Serum Concentrations of Cross-Linked N-Telopeptides of Type I Collagen: New Marker for Bone Resorption in Hemodialysis Patients

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Background: Urinary cross-linked N-telopeptide of type I collagen (NTX) is a reliable bone resorption marker in patients with metabolic bone disease. We assessed a clinically available serum NTX assay suitable for anuric patients on hemodialysis (HD).

Methods: Serum concentrations of NTX, C-terminal telopeptide of type I collagen (β-CTX), pyridinoline (PYD), and deoxypyridinoline (DPD) were determined as bone resorption markers, and those of bone alkaline phosphatase (BAP) and intact osteocalcin (OC) as bone formation markers, in 113 male HD patients (mean age, 59.3 years; mean HD duration, 67.7 months). Each patient’s bone mineral density (BMD) in the distal third of the radius was measured twice, with a 2-year interval between measurements, by dual-energy x-ray absorptiometry.

Results: Serum NTX correlated significantly with β-CTX, PYD, DPD, BAP, and intact OC. NTX, as well as β-CTX, PYD, DPD, BAP, and intact OC, correlated significantly with BMD at the time of measurement. NTX, β-CTX, and DPD correlated significantly with the annual change in BMD during the 2-year period thereafter, in contrast to PYD, BAP, and intact OC. Patients in the highest quartile of serum NTX concentrations showed the fastest rate of bone loss. The sensitivity and specificity for detecting rapid bone loss were 48% and 83%, respectively, for serum NTX.

Conclusion: Serum NTX may provide a clinically relevant serum assay to estimate bone turnover in HD patients.

Renal osteodystrophy is one of the major complications in hemodialysis (HD) patients, leading to a substantial increase in fracture rate and increased morbidity and mortality (1). Bone mineral density (BMD) is widely used to estimate bone fracture risk. In addition, evaluation of bone turnover rate is established as an independent predictor for bone fracture (2, 3) and future bone loss (4), and measurements of bone metabolic markers are increasingly used to complement BMD measurements (5, 6). Bone resorption markers are assumed to be superior to bone formation markers for predicting future changes in bone mass, well in advance of detection of BMD reductions (7). Measurement of cross-linked N-telopeptide of type I collagen (NTX) in serum was recently approved in Japan for clinical use as a bone resorption marker. Impaired urinary excretion precludes urinary measurement of bone resorption markers in HD patients, but measurement of serum NTX may provide a good marker for the bone resorption state in these patients. We recently reported (8) that the β-CrossLaps assay for serum C-terminal telopeptide of type I collagen (β-CTX) (9–11) provides values that are no less significant than those for pyridinoline (PYD) and deoxypyridinoline (DPD), 2 commonly used serum bone resorption markers, in assessing bone metabolism in HD patients. Because NTX is derived from a different part of the same type I collagen as β-CTX.

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(the NH₂ and COOH termini for NTX and β-CTX, respectively), it is important to determine the significance of serum NTX as a bone resorption marker in HD patients.

We assessed the performance of serum NTX as a bone resorption marker in HD patients, compared with other established markers of bone resorption (β-CTX, PYY, and DPD) and bone formation [bone alkaline phosphatase (BAP) and intact osteocalcin (OC)], by examining the correlation of NTX with other markers and with the rate of bone loss over a subsequent 2-year period.

Patients and Methods

Patients

A total of 113 patients maintained on HD at Shirasagi Hospital were enrolled. Informed consent to the study protocol was obtained from each patient. Participation was restricted to male patients to avoid the influence of the menstrual cycle and menopause on bone metabolism. Underlying kidney diseases were as follows: diabetic nephropathy (n = 31), chronic glomerulonephritis (n = 52), nephrosclerosis (n = 8), polycystic kidney disease (n = 3), other diseases (n = 5), and unknown diseases (n = 14). None of the patients had a past history of parathyroidectomy or renal transplantation, and all patients were free of significant acute illness during the protocol period of 2 years. They also had neither a past history of fracture nor radiographic evidence of vertebral or rib fracture, and the possibility of past exposure to aluminum was negated in all of the participants. Because bone biopsy was not performed, adynamic bone disease could not be ruled out in the study patients. The active vitamin D derivatives calcitriol or alfacalcidol were given orally to 55 patients, with the daily dose unchanged during the study period. None of the patients received any other medication that might affect calcium metabolism, such as ipriflavone, vitamin K, or aluminum hydroxide. Patients underwent HD 3 times a week in sessions of 4 h each, performed with a hollow-fiber dialyzer and dialysate containing 3.0 mEq/L calcium. BMD was measured twice for all patients, and measurement of serum markers for bone metabolism was performed at the time of the first BMD measurement.

