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Osteocalcin: A non-invasive index of metabolic bone disease in patients treated by CAPD

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Osteocalcin: A non-invasive index of metabolic bone disease in patients treated by CAPD. Serum osteocalcin has been found to correlate with bone formation. However, present literature gives only limited data on osteocalcin and bone histomorphometry in patients undergoing peritoneal dialysis. This study assessed serum osteocalcin, dialysate osteocalcin, peritoneal clearance of osteocalcin (Cl_p-osteocalcin) and mass transfer of osteocalcin (MT_p-osteocalcin), and evaluated relationships between these values and bone histomorphometry. Eighteen patients were treated by continuous ambulatory peritoneal dialysis (CAPD). Bone biopsies, serum and dialysate osteocalcin, serum levels of parathyroid hormone, alkaline phosphatase, aluminum, phosphate, Ca^{2+} and vitamin D_3 metabolites were measured at the start and in 10 of the patients a year later. Serum osteocalcin was found to be elevated. Osteocalcin was detected in the dialysate resulting in significant values of Cl_p-osteocalcin and MT_p-osteocalcin. Serum and dialysate levels of osteocalcin correlated significantly ($r = 0.66$, $P < 0.001$) and like MT_p-osteocalcin with serum levels of alkaline phosphatase and PTH. Histomorphometry showed that osteitis fibrosa was the predominant bone disease detected. Serum concentration of osteocalcin correlated with osteoid thickness, eroded and osteoclast surfaces, aluminum staining, and some of the bone dynamic parameters. Dialysate osteocalcin, MT_p-osteocalcin, PTH and alkaline phosphatase correlated with practically the same histomorphometric parameters as serum osteocalcin. No correlations were seen between Cl_p-osteocalcin and any histomorphometric parameters. Serum osteocalcin was elevated above the normal range, and significant positive correlations between serum osteocalcin and bone formation parameters were found. Serum osteocalcin correlated with almost the same histomorphometric parameters as PTH. Thus, serum levels of PTH and osteocalcin gave additional information to one another as non-invasive parameters in this group of patients. Hence, serum osteocalcin is a valuable non-invasive index of metabolic bone disease in patients treated by CAPD. The transperitoneal removal of osteocalcin does not appear to be clinically significant.

With the discovery of osteocalcin another non-invasive tool for evaluation of bone metabolism became possible [1, 2]. This protein, which constitutes 20 to 25% of non-collagen bone protein, is exclusively synthesized by osteoblasts and is a sensitive and specific humoral marker of bone formation. It is secreted into bone matrix, where it binds to hydroxyapatite and later incorporates into bone matrix [2]. However, a fraction of the newly synthesized protein escapes to serum ("spill-over") where levels have been found to correlate with histomorphometric indices of bone formation [3], a parameter otherwise not easily studied except through kinetic techniques or by an invasive approach like bone biopsy.

Osteocalcin is normally filtered by the kidneys and thereafter catabolized to its constituent amino acids [4]. An increase in circulating osteocalcin could therefore reflect increased skeletal production, decreased renal clearance, or both. In conservatively treated uremic and hemodialyzed patients, a direct relationship between serum levels of osteocalcin and histological parameters of bone formation has been observed [5–7]. Studies in adults and children have shown that serum levels of osteocalcin in patients undergoing continuous ambulatory peritoneal dialysis (CAPD) were significantly lower than corresponding concentrations in hemodialysis patients [8, 9]. This observation could be explained by the finding of higher concentrations of osteocalcin in peritoneal dialysate effluent compared to concentrations in the effluent from hemodialysis [10–12]. However, only limited data dealing with osteocalcin measurements in CAPD are available [4, 7, 8, 10–12]. Nevertheless, to the best of our knowledge, no data including serum and dialysate levels of osteocalcin, peritoneal mass transfer and clearance values of this protein in correlations to bone histomorphometry have yet been published.

The aim of the present investigation therefore was to: (1) assess values of serum osteocalcin, dialysate levels of osteocalcin as well as peritoneal clearance and mass transfer of osteocalcin in adults treated by CAPD; (2) evaluate the relationship between the values of osteocalcin and bone histomorphometry with respect to renal osteodystrophy as a non-invasive predictor of bone histology; and (3) determine the correlations, if any, between osteocalcin levels and other humoral parameters of bone metabolism in CAPD patients not receiving vitamin D_3 analogues.

Methods

Subjects

A group of 5 women and 13 men maintained on CAPD for 19.7 ± 21.3 months (range 1 to 87) was studied. Mean age was 62.0 ± 14.4 years (range 34 to 83). Primary renal diseases were diabetic nephropathy in six patients, chronic glomerulonephritis in four, interstitial nephropathy in three, hypertensive nephropathy in two, chronic pyelonephritis in one, obstructive nephropathy in one and systemic lupus in one. Clinical evidence of cardiac failure, peripheral edema or nephrotic syndrome was not present in any subject. Residual kidney function was not evaluated. Three of the five female patients were postmenopausal; the two others had developed amenorrhea during the course of their chronic

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renal failure. None of the patients was nephrectomized, parathyroidectomized, oophorectomized or had signs of liver disease or malabsorption. Moreover, none had received a kidney transplant earlier and none had been immobilized before the current study. None took vitamin D_3 analogues of any kind, calcium supplements, steroids, estrogens, coumarins, anticonvulsants or medications known to interact with calcium, vitamin D_3 and osteocalcin levels, and only the patient with systemic lupus had received steroids and cytostatic therapy earlier for his primary disease. None had peritonitis episodes for at least the preceding two months before study periods. None used drugs known to influence peritoneal permeability. No patient had ever been exposed to dialysis solutions containing acetate or chlorhexidine intraperitoneally or had undergone abdominal surgery involving the peritoneal cavity.

