Influence of PTH assay methodology on differential diagnosis of renal bone disease

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Abstract

**Background.** Determination of plasma parathyroid hormone (PTH) is routinely performed to diagnose and monitor renal bone disease. Recently, a new PTH assay (‘whole PTH’) using an antibody directed specifically against PTH(1–4) has been introduced. It was the aim of the current study to evaluate whole PTH and parameters derived from whole PTH in renal bone disease.

**Methods.** The following measurements were carried out in blood samples from 141 unselected haemodialysis patients: three intact PTH assays (Nichols, Roche Elecsys\(^1\), Scantibodies total); whole PTH (Scantibodies); bone-specific alkaline phosphatase (bAP); tartrate-resistant acid-phosphatase 5b (TRAP 5b); osteocalcin, 25-hydroxyvitamin D. Parameters derived from whole PTH were: (i) non-PTH(1–84), difference between intact PTH (Scantibodies assay) and whole PTH; (ii) whole PTH\(^{\text{u}}\)non-PTH(1–84) ratio.

**Results.** The values generated by the intact PTH assays were comparable. The mean whole PTH concentration was lower than mean intact PTH concentrations (16.9 ± 18.1 vs 26.4 ± 30.5 pmol/l, Nichols, \(P < 0.05\)). The correlation coefficients between all four PTH assays were comparable and were very high (\(r > 0.96\), ns). The rank order of values generated by the whole PTH assay was statistically not significantly different from the rank order generated by the Nichols intact PTH assay. The median non-PTH(1–84) concentration was 5.2 pmol/l (range 0–49.4). All PTH assays correlated highly significantly with non-PTH (1–84) (correlation coefficients 0.83–0.92). Corrected serum calcium was also associated with non-PTH (1–84) but the correlation was weaker (\(r = 0.28\)). Regression analysis indicated that the non-PTH(1–84) concentration could be predicted by 76.6–84.6% by the prevailing intact PTH concentrations. Other parameters contributed only marginally to prediction of non-PTH(1–84). In the entire patient group, there was no statistically significant correlation between the whole PTH/non-PTH(1–84) ratio and any of the PTH assays or biochemical bone markers. Eight of 141 patients had a whole PTH/non-PTH(1–84) ratio <1. TRAP 5b, bAP and osteocalcin had high correlations with intact PTH assays and the whole PTH assay (correlation coefficients 0.51–0.56, no significant difference). None of the PTH assays was superior to any other PTH assay in predicting serum concentrations of the bone markers. Therapy with active vitamin D metabolites (\(n = 70\)) did not alter the results of our analyses.

**Conclusions.** With respect to information about bone turnover we were not able to find differences between whole PTH and intact PTH assays. Our data also suggest that whole PTH and intact PTH assays give similar information. (i) The correlation between all PTH assays was very high. (ii) The rank order between whole PTH and Nichols intact PTH assays was comparable. (iii) The association between intact PTH assays and non-PTH(1–84) was very high. Albeit non-PTH(1–84) was mostly determined by the prevailing intact PTH concentration, diagnostic information on parathyroid activity provided by whole PTH or intact PTH, respectively, may differ in individual patients. How often this would happen cannot be answered with the currently available data. Unequivocal structural identification of the non-PTH(1–84) fraction would facilitate the answer to that question. The use of the whole PTH/non-PTH(1–84) ratio as a biochemical bone marker in renal bone disease requires further investigation.

**Keywords:** bAP; intact PTH; renal bone disease; renal failure; TRAP 5b; whole PTH
Introduction

Abnormalities of bone and mineral metabolism are one of the main complications during long-term treatment of patients with end-stage renal failure. There are large variations in form and degree of renal bone disease among dialysis patients ranging from adynamic bone disease with low bone turnover to severe hyperparathyroid bone disease with accelerated bone turnover. Most nephrologists rely on measurement of biochemical markers of calcium and bone metabolism to diagnose the different forms of renal bone disease and to initiate appropriate treatment. It has become standard to monitor plasma intact parathyroid hormone (PTH) in end-stage renal disease patients; additional biochemical markers are bone-specific alkaline phosphatase (bAP), tartrate-resistant acid-phosphatase 5b (TRAP 5b) and osteocalcin in serum.

Biologically active PTH circulates as an 84 amino acid peptide. The intact PTH(1–84) molecule is metabolized within minutes, either in the parathyroid glands or in peripheral tissues to form C-terminal, N-terminal and mid-regional PTH-fragments of varying length. The main pathway for elimination of PTH fragments is glomerular filtration followed by tubular degradation. As a consequence, excess amounts of PTH fragments are retained in renal failure. This has made it difficult to interpret results of PTH measurements in renal failure patients carried out with PTH assays employing antibodies derived against epitopes within the mid- or C-terminal regions of PTH.

The introduction of two-site assays for the intact PTH molecule has greatly improved the diagnostic accuracy of PTH measurements in renal failure. The intact PTH assays use two antibodies; one is directed against epitopes within the N-terminal end and one against epitopes within the C-terminal end of the PTH peptide. One antibody is immobilized on a solid support and binds the C-terminal portion of the PTH peptide; the second antibody binds the N-terminal region of the PTH peptide, whereas truncated PTH fragments are not bound by either antibody. It is assumed that those assays only detect biologically active PTH(1–84) and thus allow a valid interpretation of parathyroid activity in renal failure.