Biochemical Markers for Calcium and Bone Metabolism

A blood sample collected from the arteriovenous fistula immediately before a morning dialysis session on March 18 and 19, 2002, was kept on ice for 1 h and then centrifuged at 1000g for 10 min. The serum obtained was stored in aliquots at −20 °C until assayed, with measurements made immediately after thawing. NTX and other biochemical bone markers were measured in the same assay run −1 year after sample collection. Although blood collection in the early morning after overnight fasting is important in persons with normal kidney function, the influence of diurnal variation seems less in HD patients whose serum NTX concentration exceeds the normal value by 6.2-fold (as found in the current study), because impaired urinary excretion leads to NTX accumulation in serum. Furthermore, because the effect of dietary collagen intake on serum NTX is negligible (12), blood samples for determination of serum NTX were collected between 0800 and 1000 in the morning, after the patients had eaten breakfast.

Biochemical markers for calcium metabolism were measured as described previously (13–15). Serum parathyroid hormone (PTH) was measured by an IRMA (Allegro Intact PTH; Nichols Institute) (16, 17) with an intraassay CV for PTH of 4.8% (13). Serum NTX was measured by ELISA (Osteomark NTX serum; Ostex International) (18, 19) with an intraassay CV of 4.6%. Serum β-CTX was determined by an Elecsys β-CrossLapSTM serum assay (Roche Diagnostics) (9) with an intraassay CV of 2.6% (9). Serum PYY and DPD were measured by HPLC (20) with intraassay CVs of 1.3% for serum PYY and 6.8% for serum DPD (21). Serum BAP and intact OC were measured with an enzyme immunoassay (ALK-PHASE-B; Metra Biosystems) (22) and with a 2-site IRMA (Mitsubishi Kagaku Bioclinical Laboratories), respectively. The intraassay CVs for BAP and intact OC were 2.2% (13) and 6.3%, respectively (23).

BMD Measurement

Measurement of BMD in the distal third of the radius by dual-energy x-ray absorptiometry (QDR-4500; Hologic Inc.) was performed 21–24 h after completion of an HD session. Serum measurements of bone markers were performed within 6 months of the first BMD measurement. The second BMD measurement was performed ~2 years after the first measurement, and the BMD change is expressed as the mean annual change in BMD over the 2-year period.

Statistical Analysis

Data were analyzed with use of the StatView 5.0 J program (Abacus Concepts, Inc.). Correlation coefficients were calculated by simple regression analysis after logarithmic transformation of bone marker data. Comparisons of the change in BMD in the distal third of the radius between marker concentration quartiles were performed by one-way ANOVA with the Fisher protected least-significant difference multiple comparison test as a post hoc test. To determine the sensitivity, specificity, and positive and negative predictive values of serum bone markers for prediction of rapid bone loss, the patients were grouped into quartiles according to the rate of BMD change, and the correlation of increased bone marker values (the highest quartile) with the highest bone loss quartile was examined. The highest bone loss quartile was chosen for this comparison because there is no generally accepted clinical cutoff point that defines excessive bone loss in male HD patients. The Youden index was calculated by combination of the sensitivity and specificity...
(sensitivity + specificity − 1). P values <0.05 were considered statistically significant.

**Results**

**CLINICAL CHARACTERISTICS OF HD PATIENTS**

The baseline characteristics of the male HD patients are shown in Table 1. The mean (SD) age of the participants was 59.3 (11.1) years (range, 26–79 years) and the HD duration was 67.7 (32.2) months (range, 17–142 months).

**SERUM NTX AND OTHER BONE MARKERS IN HD PATIENTS**

The mean serum NTX value in the HD patients was 112.1 nmol of bone collagen equivalents (BCE) per liter (range, 15.0–675.0 nmol/L BCE), 6.2-fold higher than the upper reference value of 18.0 nmol/L BCE, as we reported previously in a study of 192 healthy men (24). Serum NTX concentrations were above the upper reference limit in 112 (99.1%) of 113 patients. The mean concentration of serum intact PTH was 1.62 μg/L, 4.4-fold higher than the value of 0.37 μg/L reported in 25 apparently healthy men (25). The mean concentrations of serum PYD and DPD were 51.5 and 9.6 nmol/L, 18.4- and 5.1-fold higher than the respective reference values of 2.8 (0.8) and 1.9 (0.4) nmol/L reported for healthy individuals (20). The mean serum BAP was 23.7 U/L, close to the reference value of 24.9 (7.0) U/L reported in healthy individuals (22). The mean serum intact OC was 44.2 μg/L, 6.5-fold higher than the value of 6.8 (2.4) μg/L reported in healthy individuals. Serum BAP and intact OC concentrations were above the respective upper reference limits in 37 (32.7%) and 87 (77.0%) of the 113 patients, respectively. The mean (SD), range, and highest quartile values for each marker are listed in Table 2.