All patients received a diet containing a daily intake of at least 1 g of protein per kg body wt and without sodium restriction. Aluminum containing phosphate binders were only prescribed when repeated measurements of serum phosphate were >2.0 mmol/liter. Six patients had to consume aluminum-containing phosphate binders.

All gave informed consent according to the Declaration of Helsinki II. No side effects or complications were observed during the investigation.

Dialysis technique

CAPD was performed by standard technique. The dialysis fluid contained 1.75 mmol/liter calcium and 0.75 mmol/liter of magnesium (Dianeal, Baxter Healthcare Corporation). The choice of dextrose solution (1.5% or 4.25%) was based on general fluid balance. None of the patients had "dry nights."

Investigations

Studies were performed during 24 hour periods with patients on their usual regime of CAPD. Peritoneal dialysate effluents and blood samples were drawn at the end of each study period when transiliac bone biopsies were also performed.

The effluent was mixed and measured. Portions were stored at −20°C with blood samples for later assay of osteocalcin.

Laboratory procedures

Plasma concentrations of alkaline phosphatase, total calcium and phosphate were determined by standard methods (SMAC, Technicon Instruments Corporation, Tarrytown, New York, USA). Ionized calcium was analyzed at pH 7.4 using an ionized calcium analyzer (ICA1, Radiometer, Copenhagen, Denmark). Immunoreactive determination of intact parathyroid hormone (PTH) was performed using the Allegro intact PTH kit (Nichols Institute Diagnostics, San Juan Capistrano, California, USA). This system has a sensitivity of 1 pg/ml (0.1 nmol/liter) and is highly specific for only the biologically active and intact PTH molecule. Normal range, intra-assay and interassay coefficients of variation were 1.1 to 4.8 pmol/liter, 1.8% and 5.6%, respectively.

Serum magnesium was determined by atomic absorption spectrophometry. Serum aluminum was analyzed in duplicate samples by Zeeman-corrected electrothermal atomic absorption spectrometry [13]. The limit of detection obtained by the method was 1.5 μg/liter. Bone aluminum concentrations were measured from the remains of plastic embedded biopsies used for the bone biopsy analysis. The methods have previously been described [14].

Plasma levels of osteocalcin were assayed using a solid phase enzyme-linked immunosorbent assay [15]. Purified osteocalcin was prepared from femoral shafts of calves, and conjugated for immunization in rabbits. The lower detection limit of this ELISA assay was less than 0.5 μg/liter (0.172 nmol/liter osteocalcin = 1 ng/ml osteocalcin). For serum osteocalcin the normal range used was 7.1 to 12.1 μg/l, without sex or age variation [15]. The intra-assay and interassay coefficients of variation were 6.0% and 5.3%, respectively. All samples were analyzed in duplicate.

$1,25(OH)_2D_3$, $25(OH)D_3$ and vitamin-D-binding protein (DBP) were determined by previously described methods [16].

Bone biopsy and histomorphometry

Eighteen patients underwent a bone biopsy at the start of the study and 10 had one performed a year later. Thus, a total of 28 bone biopsies were obtained. One of the 10 biopsies performed at the end of the study was taken postmortem. The transiliac approach at the anterior iliac crest was used and performed under local analgesia using a Bordier trocar with an internal diameter of 8 mm. Prior intravital tetracycline double-labeling with tetracycline and oxytetracycline on a 2/11/2 day basis (250 mg tetracycline twice daily for 2 days; 11 days without medication, followed by 250 mg oxytetracycline twice daily for another 2 days) was used. Biopsies were obtained three to four days after the last oral doses of oxytetracycline. The samples were fixed in 70% ethanol and prestained by the Villanurva method for 72 hours before plastic embedding [17]. Five micrometer thin sections were cut by a Jung Universal microtome 1150/autocut. Sections were examined without further staining or were stained with the Masson Trichrome for evaluation of osteoid and the ammonium aurine tricarboxylic acid (Aluminon) which stains specifically for aluminum [18]. Three groups of sections spaced approximately 200 μm apart were taken from each biopsy, and except for trabecular and cortical areas, which were measured in one section only, all variables represent an average value for all trabecular and cortical bone in three sections. The intraobserver variation for most variables was between 5 to 15% [19].

The histomorphometric analysis was made with a Morphomat 30 (Zeiss, Germany) digitizing analyzer and Olympus BH2 microscope. Conventional bone histomorphometry, including staining for aluminum, definitions and normal values of the histomorphometric data, was performed as previously described [14, 20, 21]. The measurements included trabecular bone volume as percentage of total tissue volume, osteoid volume as percentage of trabecular bone volume and osteoid thickness, osteoid surface, eroded surface and osteoclast surface all as percentage of total surface. Osteoclast surface was defined as the percentual length of osteoclast covered resorption surface. Cortical thickness was measured by dividing cortical area by cortical length. Cortical porosity was defined as cortical bone area as a percentage of total cortical area. Trabecular bone thickness was defined as twice tracecular bone area divided by trabecular bone perimeter length. Tetracycline labeled biopsies were evaluated under fluorescent light, enabling a quantitative measure of bone formation rate and bone mineralization. Tetracycline labeled surfaces were defined as the length of double tetracycline labeled surface plus one-half length of single labeled surface. In patients where no marker interval could be observed due to low bone formation, the mineralization appositional rate was conventionally defined as 0.1

μm/day and the mineralization lag time as 100 days minimum. The following diagnostic criteria were used:

Osteitis fibrosa. Osteoclast surface >0.65% (that is, length of osteoclast covered surface as a percentage of total bone surface), normal mineralization lag time (<100 days), varying degrees of dissecting resorption and peritrabecular fibrosis and increased turnover in tetracycline labeled biopsies.