Recent papers [1,2], however, have cast some doubt on that assumption. Serum pools from healthy individuals and uraemic patients were fractionated by HPLC, and the fractions were tested in several commercially available two-site assays for intact PTH. One large peak that was identified co-migrated with synthetic human PTH(1–84). Several intact PTH assays also cross-reacted with a second, more hydrophilic peak whose elution profile corresponded to that of synthetic human PTH(7–84). Some authors [3,4] have suggested that the detection antibodies used in the tested intact PTH assays were not completely specific for PTH(1–84). Large C-terminal fragments [possibly PTH(7–84)] might also be detected, and the concentration of biologically active PTH(1–84) might be overestimated. The second more hydrophilic peak was more predominant in preparations derived from uraemic sera [2,5] suggesting that the possible overestimation of the plasma PTH(1–84) concentration by intact PTH assays would be more pronounced in renal failure than in normal individuals.

Recently, a novel immunoradiometric two-site PTH assay has been developed that exclusively detected full-length PTH(1–84), but not PTH fragments [3,4]. The assay (termed ‘whole PTH assay’) uses an antibody directed specifically against the first four amino acids of the PTH molecule. In contrast to the Nichols intact PTH assay, the new ‘whole PTH’ assay recognizes only one peak in HPLC-fractionated uraemic serum pools which co-migrates with synthetic human PTH(1–84) [4].

In several studies in renal failure patients, the whole PTH assay was compared with the Nichols intact PTH assay [3–7]. The plasma PTH concentration in renal failure patients was 30–60% lower when measured by the whole PTH assay as compared with the Nichols intact PTH assay. Consequently, the need of a higher intact PTH concentration in end-stage renal failure patients as compared with normal to maintain normal bone turnover [8] has been explained in part by the overestimation of plasma PTH by standard intact PTH assays [6].

The presumed non-PTH(1–84) fraction of a given plasma sample was quantified by subtracting PTH measured by the whole PTH assay from PTH measured by a standard intact PTH assay. It was postulated that the non-PTH(1–84) fraction may have in part PTH-antagonistic properties as it co-migrates with PTH(7–84) on HPLC and may correspond to PTH(7–84). PTH(7–84) in supraphysiological concentrations has been shown by several investigators to antagonize some of the biological actions of PTH [6,9]. Recently, it was reported that a ratio of whole PTH/non-PTH(1–84) < 1 was highly correlated with low bone turnover [10].

The aim of the current study in a large group of chronic haemodialysis patients was to define the validity of several PTH assays in the differential diagnosis of renal bone disease by: (i) comparison of three intact PTH assays (Nichols intact PTH; Elecsys® intact PTH, Scantibodies total PTH) with the whole PTH assay; (ii) correlation of other biochemical bone markers (bAP, TRAP 5b, osteocalcin) with the PTH assays; (iii) analysis of presumed non-PTH(1–84) fractions in relation to other biochemical and patient parameters.

Subjects and methods

Patients

We studied 141 unselected patients (61 female, 80 male) with end-stage renal disease from four dialysis units. All patients underwent chronic haemodialysis treatment three times weekly. The median age of the patients was 65.8 years.
(range 24.5–89.9; mean ± SD 64.1 ± 12.7). The median time on dialysis was 4.2 years (range 1.0–24.3; mean ± SD 5.7 ± 4.8). The underlying renal diseases were: diabetic nephropathy, n = 46; chronic glomerulonephritis, n = 29; chronic pyelonephritis/interstitial disease, n = 12; vascular disease, n = 10; polycystic kidney disease, n = 8; analgesic nephropathy, n = 6; lupus nephritis, n = 3; vasculitis, n = 3; others, n = 3; unknown, n = 21. All patients were treated with synthetic dialysis membranes: polysulfone, n

Methods

**PTH assays.** (i) PTH Nichols: intact PTH immunoassay (Nichols Institute, San Juan Capistrano, CA). This immuno-radiometric assay (IRMA) incorporates two goat polyclonal antibodies to human PTH. One antibody directed against the mid- and C-terminal PTH-regions [PTH(39–84)] is immobilized onto plastic beads. The second 125I-labelled antibody binds only to the N-terminal PTH-region [PTH(1–34)]. Inter-assay variation in the normal and elevated concentration range is <10%.

(ii) Elecsys® PTH: intact PTH immunoassay (Roche Diagnostics, Mannheim, Germany). The Elecsys® PTH is an electrochemiluminescence immunoassay (ECLIA) performed on the fully automated immunoanalyzer Elecsys® 2010. Amino acids 26–32 are recognized by the monoclonal biotinylated (capture) antibody. Amino acids 55–64 are recognized by the monoclonal ruthenylated (tracer) antibody. Inter-assay variation is <7% in the low and upper normal range, and <5% in the high concentration range, respectively.

The PTH Scantibodies duo-PTH immunoassay (Scantibodies Laboratories, Santee, CA) was also employed. It contains two PTH assays for total (or intact) PTH and whole PTH, respectively.

(iii) Scantibodies total PTH immunoassay: intact PTH immunoassay. A polyclonal human antibody generated against PTH(39–84) is immobilized onto plastic beads. A second polyclonal antibody is directed against PTH(7–34). Inter-assay variation is <8%.