**CORRELATION OF SERUM NTX WITH OTHER BONE MARKERS**

As shown in Fig. 1 of the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol51/issue12/, serum NTX correlated significantly with serum β-CTX (r = 0.929; P <0.0001) and the 2 other bone resorption markers, PYD (r = 0.793; P <0.0001) and DPD (r = 0.919; P <0.0001). Serum NTX also showed significant correlations with intact PTH (r = 0.735; P <0.0001) and the bone formation markers BAP (r = 0.697; P <0.0001) and intact OC (r = 0.776; P <0.0001).

<table>
<thead>
<tr>
<th>Table 1. Clinical characteristics of 113 male HD patients.</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>59.3 (11.1)</td>
<td>26–79</td>
</tr>
<tr>
<td>HD duration, months</td>
<td>67.7 (32.2)</td>
<td>17–142</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>58.2 (9.0)</td>
<td>38.8–83.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165 (6)</td>
<td>148–183</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.4 (2.7)</td>
<td>15.2–32.3</td>
</tr>
<tr>
<td>Calcium, mg/L</td>
<td>90 (7)</td>
<td>72–110</td>
</tr>
<tr>
<td>Phosphorus, mg/L</td>
<td>57 (16)</td>
<td>23–102</td>
</tr>
</tbody>
</table>

* BMI, body mass index.

**CORRELATION OF SERUM NTX AND OTHER BONE MARKERS WITH BMD IN DISTAL THIRD OF THE RADIUS**

During the 2 years of the study period, the patients suffered significant bone loss in the distal third of the radius, with BMD decreasing from 0.696 (0.081) to 0.685 (0.085) g/cm² (P <0.0001) with a mean annual rate of bone loss of 0.74 (1.77)%. Lumbar spine BMD did not change appreciably, from 0.709 (0.138) to 0.677 (0.169) g/cm² (P = 0.126). Serum NTX correlated significantly in a negative manner with the first measurement of BMD in the distal third of the radius (r = −0.372; P <0.0001) when serum bone markers were determined (Fig. 2 in the online Data Supplement). BMD in the distal third of the radius was also significantly and negatively correlated with serum intact PTH (r = −0.270; P = 0.0038), β-CTX (r = −0.340; P = 0.0002), PYD (r = −0.286; P = 0.0022), DPD (r = −0.371; P <0.0001), BAP (r = −0.334; P = 0.0003), and intact OC (r = −0.300; P = 0.0013). Lumbar spine BMD did not correlate with either PTH or any of the bone markers (data not shown).

Of particular interest, serum NTX (r = −0.236; P = 0.0118), β-CTX (r = −0.285; P = 0.0022), and DPD (r = −0.188; P = 0.0465) showed a significant correlation with annual BMD change in the distal third of the radius during the 2-year study period, in contrast to the lack of significant correlation with any of the other markers: serum intact PTH (r = −0.087), PYD (r = −0.175), BAP (r = −0.181), and intact OC (r = −0.020; see Fig. 3 in the online Data Supplement).

**ANNUAL BMD CHANGE IN DISTAL THIRD OF THE RADIUS IN QUARTILES BASED ON SERUM CONCENTRATIONS OF NTX AND OTHER BONE MARKERS**

Patients were divided into quartiles on the basis of serum NTX concentrations and other bone markers, and the annual rate of BMD reduction in the distal third of the radius was compared among these quartiles (see Fig. 4 in the online Data Supplement). The quartiles for a given bone marker are referred to as Q1, Q2, Q3, and Q4, with Q1 representing the 25% of patients with the lowest concentration of that particular marker. The mean BMD change in each serum NTX quartile (P = 0.033) and each β-CTX quartile (P = 0.038) were statistically different, whereas quartiles based on the serum intact PTH concentration and on other bone markers showed no statistically significant differences.
significant differences in BMD change. The percentage reduction in BMD in the distal third of the radius was 0.37 (1.44)% per year in the lowest serum NTX quartile (Q1), but was 1.59 (2.21)% per year in the highest NTX quartile (Q4), with statistically significant differences between Q4 and all other NTX quartiles (Q1 vs Q4, P < 0.01; Q2 vs Q4, P < 0.05; Q3 vs Q4, P < 0.05).