Mixed uremic osteodystrophy. Biopsies with increased osteoclast surface (>0.65%) and increased mineralization lag time (>100 days).

Osteomalacia. Biopsies with normal osteoclast surface (<0.65%), osteoid thickness (>15 μm) and increased mineralization lag time (>100 days).

Adynamic lesion. Biopsies with increased mineralization lag time (>100 days) but normal osteoclast surface (<0.65%) and normal osteoid thickness (<15 μm).

Osteopenia. Biopsies with reduced trabecular bone volume (<15% of total bone area) but otherwise with normal bone parameters.

Normal. Biopsies with none of the above-mentioned features.

Aluminum staining was defined semiquantitatively as absent (0), light (1), moderate (2) or heavy (3). Patients with osteomalacia or adynamic lesion and histochemical aluminum deposition (light, moderate or heavy) on the mineralization front were classified as having aluminum related bone disease.

Calculations

Peritoneal clearances (Cl_p) were calculated using the standard formula:

$$Cl_p = \frac{\text{Dialysate concentration}}{\text{Serum concentration}} \times \frac{\text{Drainage volume}}{\text{Study period}}$$

Peritoneal mass transfer (MT_p) of the solutes was calculated according to the formula:

$$MT_p = C_i \times V_i - C_o \times V_o$$

where C_i is the concentration in the inflow, V_i is the volume in the inflow, C_o the concentration in the outflow and V_o the volume in the outflow. MT_p was considered positive when a gain was observed and negative in case of net removal from the body. The instilled volume per exchange was assumed to be 1050, 1550 or 2050 ml in bags containing 1000, 1500 or 2000 ml dialysis solution, respectively.

Statistical analysis

Data were analyzed parametrically and compared using Student's *t* test. Variables were correlated using conventional regression analysis and Student's *t*-test. For variables not normally distributed, comparison using the Mann-Whitney rank sum test was used. To develop an algorithm to predict the MD analysis of variance for all significantly correlated variables, stepwise variable selection using the F test was performed. The variable with the highest F-ratio was entered into the model, and the process was repeated as long as the new variable added significantly to the model. At each stage checks were made to see that previously selected variables were still significant; variables that had become insignificant were removed. Only F-ratios above 4.0 were allowed. When all significant variables had been selected, the final model was determined using the Graham-Schmidt algorithm.

Results

Biochemical parameters

Biochemical parameters including serum, dialysate, peritoneal clearance (Cl_p) and peritoneal mass transfer (MT_p) levels of the non-invasive markers of bone metabolism at start and end of the study are reported in Table 1. Mean levels of serum osteocalcin were elevated above the normal range at baseline and one year later (Table 1). Moreover, significant amounts of osteocalcin were detected in the dialysate effluent, both at baseline and after one year, resulting in substantial peritoneal clearances of osteocalcin (Cl_p-osteocalcin) and peritoneal mass transfer values of osteocalcin (MT_p-osteocalcin; Table 1). Mean values of serum levels of PTH, magnesium and aluminum were all increased at the time of both evaluations (baseline and one year later). Serum concentrations of $1,25(OH)_2D_3$ were below the normal range, adjusted for sun exposure and age (Table 1). No significant changes between parameters given in Table 1 at baseline and one year later were found. No correlations were found between dialysate osteocalcin values, inflow volume and drained volume of dialysate effluent.

Humoral parameters

Linear correlations between serum, dialysate, Cl_p-osteocalcin and MT_p-osteocalcin levels and other humoral parameters are shown in Table 2. Thus, serum osteocalcin, dialysate osteocalcin and MT_p-osteocalcin levels significantly correlated statistically with serum levels of alkaline phosphatase and PTH (Table 2). Also a statistically significant correlation between serum osteocalcin and MT_p-osteocalcin was found ($r = 0.55$, $P < 0.01$). No correlations were found between serum osteocalcin levels and serum values of calcium, Ca^{2+}, HCO_3, magnesium, aluminum and vitamin D_3 parameters. Furthermore, serum and dialysate levels of osteocalcin correlated significantly ($r = 0.66$, $P < 0.001$). Also dialysate osteocalcin was significantly correlated with serum levels of alkaline phosphatase and PTH ($r = 0.70$, $P < 0.001$ and $r = 0.69$, $P < 0.001$, respectively).

Bone histology

The distribution of bone histology is given in Figure 1. No specific etiology of renal disease was associated with any one of the bone diagnoses. Osteitis fibrosa was the predominant biopsy diagnosis. Six patients had a normal bone biopsy diagnosis, only one of these six patients had a consistent bone diagnosis at baseline evaluation and the year later (Fig. 1). Two of the five patients having adynamic bone lesion had diabetes mellitus. Only one of these patients had aluminum staining in his bone biopsy. None in the adynamic group had high serum PTH levels.

Bone histomorphometry

In Table 3 bone histomorphometric parameters at baseline, the year after for patients evaluated with repeated bone biopsies and the average values for all patients evaluated are given. Significant differences between parameters at baseline and the year after were only found for tetracycline labeled surface and trabecular bone volume ($P < 0.05$ for both).

Correlations between osteocalcin measurements, other markers of bone metabolism and the bone histomorphometric parameters

Table 1. Serum (S), dialysate (D), peritoneal clearance (Cl_p) and peritoneal mass transfer (MT_p) of non-invasive markers of bone metabolism in patients undergoing peritoneal dialysis.