(iv) ‘Whole PTH’ immunoassay: the polyclonal antibody recognizing hPTH(39–84) is identical to that in the Scantibodies total PTH assay. The whole PTH assay uses a new 125I-labelled polyclonal antibody, which is directed against the first four amino acids of the N-terminal end of the PTH peptide. The assay does not cross-react with synthetic human PTH(7–84). The whole PTH assay has also been labelled CAP (cyclase activating PTH) assay. Inter-assay variation is <8%.

(v) Non-PTH(1–84): the non-PTH(1–84) concentration was quantified by subtracting the PTH value obtained by the whole PTH assay from the PTH value obtained by the Scantibodies intact PTH assay (Scantibodies total PTH). The non-PTH(1–84) fraction has also been termed CIP (cyclase inactive PTH). In two patients, whole PTH concentration was higher than intact PTH concentration. In additional calculations (as indicated) non-PTH(1–84) was determined by subtraction of whole PTH from Nichols intact PTH.

Biochemical markers of calcium and bone metabolism. (i) TRAP 5b in serum; TRAP 5b (Suomen Bioanalytikka, Oulo, Finland) is a solid-phase immunofixed-enzyme assay for the detection of osteoclast-derived TRAP 5b. TRAP 5b is a specific marker of bone resorption [11]. The inter-assay coefficient of variation is <8%.

(ii) bAP: access-Ostase® immunoassay (Beckman-Coulter Inc.) is a chemiluminescence immunoassay run on the fully automated ACCESS immunoassay system for the detection of bAP. bAP is a marker of bone formation. The inter-assay coefficient of variation is <6.5%.

(iii) N-mid® Osteocalcin (Roche Diagnostics, Mannheim, Germany) in serum: N-mid® Osteocalcin is an electrochemiluminescence sandwich immunoassay run on the fully automated analyzer Elecsys® 2010. The inter-assay coefficient of variation is <3%.

(iv) The radioimmunoassay for determination of serum 25-Hydroxyvitamin D (25(OH)D) has been described previously [12]. The inter-assay coefficient of variation is <12%.

Routine parameters. Serum calcium and phosphate were measured by the automated clinical chemistry analyzer Hitachi 740 using reagents obtained from Roche Diagnostics, Mannheim, Germany. Serum calcium was corrected for serum total protein according to the formula of Husdan et al. [13]. Corrected serum calcium is given throughout the paper.

All blood samples were drawn for routine measurements immediately before the beginning of a dialysis treatment after the long interval. The study was carried out in December. Plasma and serum samples were frozen immediately and stored at −70°C until measurement. All samples were measured simultaneously in each assay to avoid inter-assay variation. All PTH tests were performed on the same day.

Statistics

Statistical analysis was carried out with the SPSS® 10.0 program (SPSS Inc., Chicago, IL). The data are shown as mean ± SD. All statistical tests were two-sided. Differences between groups were calculated by the Mann–Whitney U-test, the χ2 analysis or ANOVA (followed by the Newman–Keuls test) where appropriate. Correlation coefficients were calculated by the method of Pearson. The comparison of ranks between the whole PTH and Nichols intact PTH values was performed by t-test.

Single regression analysis was used to predict non-PTH (1–84) and bone markers by PTH assays. In a further analysis, whole PTH was added as a second variable to intact PTH to assess if inclusion of PTH would improve prediction of bone markers by intact PTH. Additionally, multiple regression analysis was applied in order to predict non-PTH(1–84) and bone markers (dependent variables) by PTH assays. Whole PTH was used as initial independent variable in this analysis and the other PTH assays where entered stepwise into the model. The criteria for entering independent variables into the equation were P = 0.05 and P = 0.10 for removal. Additional tests were computed for R2 change and their significance for sets of independent variables. The total sample was split into two equal-sized subgroups using the
median of PTH to compare the predictability of bone markers by the various PTH assays.

Results

PTH

Concentration. Results of biochemical measurements are summarized in Table 1.

The three intact PTH assays yielded comparable mean intact PTH values: Nichols 26.4 ± 30.5 pmol/l; Elecsys® 29.6 ± 33.3 pmol/l; Scantibodies 24.8 ± 25.7 pmol/l. In contrast, mean plasma PTH was significantly lower when measured with the whole PTH assay (16.9 ± 18.1 pmol/l, P < 0.05 vs all other intact PTH assays). The mean difference between whole PTH and Nichols intact PTH was 35.9%. Whole PTH values were, on average 42.9% lower than Elecsys® intact PTH and 31.9% lower than Scantibodies intact PTH.

Correlations. The whole PTH assay had a very high correlation with each of the other PTH assays (Table 2).

The correlation coefficients were between 0.967 and 0.983. The association between whole PTH and Nichols intact PTH is shown in Figure 1. The associations between whole PTH and Elecsys intact PTH (r = 0.967, y = 1.54 + 0.53x) and between whole PTH and Scantibodies intact PTH (r = 0.983, y = 0.27 + 0.69x) were similar (data not shown).

Likewise, the three intact PTH assays correlated very highly with each other (Table 2). The correlation coefficients were between 0.977 and 0.983. A statistically significant difference between the correlation coefficients was not present.

The calculation of the correlation coefficients between PTH assays was repeated for the patient group undergoing therapy with active vitamin D metabolites (n = 70) and for the patient group without vitamin D therapy (n = 71). Vitamin D therapy did not influence the associations between the various PTH assays: (i) vitamin D therapy: correlation coefficients were similar (data not shown).