**Sensitivity and Specificity of the Serum NTX Assay**

Shown in Table 3 are the sensitivity, specificity, and positive and negative predictive values of the highest quartile concentrations of each marker for identification of HD patients in the highest quartile of BMD reduction in the distal third of the radius (loss ≥1.66% per year). The sensitivities of the highest quartiles as cutoff points for identification of those HD patients who had lost bone mass in the distal third of the radius at a rate of more than 1.66%/year during the preceding 2 years were 48% for NTX, 45% for β-CTX, 41% for PYD, and 38% for DPD. The specificities for identification of those HD patients who had lost bone mass at no more than 1.66%/year were high: 83% for NTX, 82% for β-CTX, 81% for PYD, and 80% for DPD. The Youden index obtained for NTX (0.31) was greater than the indexes for β-CTX (0.27), PYD (0.22), and DPD (0.18). The positive predictive values of serum bone marker concentrations in the highest quartile were 50% for NTX, 46% for β-CTX, 43% for PYD, and 39% for DPD, and the positive predictive values for increased bone formation markers were 39% for BAP and 32% for intact OC. The negative predictive values for serum marker concentrations below the highest quartile were 82% for NTX, 81% for β-CTX, 80% for PYD, 79% for DPD, 79% for BAP, and 76% for intact OC.

**Discussion**

We compared serum NTX, a newly identified biochemical marker for bone resorption, with established biochemical markers of bone resorption (PYD, DPD, and β-CTX) and bone formation (BAP and intact OC) as indicators for BMD loss in male HD patients. We observed a good correlation of serum NTX with serum concentrations of intact PTH and other bone markers (Fig. 1 in the online Data Supplement) and a significant and negative correlation of serum NTX with BMD in the distal third of the radius, as with other bone markers (Fig. 2 in the online Data Supplement). Of interest, only serum NTX, β-CTX, and DPD exhibited a significant correlation with annual BMD reduction in the distal third of the radius over a 2-year period. Because serum bone markers theoretically reflect systemic bone metabolism and the bone surface at the distal radius is quite small because of the preferential distribution of cortical bone at the indicated site, the correlation of serum markers with BMD or its annual change at the distal third of the radius should be weak. Furthermore, when the patients were divided into 4 groups on the basis of serum concentrations of bone markers, increased concentrations of serum NTX and β-CTX, but not other bone markers, were significantly associated with a faster rate of bone loss in the distal third of the radius during the 2-year period (Fig. 4 in the online Data Supplement). Serum NTX and β-CTX differed significantly between the groups of patients with fast and slow bone loss, whereas other bone markers did not (Fig. 5 in the online Data Supplement), suggesting that serum NTX, in addition to serum β-CTX, is the best marker for assessing the bone metabolic state and thus is the best predictor for the rate of bone loss or fracture risk.

It is recommended that BMD be measured at the forearm in patients with uremic hyperparathyroidism because of the preferential effect of PTH on cortical bone.

**Table 3. Sensitivity, specificity, Youden index, and positive and negative predictive values of highest quartile marker concentrations for identification of male HD patients with BMD loss >1.66% per year in the distal third of the radius (highest quartile for rate of BMD loss).**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Youden Index</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTX</td>
<td>48%</td>
<td>83%</td>
<td>0.31</td>
<td>50%</td>
<td>82%</td>
</tr>
<tr>
<td>β-CTX</td>
<td>45%</td>
<td>82%</td>
<td>0.27</td>
<td>46%</td>
<td>81%</td>
</tr>
<tr>
<td>PYD</td>
<td>41%</td>
<td>81%</td>
<td>0.22</td>
<td>43%</td>
<td>80%</td>
</tr>
<tr>
<td>DPD</td>
<td>38%</td>
<td>80%</td>
<td>0.18</td>
<td>39%</td>
<td>79%</td>
</tr>
<tr>
<td>BAP</td>
<td>38%</td>
<td>80%</td>
<td>0.18</td>
<td>39%</td>
<td>79%</td>
</tr>
<tr>
<td>Intact OC</td>
<td>38%</td>
<td>77%</td>
<td>0.08</td>
<td>32%</td>
<td>76%</td>
</tr>
<tr>
<td>Intact PTH</td>
<td>31%</td>
<td>75%</td>
<td>0.03</td>
<td>28%</td>
<td>75%</td>
</tr>
</tbody>
</table>

*Proportion of male HD patients with rapid bone loss (≥1.66% per year in the distal third of the radius) with marker concentrations in the highest quartiles of bone marker concentrations.