Parameters		Time 0 year	Time 1 year
S-osteocalcin *7.1–12.1 μg/liter*	Mean ± SD:	21.4 ± 16.8	18.8 ± 16.1
	Range:	1.9–50.5	1.0–56.5
	Median:	16.7	15.6
	Quartiles:	6.0–39.0	9.7–19.1
D-osteocalcin *μg/liter*	Mean ± SD:	7.5 ± 6.7	7.5 ± 4.5
	Range:	1.0–23.8	2.0–15.2
	Median:	6.0	5.5
	Quartiles:	3.3–9.6	4.7–11.2
Cl_p-osteocalcin *ml/min*	Mean ± SD:	2.3 ± 2.9	3.9 ± 3.8
	Range:	0.5–12.2	1.4–12.8
	Median:	1.2	2.6
	Quartiles:	1.0–1.9	1.7–4.5
MT_p-osteocalcin *μg/24 h*	Mean ± SD:	−48.3 ± 47.2	−54.0 ± 33.2
	Range:	−5.2–(−) 202.8	−18.4–(−) 112.5
	Median:	−33.3	−42.0
	Quartiles:	−19.5–(−) 57.5	−30.6–(−) 78.0
S-calcium *2.17–2.57 mmol/liter*	Mean ± SD:	2.18 ± 0.18	2.22 ± 0.24
	Range:	1.90–2.49	1.88–2.68
	Median:	2.23	2.21
	Quartiles:	1.99–2.31	2.03–2.33
S-Ca^{2+} *1.15–1.35 mmol/liter*	Mean ± SD:	1.14 ± 0.07	1.11 ± 0.09
	Range:	1.04–1.27	1.02–1.23
	Median:	1.14	1.10
	Quartiles:	1.08–1.21	1.04–1.18
S-phosphate *0.79–1.51 mmol/liter*	Mean ± SD:	1.84 ± 0.52	1.98 ± 0.61
	Range:	1.03–3.19	1.25–3.23
	Median:	1.75	1.88
	Quartiles:	1.63–2.01	1.60–2.22
S-alkaline phosphatase *80–275 U/liter*	Mean ± SD:	230 ± 80	243 ± 63
	Range:	126–447	136–380
	Median:	200	242
	Quartiles:	171–302	199–265
S-PTH *1.1–4.8 pmol/liter*	Mean ± SD:	22.8 ± 25.8	18.8 ± 8.9
	Range:	0.5–131.6	9.7–40.3
	Median:	16.7	16.4
	Quartiles:	6.5–28.1	14.9–20.2
S-HCO_3 *23–35 mmol/liter*	Mean ± SD:	24 ± 3	23 ± 2
	Range:	20–32	20–26
	Median:	24	23
	Quartiles:	22–26	22–24
S-magnesium *0.61–0.85 mmol/liter*	Mean ± SD:	1.14 ± 0.21	1.10 ± 0.12
	Range:	0.80–1.66	0.98–1.35
	Median:	1.10	1.08
	Quartiles:	0.99–1.27	0.99–1.15
S-aluminum *0.0–0.6 μmol/liter*	Mean ± SD:	1.6 ± 2.0	1.8 ± 2.5
	Range:	0.1–9.6	0.2–7.4
	Median:	0.85	0.7
	Quartiles:	0.7–1.9	0.4–1.9
S-1,25$(OH)_2D_3$ *24–158 pmol/liter*	Mean ± SD:	18 ± 6	—
	Range:	12–33	
	Median:	16	
	Quartiles:	15–21	
S-25$(OH)D_3$ *18.5–82.0 nmol/liter*	Mean ± SD:	29.8 ± 20.7	—
	Range:	3.6–76.0	
	Median:	21.5	
	Quartiles:	14.7–50.0	
S-DBP *4.1–7.5 μmol/liter*	Mean ± SD:	6.0 ± 1.1	—
	Range:	4.6–8.4	
	Median:	5.9	
	Quartiles:	5.4–6.2	

No significant differences between levels found at baseline and at one year was found. Abbreviation is DBP, vitamin D_3 binding protein. Quartiles are the 25th and 75th percentiles.

are reported in Table 4. Serum osteocalcin correlated best with eroded surface and osteoclast surface ($P < 0.001$ and $P < 0.05$, respectively; Table 4) and with bone dynamic parameters (mineral appositional rate, labeled surface, bone formation rate per unit of bone surface, bone volume and tissue volume, respectively). The extent of aluminum deposits in bone (aluminum bone content)

Table 2. Correlations between osteocalcin measurements and other biochemical markers of bone metabolism

	Serum osteocalcin	Dialysate osteocalcin	Cl_p-osteocalcin	MT_p-osteocalcin
S-calcium	NS	NS	NS	NS
S-Ca^{2+}	NS	NS	NS	NS
S-phosphate	NS	NS	NS	NS
S-alk. phos.				
r =	0.43	0.70	NS	0.55
t =	2.1	4.5		2.9
P =	0.05	<0.001		<0.05
S-PTH				
r =	0.55	0.69	NS	0.46
t =	2.3	4.5		2.9
P <	0.05	<0.001		<0.05
S-HCO_3	NS	NS	NS	NS
S-magnesium	NS	NS	NS	NS
S-aluminum	NS	NS	NS	NS
S-1,25$(OH)_2D_3$	NS	NS	NS	NS
S-25$(OH)D_3$	NS	NS	NS	
r =				0.57
t =				2.4
P <				0.05
S-DBP	NS	NS	NS	NS

Data at baseline and at one year were added.

Abbreviations are: S, serum; D, dialysate; Cl_p, peritoneal clearance; MT_p, peritoneal mass transfer; DBP, vitamin D_3 binding protein; NS, not significant.