Table 2. Correlation coefficients

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<th>Ratio</th>
<th>Scanti</th>
<th>Elecsys</th>
<th>Nichols</th>
<th>TRAP5b</th>
<th>bAP</th>
<th>OC</th>
<th>25(OH)D</th>
<th>Calcium</th>
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Significance levels: aP < 0.001; bP < 0.01; cP < 0.05.

Whole, whole PTH; Non1-84, non-PTH(1–84); Ratio, whole PTH/non-PTH(1–84) ratio; Scanti, Scantibodies intact PTH; Elecsys, Elecsys® intact PTH; Nichols, Nichols intact PTH; OC, osteocalcin; PO₄, phosphate.
between 0.969 and 0.985; (ii) no vitamin D therapy: correlation coefficients between 0.964 and 0.986.

Comparison of ranks. The ranks formed by the whole PTH and Nichols intact PTH assays were compared in an additional analysis. The whole PTH concentrations were divided into the lower half and the upper half of the values and compared with the ranks of the corresponding Nichols intact PTH values. There was no statistically significant difference between the ranks of the two assays. In the lower half, the mean rank sum for whole PTH was 35.5; the mean rank sum for Nichols intact PTH was 36.2 ($P = 0.565$). In the upper half, the mean rank sum for whole PTH was 106; the mean rank sum for Nichols intact PTH was 105.3 ($P = 0.647$).

Non-PTH(1–84) concentration

Concentration. The presumed non-PTH(1–84) concentration was calculated by subtracting the whole PTH concentration from the result of an intact PTH assay (Scantibodies total PTH from the same manufacturer). Mean non-PTH(1–84) concentration was 8.0 pmol/l. There were two patients without detectable non-PTH(1–84) molecules; the highest concentration of non-PTH(1–84) was 49.4 pmol/l. Mean non-PTH (1–84) was comparable in the patients receiving active vitamin D metabolites (7.8 ± 8.1) and in the patients without vitamin D therapy (8.1 ± 8.9).

Correlations. The non-PTH(1–84) concentration was significantly correlated both with the whole PTH concentration and with the intact PTH assay concentrations (Table 2). The correlation coefficients were between 0.831 and 0.920. A graphic representation between non-PTH(1–84) and Nichols intact PTH is shown in Figure 2. The associations between non-PTH(1–84) and Elecsys intact PTH ($r = 0.882$, $y = 1.31 + 0.23x$) and between non-PTH(1–84) and Scantibodies intact PTH ($r = 0.920$, $y = 0.32 + 0.31x$) were similar (data not shown). As we used the Scantibodies intact PTH for calculation of the non-PTH(1–84) concentration, this assay had the highest correlation with whole PTH. Vitamin D therapy did not change the correlation coefficients between non-PTH(1–84) and the PTH assays. The results were comparable for the patient groups with ($r = 0.852–0.930$) and without ($r = 0.828–0.922$) vitamin D therapy.

Regression analysis. Because of the relatively high variability of non-PTH(1–84), regression analysis was performed to assess which parameters could predict the non-PTH(1–84) concentration. Simple regression analysis with non-PTH(1–84) as the dependent variable and Scantibodies intact PTH as the independent variable indicated that the Scantibodies intact PTH concentration could explain 84.6% of the variability in non-PTH(1–84) ($R^2 = 0.846$). In addition to Scantibodies intact PTH, other parameters [bone markers, calcium, phosphate, 25(OH)D, age] were included as independent variables into a multiple regression analysis with non-PTH(1–84) as the dependent variable. The results indicated that all factors combined contributed 85.4% to the explanation of the variability.
of non-PTH(1–84) (multiple regression coefficient = 0.924; $R^2 = 0.854$).

As non-PTH(1–84) was derived from Scantibodies intact PTH, the calculation of a regression analysis was repeated both for Nichols intact PTH and for Elecsys intact PTH as the independent variable. Nichols intact PTH explained 76.6% of the variability of non-PTH(1–84) ($R^2 = 0.766$); Elecsys intact PTH explained 78.2% of the variability of non-PTH(1–84) ($R^2 = 0.782$). The combination of other factors did not further contribute to prediction of non-PTH(1–84).

### Whole PTH/non-PTH(1–84) ratio

**Concentration and correlations.** The whole PTH/non-PTH(1–84) ratio was calculated for all patients. The mean whole PTH/non-PTH(1–84) ratio in all patients was 2.4. There was no significant correlation between the whole PTH/non-PTH(1–84) ratio and any of the other parameters (Table 2). In the entire group, the whole PTH/non-PTH(1–84) ratio was related neither to any PTH assay nor to any biochemical bone marker (bAP, TRAP 5b, osteocalcin).

Additional correlation coefficients between the whole PTH/non-PTH(1–84) ratio and all laboratory and clinical parameters were then calculated for the patient groups forming the lowest and the highest quartiles. The whole PTH/non-PTH(1–84) ratio was not associated with any parameter in the patient group with a whole PTH/non-PTH(1–84) ratio above the 75th percentile. In the group with a whole PTH/non-PTH(1–84) ratio below the 25th quartile, there were weak correlations between the whole PTH/non-PTH(1–84) ratio and whole PTH ($r = 0.336, P < 0.05$) and between the whole PTH/non-PTH(1–84) ratio and TRAP 5b ($r = 0.351, P < 0.05$). Intact PTH assays, bAP and osteocalcin were not significantly correlated with the whole PTH/non-PTH(1–84) ratio in this group.

