmbH, Wedel, Germany), a solid-phase immunofixed-enzyme assay for the detection of the active isoform 5b of the tartrate-resistant acid phosphatase. The inter-assay CV is <10%.

BALP was measured with *Tandem®-R Ostase®* (Beckman Coulter™, Fullerton, CA), an immunoradiometric assay for the measurement of skeletal alkaline phosphatase in human serum. The inter-assay CV is <6%. 100 U/l of skeletal enzymatic activity in serum approximately correspond to 38.4 ± 8.1 μg/l in the Tandem-R Ostase assay.

P1NP and CTXS were performed by CLIA on the Elecsys 2010 (Roche Diagnostics GmbH, Mannheim, Germany). The inter-assay CV of these tests is also <6%.

Homocystein (HCY) was performed with the Abbott IMX (Abbott Laboratories, IL, USA). Inter-assay CV is <5%.

Serum phosphate, calcium and albumin were measured by the automated clinical chemistry analyzer Modular using reagents obtained from Roche Diagnostics (Mannheim, Germany). Inter-assay CV for both tests is <10%.

Folate and vitamin B12 determinations were performed on Elecsys 2010 (Roche Diagnostics GmbH, Mannheim, Germany). The inter-assay CV is <15%.

2.3. Statistical analysis

Statistical analysis was performed with the MedCalc® software version 8.1.0.0 (Mariakerke, Belgium). All statistical tests were two-sided. Differences between groups were calculated by the Mann-Whitney *U*-test. Correlation coefficients were determined by the Pearson method. Methods' comparison was determined with the Bland-Altman test.

The criteria for considering independent variables were $p < 0.05$.

3. Results

Means, medians, ranges and reference limits used in our laboratory are shown in Table 1 for each investigated biochemical marker.

We observed a relatively high proportion of secondary hyperparathyroidism according to Salusky et al. [5] with 32 patients (44%) having levels of iPTH above 250–300 pg/ml.

A correlation matrix of the main variables is shown in Table 2. It appears that there were very strong correlations between bone markers concentrations, particularly between BALP and P1NP ($r = 0.953$).

The presumed non-(1–84) PTH represented 41.7% of the iPTH in our patients group; the different PTH moieties correlated very well together, as well as with the other bone markers. The whole PTH/non-(1–84) PTH ratio correlated positively with wPTH and TRAP 5b and negatively with total