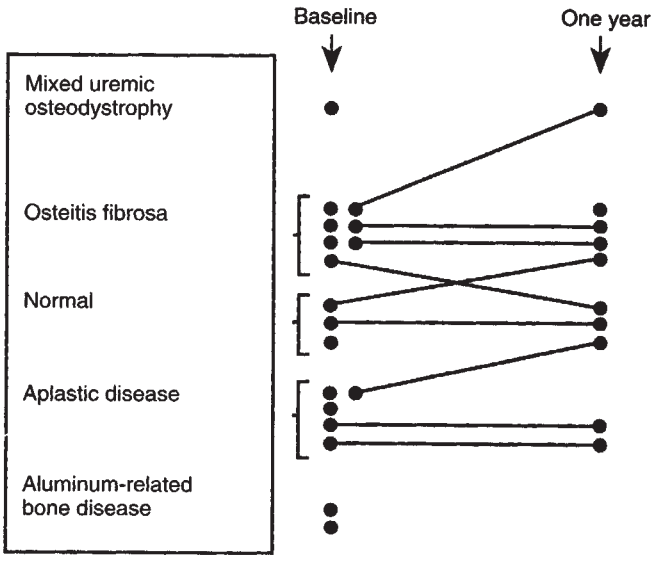

Fig. 1. *The distribution of bone biopsy diagnosis at baseline, one year later and the changes over time.* Each dot represents one patient. Patients biopsied twice are indicated with two dots connected with a line, while a single dot represents patients only having one bone biopsy taken.

did not correlate with any of the measured bone markers, although the qualitative assessment of aluminum deposits in bone (aluminum staining) correlated negatively with serum osteocalcin and serum total calcium (Table 4). Tetracycline labeled surface correlated to a very high extent with serum and dialysate levels of osteocalcin and serum PTH (tetracycline labeled surface = $-3.15 + 0.145 \times$ serum osteocalcin $+ 0.409 \times$ dialysate osteocalcin $+ 0.184 \times$ serum PTH; $r = 0.98$) as shown in Figure 2, where measured tetracycline labeled surface is illustrated in relation to the predicted tetracycline labeled surface. Dialysate osteocalcin, MT_p-osteocalcin, serum levels of PTH and alkaline phosphatase correlated almost with the same histomorphometric parameters as serum osteocalcin (Table 4). No correlations were seen between Cl_p-osteocalcin as well as serum aluminum levels and any of the histomorphometric parameters listed (Table 4). Finally, a negative correlation was found for osteoclast surface versus length of uremia ($r = 0.49$, $P < 0.05$). Age correlated positively with serum Ca^{2+} ($P < 0.05$) and negatively with serum phosphate ($P < 0.05$), serum aluminum ($P < 0.05$), serum 1,25$(OH)_2D_3$ ($P < 0.05$), corrected apposition rate ($P < 0.01$), and bone formation rate per unit of bone surface, bone volume and tissue volume, respectively ($P < 0.05$ for all three).

Discussion

Many procedures have been used to assess bone metabolism including humoral substances, radiological, radionuclear and densitometric evaluations as well as scanning and bone histomorphometry. Although these procedures have provided important information each method has limitations. Therefore, a sensitive and specific non-invasive biochemical marker for bone metabolism is needed. Alkaline phosphatase has been widely used as such a marker. However, osteocalcin has the important advantage over alkaline phosphatase in that it is a specific marker for bone metabolism only. Unlike alkaline phosphatase, its plasma level is not contributed to by any other cell type than the osteoblast. In addition, it has been shown that alkaline phosphatase was unable to differentiate between patients with low bone turnover and those with high bone turnover [22]. Some authors have claimed that osteocalcin has the same sensitivity but higher specificity than PTH for prediction of bone formation status [22]. We found that serum osteocalcin and PTH levels gave supplementary information to one another. As previously observed [5, 7, 9, 10, 22], major correlations between serum, dialysate, Cl_p-osteocalcin and MT_p-osteocalcin levels and other humoral parameters related to bone metabolism were found. In agreement with others [5, 7, 10, 22], serum osteocalcin was significantly correlated with serum alkaline phosphatase and serum PTH. Moreover, we observed that dialysate osteocalcin and MT_p-osteocalcin also correlated with serum alkaline phosphatase and serum PTH. However, unlike to Klein et al [10], we were unable to find any correlation between serum osteocalcin and serum calcium.

Another important topic has been the probable influence of the dialysis modality (peritoneal vs. hemodialysis) on osteocalcin concentrations. Some authors have reported no differences in serum osteocalcin levels in patients undergoing CAPD versus hemodialysis [9]. Others found lower serum levels in CAPD patients due to a significant peritoneal removal of osteocalcin [4, 8]. This is consistent with the view that some proteins with a molecular weight in the range of osteocalcin's and PTH are not removed as effectively by hemodialysis as by CAPD [23]. We found that the transperitoneal removal of osteocalcin did not appear to alter osteocalcin levels in blood.