**Whole PTH/non-PTH(1–84) ratio < 1.** It was recently reported that a whole PTH/non-PTH(1–84) ratio < 1 was highly specific for low bone turnover [10]. Eight of the 141 patients had a whole PTH/non-PTH(1–84) ratio < 1; 133 patients had a whole PTH/non-PTH(1–84) ratio > 1. Those two groups were also compared (Table 3).

Because of the large difference in group sizes, statistics were not calculated. The eight patients with a whole PTH/non-PTH(1–84) ratio < 1 had lower whole PTH and intact PTH concentrations than the patients with a whole PTH/non-PTH(1–84) ratio > 1 ($6.3 \pm 5.6$ vs $17.7 \pm 18.4$ pmol/l). The mean non-PTH(1–84) concentration was similar in the two groups ($6.5$ pmol/l in whole PTH/non-PTH(1–84) ratio < 1 vs $8.0$ pmol/l whole PTH/non-PTH(1–84) ratio > 1). Mean bAP, TRAP 5b, and osteocalcin concentrations were higher in the group with a whole PTH/non-PTH(1–84) ratio > 1.

**Influence of therapy with active vitamin D metabolites.** Mean whole PTH/non-PTH(1–84) ratio was comparable in the patients receiving active vitamin D metabolites ($2.7 \pm 2.3$) and in the patients without vitamin D therapy ($2.2 \pm 1.3$). In the vitamin D treated group, three patients had a whole PTH/non-PTH(1–84) ratio < 1. In the group without vitamin D, five patients had a whole PTH/non-PTH(1–84) ratio < 1.

In the vitamin D-treated patient group, the whole PTH/non-PTH(1–84) ratio was not related to any PTH assay or to any bone marker. In the patient group without vitamin D, the whole PTH/non-PTH(1–84) ratio was not related to any PTH assay or to TRAP 5b and osteocalcin, respectively. There was a positive correlation ($r = 0.320, P < 0.02$) between the whole PTH/non-PTH(1–84) ratio and bAP in the group without vitamin D treatment.

**Calculation of the whole PTH/non-PTH(1–84) ratio with the Nichols intact PTH assay.** For an additional analysis, the whole PTH/non-PTH(1–84) ratio was also calculated using the Nichols intact PTH assay (according to [10]). In that analysis, seven patients had a whole PTH/non-PTH(1–84) ratio < 1. Four of those patients also had a whole PTH/non-PTH(1–84) ratio < 1 with the Scantibodies total PTH assay; the remaining three patients had a mean whole PTH/non-PTH(1–84) ratio > 1.

### Table 3. Comparison whole PTH/non-PTH(1–84) ratio < 1 vs whole PTH/non-PTH(1–84) ratio > 1

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Whole PTH/non-PTH(1–84) ratio &lt; 1</th>
<th>Whole PTH/non-PTH(1–84) ratio &gt; 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole PTH/non-PTH(1–84) ratio</td>
<td>0.76 ± 0.16</td>
<td>8</td>
</tr>
<tr>
<td>Whole PTH (pmol/l)</td>
<td>6.3 ± 5.6</td>
<td>0.41–18.0</td>
</tr>
<tr>
<td>Non-PTH(1–84) (pmol/l)</td>
<td>6.5 ± 6.1</td>
<td>0.6–19.0</td>
</tr>
<tr>
<td>PTH Scantibodies (pmol/l)</td>
<td>11.3 ± 10.4</td>
<td>0.5–13.7</td>
</tr>
<tr>
<td>PTH Elecsys (pmol/l)</td>
<td>15.3 ± 13.9</td>
<td>1.0–47.0</td>
</tr>
<tr>
<td>PTH Nichols (pmol/l)</td>
<td>11.5 ± 11.8</td>
<td>0.6–37.0</td>
</tr>
<tr>
<td>TRAP 5b (U/l)</td>
<td>2.9 ± 1.1</td>
<td>1.1–4.7</td>
</tr>
<tr>
<td>bAP (µg/l)</td>
<td>7.1 ± 1.9</td>
<td>4.0–9.7</td>
</tr>
<tr>
<td>Osteocalcin (µg/l)</td>
<td>142.7 ± 92.8</td>
<td>28.2–297.4</td>
</tr>
</tbody>
</table>

*Two patients with undetectable non-PTH(1–84) were omitted from the calculation of the ratio.*
PTH/non-PTH(1–84) ratio of 2.6 with the Scantibodies total PTH assay. The associations between the whole PTH/non-PTH(1–84) ratio and non-PTH(1–84), respectively, and the other parameters were unaltered when the whole PTH/non-PTH(1–84) ratio was calculated with the Nichols intact PTH assay.

Biochemical bone markers

Concentration. In the whole patient group, mean TRAP 5b serum concentration was elevated (Table 1); there was a wide variation of TRAP 5b concentrations. Likewise, the serum concentrations of bAP and osteocalcin varied widely.

Correlations. There was a strong association between the serum concentrations of biochemical bone markers (Table 2). The highest correlation coefficient was present between bAP and TRAP 5b ($r = 0.636$). The correlations between TRAP 5b and whole-PTH and the three intact PTH assays (Nichols, Elecsys®, Scantibodies), respectively, were highly significant. The correlation coefficients ranged from 0.532 to 0.565 (Table 2). There was no statistically significant difference between the correlation coefficients. TRAP 5b was also significantly correlated with non-PTH (1–84) ($r = 0.515$) and osteocalcin ($r = 0.510$).