2. PPV, positive predictive value; NPV, negative predictive value.

3. Incidence of rapid bone loss among male HD patients with marker concentrations in the highest quartiles of bone marker concentrations.

4. Incidence of slow bone loss among male HD patients with marker concentrations below the highest quartiles of bone marker concentrations.
Although lumbar spine BMD is usually measured in anterior–posterior projection in osteoporotic patients because of superior precision and reproducibility, it may be complicated by the frequent occurrence of vascular calcification of the abdominal aorta in HD patients (28). Indeed, the patients in the present study showed a significant annual bone loss of 0.74% at the forearm but not at the lumbar spine BMD (data not shown), as described previously (29, 30).

Type I collagen is degraded during the bone resorption process, liberating small fragments, such as NTX, β-CTX, PYD, and DPD, into the blood and then into the urine (5, 31) and making serum and urinary concentrations of these fragments available as biochemical markers of bone resorption. In patients with anuria, fragments derived from cleavage of type I collagen accumulate in serum because of impaired urinary excretion. Indeed, the mean serum NTX concentration in male HD patients was 6.2-fold higher than the upper reference limit for healthy individuals. Some bone formation markers, such as BAP, remained within the reference intervals (32), but serum N-terminal propeptide of type I collagen, another bone formation marker, showed a 1.34-fold increase (33). Although bone markers accumulate in serum, except for serum BAP, because of impaired secretion into urine, a significant correlation persists between bone markers and histomorphometric indexes in HD patients (34, 35), clearly indicating that the rate-limiting step in determining serum concentrations of bone markers is the release of these molecules from bone, supporting the clinical utility of these molecule as bone markers in hemodialyzed patients.

The results of the current study indicate that only serum NTX, β-CTX, and DPD are correlated significantly with the annual change in BMD in the distal third of the radius. The NTX and β-CTX epitopes, which are preferentially liberated from type I collagen in bone by osteoclastic hydrolysis (36), suggest bone specificity. PYD occurs in many tissues outside bone, and DPD is also found in other tissues, such as dentine, skeletal muscle, and the aorta. Furthermore, assaying of serum PYD and DPD is problematic with regard to sample preparation and the stability of internal standards. Because NTX can be measured by ELISA with much smaller assay CVs, the stable assay method may also improve the precision of NTX measurements. Indeed, in clinical practice in osteoporosis, serum NTX is reported to be superior to other markers because of its small assay CV and greater suppression rate after treatment with bone antiresorptive drugs (37).

Few studies have examined the clinical usefulness of serum NTX compared with other bone metabolic markers in HD patients. Because serum BAP is unaffected by renal dysfunction and is thus useful in HD patients, serum BAP in HD patients remained within the reference interval (Table 2). However, the significant correlations of serum NTX with serum intact PTH and other bone markers (see Fig. 1 in the online Data Supplement) indicate that the increase in serum NTX is also related to enhanced bone turnover attributable to secondary hyperparathyroidism.

Biochemical markers of bone turnover are used to predict future bone loss in osteoporotic patients (4, 38, 39). We found that serum NTX and β-CTX, but not intact PTH or other bone markers such as PYD, DPD, BAP, and intact OC, correlated significantly with the rate of forearm bone loss during a subsequent 2-year period in HD patients (see Fig. 3 in the online Data Supplement). Furthermore, we found a strong relationship between the quartile distribution of serum NTX concentrations and faster rates of cortical bone loss, with patients in the highest quartile of NTX concentrations showing a significantly faster cortical bone loss than those in the lowest quartile (see Fig. 4 in the online Data Supplement).

The sensitivity, specificity, and positive and negative predictive values of serum NTX were as high as those of serum intact PTH and other bone markers (Table 3). These data indicate that serum NTX might provide a more relevant prediction of the rate of cortical bone loss in HD patients. For all bone markers, including NTX, the sensitivity and positive predictive value were relatively lower but the specificity and the negative predictive value were good. The Youden index for NTX was 1.5-fold better than the indexes for PYD, DPD, and BAP, and more than 4-fold better than the indexes for intact OC and intact PTH, further suggesting that measurement of NTX might be more useful than other bone resorption markers for predicting cortical bone loss in HD patients.

In summary, we found that serum NTX, a newly identified biochemical marker of bone resorption, is equivalent to or better than other established serum bone markers for predicting the rate of forearm bone loss over a subsequent 2-year period in male HD patients. Use of serum NTX as a bone resorption marker for detection of rapid cortical bone loss may also be an improvement over use of intact PTH.

References