There is still controversy over whether increased serum osteocalcin reflects increased osteoblast production or decreased renal clearance of this protein. Unfortunately, residual kidney function and urine output was not monitored in the present study. Thus, a possible renal excretion of osteocalcin could not be evaluated. However, as the majority of our bone biopsies showed high

Table 3. Bone histomorphometric parameters (mean ± SD with ranges given in parentheses) at baseline and one year later in patients on peritoneal dialysis biopsied twice

Parameters *units*	All patients	Baseline	One year
Osteoid	—	—	—
Osteoid thickness *μm*	9.7 ± 3.0	9.8 ± 2.9 (6.0–15.4)	9.8 ± 3.4 (6.0–16.3)
Osteoid surface %	25.0 ± 15.5	23.3 ± 15.4 (1.8–54.3)	23.7 ± 16.5 (6.1–51.4)
Osteoid volume %	4.51 ± 3.01	4.05 ± 3.03 (0.16–9.20)	4.74 ± 3.89 (0.76–12.50)
Resorption	—	—	—
Eroded surface %	2.53 ± 1.62	2.58 ± 1.77 (0.30–6.74)	2.45 ± 1.40 (0.90–5.10)
Osteoclast surface %	0.55 ± 0.34	0.55 ± 0.34 (0.03–1.25)	0.52 ± 0.49 (0.00–1.41)
Trabecular bone	—	—	—
Trabecular volume %	17.7 ± 4.65	18.6 ± 4.7[a] (11.0–28.0)	14.6 ± 4.6[a] (7.9–20.8)
Trabecular thickness *μm*	130 ± 19	130 ± 16 (104–164)	118 ± 16 (96–141)
Cortical bone	—	—	—
Cortical thickness *cm*	0.69 ± 0.22	0.68 ± 0.27 (0.27–1.24)	0.74 ± 0.40 (0.37–1.57)
Cortical porosity %	90.3 ± 3.1	90.5 ± 4.4 (80.1–96.3)	88.5 ± 4.1 (84.8–96.0)
Bone dynamics	—	—	—
Mineral appositional rate *μm/day*	0.59 ± 0.59	0.54 ± 0.57 (0.00–1.89)	0.83 ± 0.52 (0.00–1.37)
Tetracycline labeled surface *mm*	5.23 ± 5.78	4.00 ± 5.45[a] (0.00–15.20)	7.79 ± 6.47[a] (0.00–16.80)
Adjusted apposition rate *μm/day*	0.24 ± 0.32	0.23 ± 0.34 (0.00–1.32)	0.26 ± 0.28 (0.00–0.78)
Mineralization lag time *days*	446.2 ± 462.1	418.5 ± 478.8 (0.0–1000.0)	261.0 ± 421.5 (9.5–1000.0)
Bone formation rate per unit of bone surface $\mu m^3/\mu m^2/yr$	13.50 ± 15.71	11.60 ± 16.89 (0.00–52.30)	16.97 ± 15.09 (0.00–46.10)
Bone formation rate per unit of bone volume *%/yr*	26.47 ± 30.56	22.12 ± 31.93 (0.00–95.00)	34.48 ± 28.64 (0.00–83.40)
Bone formation rate per unit of bone tissue *%/yr*	5.08 ± 6.31	4.71 ± 7.15 (0.00–21.30)	5.57 ± 5.49 (0.00–17.30)
Aluminum staining (0–3)	0.5 ± 0.7	0.3 ± 0.7 (0–2)	0.6 ± 0.7 (0–2)
Aluminum content *ppm*	20.9 ± 14.3	21.1 ± 15.0 (6.8–52.9)	—

Significant differences were only found between trabecular bone volume and tetracycline labeled surface at baseline and the year later ([a] $P < 0.05$). In the second column from left the average values for all evaluated patients (pooled data) are presented.

turnover bone disease, it seems likely that increased osteoblast production was involved in the increased levels of serum osteocalcin and that decreased renal clearance of osteocalcin was not significant. Moreover, administration of $1,25(OH)_2D_3$ has been shown to induce a fall in serum osteocalcin and PTH in patients with predialysis renal failure [6]. It was therefore concluded that elevated serum osteocalcin in uremics mainly reflect the extent of the osteoblastic pool, which to a larger degree is dependent on the duration and severity of the underlying renal bone disease [6]. As vitamin D_3 influences serum osteocalcin levels [24] by increasing the transcription of the osteocalcin gene, it is important to emphasize that none of our patients received any vitamin D_3 analogues during the entire study.

Our patients were evaluated on their usual regime of CAPD since peritoneal clearance and mass transfer of osteocalcin are independent of dialysate osmolalities [12]. Klein et al [10] found notably higher serum osteocalcin levels in adult CAPD patients than in the present study. While their MT_p-osteocalcin was comparable to ours [10], we found higher Cl_p-osteocalcin values. However, our results indicate no correlations between Cl_p-osteocalcin and any of the measured histomorphometric parameters (Table 4). Cl_p-osteocalcin is therefore of no interest as a non-invasive marker of metabolic bone disease in patients treated by CAPD. As the significant correlations involving dialysate osteocalcin (Table 4) were almost the same as serum osteocalcin, a kind of secondary phenomenon to the dialysis treatment *per se* was most likely.

Notable levels of osteocalcin in serum and dialysate have been reported in children receiving peritoneal dialysis with osteocalcin concentrations as much as 10% higher in the peritoneal dialysate than in serum [8]. The difference between serum and peritoneal dialysate levels in children and adults with end-stage renal failure could be caused by differences in skeletal turnover. It is also of relevance to note the possibilities that children with early onset renal failure have a higher incidence of bone disease. Moreover, children have a relatively high clearance per kg of body wt, which could also influence the clearance values. In the present study serum and dialysate levels of osteocalcin correlated significantly, while no correlations were found between osteocalcin values in the dialysate, inflow and drained volumes of dialysate effluent. In contrast, Klein et al [10] did not observe any correlation between dialysate and serum osteocalcin concentrations, whereas they, like us, observed a significant correlation between serum osteocalcin and MT_p-osteocalcin [10]. Based on this correlation Klein et al concluded that serum osteocalcin levels among CAPD patients should always be interpreted with MT_p-osteocalcin levels [10].