There was a high correlation between bAP and whole PTH and the three intact PTH assays, respectively. The correlation coefficients ranged from 0.511 to 0.530 and did not differ significantly (Table 2). bAP also was correlated with non-PTH(1–84) ($r = 0.427$) and osteocalcin ($r = 0.552$).

Osteocalcin also had comparable high correlations with whole-PTH and the three intact PTH assays; the correlation coefficients ranged from 0.521 to 0.542 (Table 2). Osteocalcin was also correlated with non-PTH(1–84) ($r = 0.490$).

The correlation coefficients between the respective PTH assays and bone markers were comparable in the group receiving vitamin D metabolites, and they were also comparable in the group without vitamin D therapy (data not shown).

Regression analysis. We also examined whether any of the four PTH assays were superior to the other PTH assays in predicting the serum concentrations of bone markers. A separate multiple regression analysis was carried out for each bone marker as the dependent variable with the four PTH assays as independent variables. The results indicated that a relatively high percentage of the variability of concentration of bone markers could be predicted by the PTH assays: (i) bAP 30.1% (multiple regression coefficient $= 0.549$; $R^2 = 0.301$); (ii) TRAP 5b 35.0% (multiple regression coefficient $= 0.592$; $R^2 = 0.350$); (iii) osteocalcin 29.2% (multiple regression coefficient $= 0.540$; $R^2 = 0.292$). The multiple regression coefficients between PTH values and bAP, TRAP 5b and osteocalcin, respectively, were statistically not different from the single correlation coefficients (Table 2).

We also estimated whether, in a regression analysis, inclusion of whole PTH to any of the three intact PTH concentrations would improve prediction of bone markers. The change in prediction of variability of the serum concentration of bone markers by addition of whole PTH to any of the three standard intact PTH assays was for (i) bAP 2.7–3.4%, for (ii) TRAP 5b 0.9–1.1% and for (iii) osteocalcin 0–2.0%. These results did not have statistical significance.

Another possibility was that the capacity of whole PTH to predict bone markers could depend on the degree of elevation of PTH. Therefore, the calculations were repeated for the patient group with the highest 50% PTH values. Significant differences between the PTH assays to predict bone markers were also not present (data not shown).

Vitamin D status

Vitamin D status was monitored by measuring serum 25(OH)D. Mean 25(OH)D was in the normal range. There were no significant correlations with any of the other laboratory parameters except a weak negative correlation with serum phosphate ($r = -0.240$).

Calcium and phosphate

Serum calcium and phosphate were within the expected range for haemodialysis patients. Corrected serum calcium correlated weakly with the three intact PTH assays (correlation coefficients 0.189–0.193), but not with whole PTH. There was a positive correlation between corrected serum calcium and non-PTH(1–84) ($r = 0.279$) and a weaker negative correlation between serum calcium and whole PTH/non-PTH(1–84) ratio ($r = -0.233$). Serum calcium could not be predicted by non-PTH(1–84) (explained variability 6.7%). Serum phosphate was not correlated with any of the PTH assays or biochemical bone markers except a weak correlation with osteocalcin ($r = 0.174$).

Discussion

We compared three standard intact PTH assays with the whole PTH assay in 141 patients on maintenance haemodialysis. We did not apply any patient selection criteria and studied all patients from four haemodialysis units. Thus, we had a fairly representative group of haemodialysis patients.

In accordance with other studies, mean whole PTH concentration was significantly lower than mean PTH concentration in the intact PTH assays. The mean difference between Nichols intact PTH and whole PTH, i.e. the mean proportion of presumed non-PTH(1–84), was 35.9%. Almost identical data were reported in two other studies of ESRD patients [6,7]. Other authors, however, found a higher fraction of non-PTH(1–84) in the Nichols assay. Brossard et al. [1] reported that non-PTH(1–84) constituted 40–60% of the total immunoreactive PTH in uraemic samples.
John et al. [3] found a proportion of almost 60% non-PTH(1–84) in ESRD patients. The reasons for these in part considerable variations may comprise different patient characteristics and inter-assay variation. The two other intact PTH assays which we used in this study (Elecsys® and Scantibodies) yielded results, which were comparable to the Nichols intact PTH assay.

The correlation coefficients among the three intact PTH assays were strikingly high ($r > 0.96$). We also confirmed the strong associations between circulating biochemical bone markers, i.e. bAP, TRAP 5b and osteocalcin, and the strong associations between intact PTH and biochemical bone markers.

The correlation coefficients between whole PTH and each of the intact PTH assays were also very high ($r > 0.96$). Our data correspond to those of Gao et al. [4] who reported a high correlation between the whole PTH and Nichols intact PTH assays ($r = 0.977$). Taken together, we found a virtually identical correlation between all four PTH assays. Likewise, the correlations between whole PTH and bAP, TRAP 5b and osteocalcin, respectively, were virtually identical to the correlations between intact PTH assays and the biochemical bone markers.

We also examined for each intact PTH assay whether prediction of the serum concentration of the three bone markers by intact PTH was improved by inclusion of whole PTH into the analysis. The results in the whole patient group and in the group with the higher PTH values (upper 50%) show that the prediction for each of the three intact PTH assays is virtually identical if whole PTH is included in the calculation. Taken together, we were not able to demonstrate that whole PTH was superior to any of the three intact PTH assays in predicting the serum concentration of established biochemical bone markers.

Nakanishi et al. [7] reported that whole PTH correlated better than intact PTH with bAP in ESRD patients. We cannot confirm this observation since correlation coefficients in our hands were almost identical. The correlation coefficients between PTH assays and bAP were also considerably higher in our study than in the study by Nakanishi et al. [7]. The non-PTH(1–84) concentration was variable in our study. We attempted to delineate factors which could predict the variability of the non-PTH(1–84) concentration. A number of analyses clearly indicated that the intact PTH concentration was, by far, the strongest predictor of non-PTH(1–84). The variability in non-PTH(1–84) could be predicted by > 75% by the intact PTH assays which were not used for calculation of non-PTH(1–84) (Nichols and Elecsys).

A clear identification of additional factors regulating non-PTH(1–84) was not possible. It has been shown that degradation of PTH(1–84) is enhanced under conditions of hypercalcaemia [14]. Slatopolsky et al. [6] reported a high association between serum calcium and non-PTH(1–84) concentrations (correlation $r = 0.638$) in 20 dialysis patients. In our study, a weaker positive correlation ($r = 0.279$) between serum calcium and the non-PTH(1–84) concentration was found. It was, however, not possible to predict serum calcium from non-PTH(1–84). Monier-Faugere et al. [10] reported that hypercalcaemia resulted in a decrease of intact PTH, whole PTH and the whole non-PTH(1–84) ratio, while no information on non-PTH(1–84) during hypercalcaemia is given. Taken together, there is some evidence that higher serum calcium is associated with higher non-PTH(1–84) but there is no evidence that hypercalcaemia is a major up-regulator of non-PTH (1–84) concentration.

Several authors [6,10] have suggested that the non-PTH(1–84) fraction (i.e. the difference between the intact PTH and whole PTH concentrations) contains mostly PTH(7–84). This assumption was based on the observation that the Nichols intact PTH assay recognized a peak in HPLC studies which co-migrated with chemically synthesized PTH(7–84) [1,2]. Synthetic PTH(7–84) had no stimulatory or inhibitory effect on cAMP production by osteoblast-like cells in vitro. PTH(7–84) in supraphysiological concentrations inhibited the calcaemic and phosphaturic responses to PTH(1–84) in rats in vivo [6]. Further PTH-antagonistic properties of synthetic PTH(7–84) were reported previously [9]. A distinct receptor in bone and renal cells was identified which specifically recognized C-terminal PTH fragments [9,15]. The PTH-antagonistic properties of PTH(7–84) and other C-terminal fragments may be mediated by binding to such a receptor. As suggested by Fournier et al. [16] it would then be logical to expect that patients with low bone turnover have a higher proportion of non-PTH(1–84) with putative PTH-antagonistic properties than patients with high bone turnover. The fraction of non-PTH (1–84) [i.e. the percentage of non-PTH(1–84) relative to intact PTH] was 38.3% in the patients in the lowest bAP quartile (mean bAP 6.0 µg/l), and it was 30.4% in the patients in the highest bAP quartile (mean bAP 21.2 µg/l) (data not shown). The latter group of patients had a higher absolute non-PTH(1–84) concentration (11.8 vs 4.9 pmol/l) but also a higher intact PTH (38.8 vs 12.8 pmol/l). The data do not support, in our opinion, a dominant PTH-antagonistic role for non-PTH(1–84) in the development of low bone turnover. It is, however, not possible to exclude a role of non-PTH(1–84) in bone metabolism in individual patients.

The interference of PTH(7–84) in the Elecsys® intact PTH assay was estimated recently in a quality control survey where synthetic PTH(7–84) was added to the assays. PTH(7–84) was recognized in 67.9% of the added amount by the Elecsys® intact PTH assay (H. Ellis, personal communication). The difference in the Nichols assays, which recognize almost all PTH(7–84) added can be explained by a different specificity of the N-terminal antibody in the Elecsys® assay. The data from the quality control survey suggest that the non-PTH(1–84) fraction must contain at least 30–40% other peptides than PTH(7–84).

Thus, the assumption that PTH(7–84) constitutes most if not all of non-PTH(1–84) is, in our opinion,
Non-PTH(1–84) could contain a mixture of C-terminal PTH fragments including PTH(7–84). Then, the question if and what proportion of non-PTH (1–84) has PTH-antagonistic properties is unclear. One can also speculate that the non-PTH(1–84) fraction contains chemically modified PTH(1–84) molecules with or without conserved biological activity, which co-migrate with synthetic PTH(7–84) on HPLC. The latter possibility is currently under investigation.

Monier-Faugere et al. [10] reported that a whole PTH/non-PTH(1–84) ratio > 1 predicted normal or high bone turnover with a sensitivity of 100%, whereas a whole PTH/non-PTH(1–84) ratio < 1 indicated a high probability (sensitivity 87.5%) of low bone turnover. Receiver-operator characteristics curves showed that a cut-off point of 1 for the whole PTH/non-PTH(1–84) ratio discriminated best between low and normal high bone turnover. Twenty-eight out of 51 ESRD patients had a whole PTH/non-PTH(1–84) ratio < 1 and 23 patients had a whole PTH/non-PTH(1–84) ratio > 1 [10]. This is a discrepancy in our study where only eight of 141 patients had a whole PTH/non-PTH(1–84) ratio < 1. Nakaniishi et al. [7] found one patient among 99 long-term haemodialysis patients with a whole PTH/non-PTH(1–84) ratio < 1. Dorsch et al. [17] studied 40 haemodialysis patients and found three patients with a whole PTH/non-PTH(1–84) ratio < 1. Two of those patients had a low bAP concentration, and one patient had a high bAP concentration.