During the last decade the spectrum of bone histological features in patients with renal osteodystrophy has changed, resulting in an increase in low turnover bone disease. In the present study, osteitis fibrosa was found to be the predominant biopsy diagnosis. Seven patients had adynamic lesions although none had "status post-parathyroidectomy" or had received intensive vitamin D_3 therapy. None of our patients had extensive accumulation of aluminum in the bone specimens. However, aluminum staining increased during the year of the study, in agreement with the finding that quantitative evaluation of bone aluminum content is a less reliable and reproducible variable than histochemical evaluation of aluminum deposition along the mineralization front [21]. Although older age is a known risk factor for development of low turnover bone disease, older patients in the present study had increased bone turnover. Likewise, we found that increased time as an uremic resulted in increased bone turnover (high osteoclast surface and tetracycline labeled surface) probably due to calcium depletion. This is indicated by the correlations between the

Table 4. Correlations between osteocalcin (ost) measurements and other markers of bone metabolism and bone histomorphometric parameters

Histomorphometric parameters	S-ost	D-ost	Cl_p-ost	MT_p-ost	PTH	Ca^{2+}	Calcium	Alk. phos.	Al
O.Th									
r	0.73	NS	NS	NS	0.73	NS	NS	NS	NS
P	<0.01				<0.01				
OS									
r	NS	NS	NS	NS	NS	0.57	NS	NS	NS
P						<0.05			
OV									
r	NS	NS	NS	NS	NS	0.58	NS	NS	NS
P						<0.05			
ES									
r	0.78	0.55	NS	0.61	0.51	NS	0.57	0.47	NS
P	<0.001	<0.05		<0.01	<0.05		<0.05	<0.05	
Oc.S									
r	0.52	NS	NS	0.47	NS	NS	NS	NS	NS
P	<0.05			0.05					
TBV	NS	NS	NS	NS	NS	NS	NS	NS	NS
Tb.Th.	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ct.Th.									
r	NS	NS	NS	NS	NS	0.60	NS	NS	NS
P						<0.05			
CP	NS	NS	NS	NS	NS	NS	NS	NS	NS
MAR									
r	0.62	0.61	NS	0.67	0.82	NS	0.51	0.53	NS
P	<0.01	<0.05		<0.01	<0.001		<0.05	<0.05	
LS									
r	0.87	0.79	NS	0.73	0.86	NS	NS	0.74	NS
P	<0.001	<0.001		<0.001	<0.001			<0.001	
Aj.AR									
r	NS	0.46	NS	0.50	0.84	NS	NS	NS	NS
P		0.05		<0.05	<0.001				
MLT									
r	NS	neg.	NS	neg.	neg.	NS	neg.	neg.	NS
P		<0.01		<0.01	<0.01		<0.01	<0.01	
BFR/BS									
r	0.75	0.71	NS	0.72	0.82	NS	NS	0.65	NS
P	<0.001	<0.01		<0.001	<0.001			<0.01	
BFR/BV									
r	0.75	0.75	NS	0.74	0.79	NS	NS	0.70	NS
P	<0.001	<0.001		<0.001	<0.001			<0.01	
BFR/TV									
r	0.79	0.65	NS	0.65	0.81	NS	NS	0.60	NS
P	<0.001	<0.01		<0.01	<0.001			<0.01	
AlS									
r	neg.	NS	NS	NS	NS	NS	neg.	NS	NS
P	<0.05						<0.05		
AlC	NS	NS	NS	NS	NS	NS	NS	NS	NS

Abbreviations are: Aj.AR, adjusted apposition rate; Al, serum aluminum; AlC, aluminum content; Alk. phos., alkaline phosphatase; AlS, aluminum staining; BFR/BS, bone formation rate per unit of bone surface; BFR/BV, bone formation rate per unit of bone volume; BFR/TV, bone formation rate per unit of tissue volume; Calcium, total calcium; Ca^{2+}, ionized calcium; Cl_p-ost, peritoneal clearance; CP, cortical porosity; Ct.Th., cortical thickness; D-ost, dialysate osteocalcin; ES, eroded surface; LS, labeled surface; MAR, mineral appositional rate; MLT, mineralization lag time; MT_p-ost, peritoneal mass transfer; NS, non-significant; Oc.S, osteoclast surface; OS, osteoid surface; O.Th, osteoid thickness; OV, osteoid volume; PTH, intact parathyroid hormone; r neg., negative correlation; S-ost, serum osteocalcin; TBV, trabecular bone volume; Tb.Th., trabecular bone thickness.

histomorphometric parameters, serum calcium and serum Ca^{2+} given in Table 4.

One of the aims of the present investigation was to evaluate the relationship between osteocalcin and bone histomorphometry for a non-invasive prediction of bone histology. We found that bone biopsies taken twice with one year's interval showed a significant increase in labeled surfaces and decrease in trabecular bone. While it is not surprising that patients lost bone mass during the investigation, it is not certain that there was a real increase in bone turnover during the year, as other measures of turnover showed no significant change. Thus, the present investigation has therefore not shown any sure biochemical or histological changes in bone turnover. This may be due either to the relatively small number of patients undergoing two biopsies or the short time interval. Moreover, we found that serum PTH correlated to almost the same histomorphometric parameters as serum osteocalcin. Multiple stepwise regression showed that serum osteocalcin and PTH levels gave supplementary information to one another although PTH had lower correlation coefficients than serum osteocalcin in respect to eroded surface area. In contrast to this finding, Sebert at al [22] found higher correlation coefficients between serum osteocalcin and bone formation rate than PTH in