The prevalence of adynamic bone disease and low bone turnover was between 30 and 40% in most studies. The percentage of patients with a whole PTH/non-PTH(1–84) ratio < 1 was 5.6% in our study, while it was even lower in a previous study [7]. In our study, the sensitivity to diagnose patients with low bone turnover would most likely have been quite low if a whole PTH/non-PTH(1–84) ratio < 1 had been used as cut-off point.

A high correlation of bAP serum concentration with histological indices of bone formation and bone resorption in renal bone disease has been shown [18]. Likewise, serum osteocalcin concentration is well correlated with bone histological indices in ESRD patients [19]. The serum concentration of TRAP 5b had a high association with bAP and osteocalcin [20]. In this study, we confirmed the high correlations between bAP, TRAP 5b and osteocalcin, respectively, and the high correlations between the biochemical bone markers and all PTH-assays, respectively. We did not find a correlation between the whole PTH/non-PTH(1–84) ratio and any of the biochemical bone indices of bone turnover or any of the PTH assays when the whole patient group was analysed. Similar data for bAP and the whole PTH/non-PTH(1–84) ratio were recently reported by Nakaniishi et al. [7] who found no significant correlation between bAP and whole PTH/non-PTH(1–84) ratio.

Possible associations between the whole PTH/non-PTH(1–84) ratio and biochemical markers might have been present only in a subgroup of patients. Therefore, correlations between the whole PTH/non-PTH(1–84) ratio and bAP, TRAP 5b, osteocalcin, whole PTH and the three intact PTH assays, respectively, were calculated separately for each subgroup with the lowest 25% or the highest 25% of values. None of these calculations in the various patient subgroups showed a statistically significant association between the whole PTH/non-PTH(1–84) ratio and biochemical markers of bone turnover except weak correlations between the whole PTH/non-PTH(1–84) ratio and whole PTH and TRAP 5b, respectively, in the lowest quartile. These weak correlations have, in our opinion, no clinical significance.

We obtained virtually identical results when the whole PTH (non-PTH(1–84) ratio was calculated with the Nichols intact PTH.

The lack of association between the whole PTH/non-PTH(1–84) ratio and established biochemical bone markers is in clear discrepancy to the high correlation between PTH assays, bAP, TRAP 5b and osteocalcin, respectively. Our data confirm that a low whole PTH (non-PTH(1–84) ratio is associated with lower parathyroid activity, but we believe that further studies on the whole PTH/non-PTH(1–84) ratio and possible cut-off points for diagnosis of low bone turnover are necessary to fully establish the clinical feasibility of the whole PTH/non-PTH(1–84) ratio as marker of bone turnover.

Approximately half of the patients were treated with active vitamin D metabolites. As calcitriol or alfa-calcidol could affect the whole PTH, the non-PTH(1–84), the whole PTH/non-PTH(1–84) ratio or the relationship between PTH and bone markers, data were also analysed in the patient groups with and without vitamin D therapy. We were not able to identify differences between the two groups with respect to a different behaviour of the whole PTH, the non-PTH(1–84) or the whole PTH/non-PTH(1–84) ratio.

Two different aspects of diagnostic information are expected from a PTH measurement: (i) direct information about parathyroid activity, and (ii) indirect information about bone turnover. With respect to information about bone turnover, we were not able to find differences between whole PTH and intact PTH assays. (i) The correlation between biochemical bone markers, i.e. bAP, TRAP 5b and osteocalcin and all PTH assays was comparable. (ii) The capacity of all PTH assays to predict serum concentrations of biochemical bone markers was comparable. One drawback of this analysis was that we were not looking directly at bone histology, but had to rely on indirect information provided by serum concentrations of biochemical bone markers. As those bone markers are closely associated with bone turnover our analysis should be valid.

With respect to information about parathyroid activity, some of our data also suggest that whole PTH and intact PTH assays give similar information. (i) The correlation between all PTH assays was very high. (ii) The rank order between whole PTH and Nichols intact PTH assays was comparable.
(iii) The association between intact PTH assays and non-PTH(1–84) was very high.

On the other hand, variability in the non-PTH(1–84) concentration was fairly high. Albeit non-PTH(1–84) was highly correlated with prevailing intact PTH, diagnostic information on parathyroid activity provided by whole PTH or intact PTH, respectively, may differ in individual patients. How often this would happen cannot be answered with the currently available data. Identification of the composition and structure of the non-PTH(1–84) fraction would facilitate the answer to that question.

The variability of plasma PTH in ESRD patients at a given level of bone turnover can be explained in part by a different target organ response to PTH at the PTH receptor and post-receptor level. The factors regulating this variability are unknown and will limit any attempt to predict bone turnover solely by plasma PTH concentration or parameters derived from plasma PTH concentration. In our opinion, it is a good strategy to combine a PTH assay with a direct bone marker like bAP or TRAP 5b in assessing bone and calcium metabolism in ESRD.

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