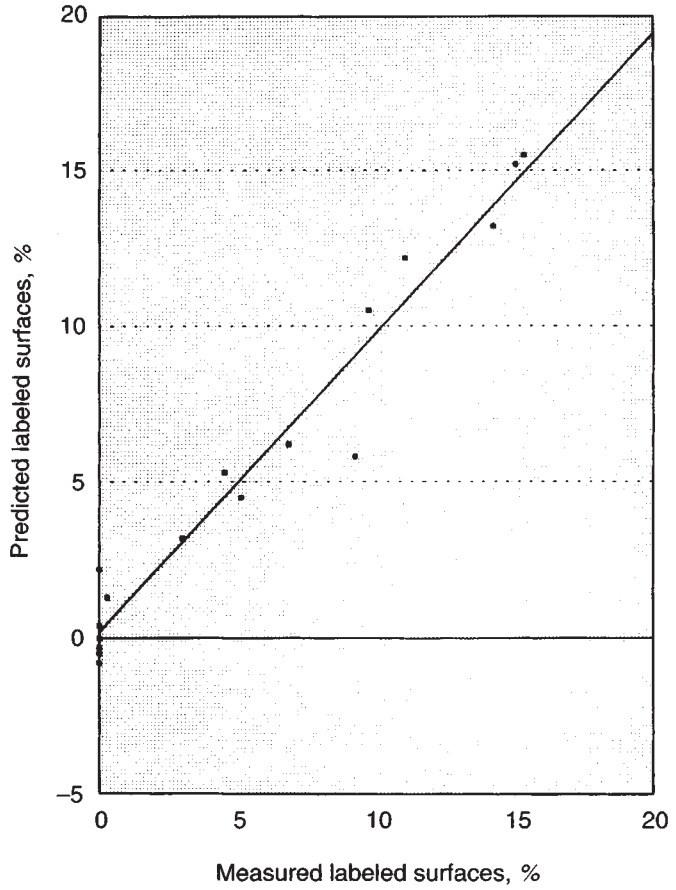

Fig. 2. *Correlation between measured tetracycline labeled surfaces and predicted tetracycline labeled surfaces in patients undergoing CAPD.* Tetracycline labeled surfaces are given with the equation: tetracycline labeled surfaces = −3.15 + 0.145 × serum osteocalcin + 0.409 × dialysate osteocalcin + 0.184 × serum PTH; $r = 0.98$).

hemodialysis patients. In another study consisting of hemodialyzed patients, serum osteocalcin ≥8 nmol/liter and a mineralized surface ≥3 gave a specificity of 94% and a sensitivity of 57% for having high turnover osteopathy [25]. Likewise, serum osteocalcin levels ≤4 nmol/liter seemed to reflect low turnover osteopathy with a specificity and a sensitivity of 100% and 17%, respectively [25]. Motz [26] studied hemodialysis patients with serum osteocalcin measurements and bone scintigraphies. It was shown that 70% of the patients with serum osteocalcin >30 ng/ml showed moderate to severe scintigraphic findings of bone disease [26]. However, the literature gives only very limited data on the correlations of osteocalcin measurements and bone histomorphometry in patients undergoing CAPD or peritoneal dialysis in general. Malluche et al [7] evaluated 30 chronically dialyzed patients, four of whom were on CAPD. Only patients who had a diagnostic bone biopsy were enrolled and the data of the CAPD patients were not separated from the remaining population of hemodialyzed patients [7]. As in the present study, Malluche et al [7] found significant positive correlations between serum osteocalcin, bone formation and mineral apposition rate. Rottembourg et al [27] evaluated 16 CAPD patients through blood studies and bone biopsy. All patients received calcitriol and eight of them had adynamic bone disease. Unfortunately, Rottembourg et al [27] did not publish any data concerning possible correlations between bone histomorphometry and osteocalcin levels. Cole, Carpenter and Gundberg [8] evaluated serum osteocalcin concentrations in children with metabolic bone disease. Only one patient with renal failure receiving hemodialysis was evaluated by radiography [8]. In a study where eight patients were treated by CAPD, Epstein et al [9] found serum and dialysate levels of osteocalcin significantly higher than in our patients. However, all of their patients were taking vitamin D_3 metabolites. In that study no bone biopsy specimens were available to confirm the presence of renal osteodystrophy or to correlate levels of osteocalcin with bone histomorphometry. Likewise, Gundberg et al [4] examined osteocalcin in infants and adolescents undergoing CAPD. As earlier adduced, children have higher levels of osteocalcin than adults due to growth. Moreover, no bone biopsies were performed [4]. Thus, to the best of our knowledge the present study is the first and most comprehensive investigation of serum and dialysate osteocalcin as well as Cl_p-osteocalcin and MT_p-osteocalcin levels where these concentrations are correlated to bone histomorphometry in patients not receiving vitamin D_3 analogues.

Conclusion

Serum osteocalcin was elevated at baseline and one year later. Osteocalcin was detected in the dialysate resulting in measurable values of Cl_p-osteocalcin and MT_p-osteocalcin but this appears to be without clinical significance. No correlations between Cl_p-osteocalcin and any of the measured histomorphometric parameters were seen. Serum and dialysate levels of osteocalcin correlated significantly. Serum osteocalcin was significantly correlated to serum alkaline phosphatase and serum PTH. Dialysate osteocalcin and MT_p-osteocalcin also correlated to serum alkaline phosphatase and serum PTH. Serum osteocalcin correlated more or less to the same histomorphometric parameters as PTH, but the latter had lower correlation coefficients than serum osteocalcin in respect to eroded surface. Significant correlations between serum osteocalcin, bone formation and mineral apposition rate were found. Thus, osteocalcin is a valuable non-invasive index of metabolic bone disease in patients treated by CAPD.

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