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Longitudinal study on osteodystrophy in primary biliary cirrhosis (PBC) and a pilot study on calcitonin treatment

A. Floreani[1], M. Chiaramonte[1], S. Giannini[2], L. Malvasi[2], M.G. Lodetti[2], R. Castrignano[2], A. Giacomini[1], A. D'Angelo[2] and R. Naccarato[1]

[1]*Department of Gastroenterology and* [2]*Pathologia Medica I, Institute of Internal Medicine, University of Padova, Padova, Italy*

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The aims of this study were to evaluate bone metabolism in primary biliary cirrhosis (PBC) and the effect of ADFR (activate, depress, free, repeat) therapy with vitamin D, calcium and calcitonin in preventing bone resorption. Sixty-nine female subjects entered the study: 38 PBC (AMA+ve) patients, 11 AMA-negative chronic liver disease patients and 20 age-matched healthy controls. Bone metabolism was evaluated by biochemical parameters and dual-photon absorptiometry of the lumbar spine at time 0, 6 and 18 months. Both PBC and chronic liver disease (CLD) patients showed low levels of serum 25-hydroxyvitamin D, osteocalcin and bone mineral content expressed as AAD (average area density) compared to healthy controls. Serum parathyroid hormone in PBC patients was at the lower limit of the normal range and was significantly lower than patients with chronic liver disease. At a 6-month interval, AAD significantly decreased in PBC patients ($p < 0.005$). At the 6-month period PBC patients were allocated into two groups according to a cut-off AAD of 0.800 g/cm^2: group A (no treatment, AAD >0.800, $n = 11$), group B (treatment, AAD <0.800, $n = 13$). The latter group received a 4-week course with oral calcium carbonate (1500 mg daily) + oral 1,25-dihydroxyvitamin D (0.5 μg twice a day for 5 days) + carbocalcitonin (40 U MRC) i.m. thrice a week. The treatment was repeated with the same protocol at 2-month intervals for 12 months. At the end of the study, we found a significant decrease in AAD in untreated as compared to treated patients ($\Delta = -4.93 \pm 8$ in group A vs. -0.91 ± 7 in group B, $p < 0.05$). In conclusion, these results suggest an early process of bone loss in PBC. Calcium, vitamin D and calcitonin supplements are effective in controlling bone resorption in PBC patients with severe osteodystrophy.

Osteodystrophy is a major complication in primary biliary cirrhosis (PBC) and a real problem in patients for whom liver transplant is indicated. Using transialic bone biopsies after double tetracycline labelling, several reports have documented decreased mean trabecular bone volume in PBC patients compared to sex- and age-matched controls (1–4). Osteomalacia is less common, although Long et al. (5) found a surprisingly high prevalence of osteomalacia (68.7%) in a series of 32 patients with chronic cholestatic liver disease.

Osteoporosis is characterized by reduced total bone mass with a simultaneous reduction of matrix and mineral content. There are two different mechanisms which account for osteoporosis. In 'low-turnover' osteoporosis, a normal degree of resorption is accompanied by reduced synthesis of matrix that is slowly mineralized. In 'high-turnover' osteoporosis, the activity of osteoclasts — modulated by the parathyroid hormone — is in favour of resorption. In PBC both mechanisms have been suggested (4,6), but it is not clear whether the severity of osteoporosis is related to oestrogen deficiency alone or to other mechanisms.

Correspondence: Professor Maria Chiaramonte, Division Gastroenterologia 'R. Farini', Via Giustiniani 2, 35100 Padova, Italy.

Dual-photon absorptiometry of the spine is usually considered the examination of choice to assess osteoporosis (7).

Finally, treatment of osteodystrophy in cholestatic liver disease is a problem that remains unsolved.

There is experimental evidence that calcitonin effectively prevents osteoclastic-stimulated bone resorption (8), and results of a therapeutic trial in patients with post-menopausal osteoporosis suggest beneficial effects of calcitonin on this type of osteoporosis (9). It is not known whether calcitonin may act as a protective hormone in PBC where a state of relative vitamin D deficiency is often found (1,3,10).

The purpose of this study was to evaluate bone metabolism in PBC in a longitudinal investigation and the effect of ADFR (activate, depress, free, repeat) (11) therapy with vitamin D, calcium and calcitonin in the prevention of bone resorption in subjects with severe osteopenia.

Materials and Methods

PBC patients studied

Thirty-eight female patients suffering from PBC entered the study. The mean age was 53 years (range 36–66 yrs). The diagnosis of PBC was confirmed using conventional clinical, immunological and histological criteria (11). According to the histological classification of Scheuer (12), nine of the patients had stage II, 13 stage III and 16 stage IV. All were AMA positive.

At the time they entered the study, 19 patients were taking cholestyramine for pruritus, two patients had been receiving steroids for 2 years before entering the study because of autoimmune-associated conditions (7.5 mg of prednisolone daily). None of the patients were receiving vitamin D or calcium supplementation.

All were asymptomatic for bone disease. Fifteen patients were pre- and 23 post-menopausal.

Controls

Group 1 consisted of 11 AMA-negative patients suffering from osteodystrophy associated to chronic liver disease (two pre- and nine post-menopausal). Osteodystrophy was diagnosed by clinical and/or radiological signs. The mean age was 57 years (range 42–72 yrs). The diagnosis of chronic liver disease was, in each case, based on conventional clinical, biochemical and histological grounds. Five had cryptogenic, five autoimmune and one cholestatic chronic active hepatitis. The five patients with autoimmune CAH were receiving long-term maintenance therapy with steroids (mean treatment time 48 months, dose ranging from 10 to 15 mg of prednisolone daily). This group was studied with the same protocol as PBC patients.

Group 2 was made up of the negative controls (20 age-matched healthy female subjects). The mean age was 55 years (range 40–66 yrs). They underwent dual photon-absorptiometry initially and at 6 months, only.

Study design

The study was conducted over an 18-month period:

Basal evaluation of bone metabolism in PBC patients and in controls. Clinical examination, biochemical tests and lumbar dual-photon absorptiometry were carried out in PBC patients, symptomatic chronic liver disease and in healthy sex- and age-matched controls.

Longitudinal study. In the 6th month of study, clinical examination, biochemical tests and dual-photon absorptiometry were carried out in the PBC and chronic liver disease patients. In the healthy controls only the dual-photon absorptiometry was repeated. No vitamin D or calcium supplementation was given during this 6-month period.

Evaluation of treatment in PBC. In the 6th month, PBC patients were allocated into two groups using the average area density (AAD) cut-off (0.800 g/cm^2) as a dividing criterion: group A, AAD $>$ 0.800 g/cm^2 (no treatment); group B, AAD $<$ 0.800 g/cm^2 (treatment).

The therapeutic protocol was: oral 1,25-dihydroxyvitamin D (0.5 μg twice a day for 5 days); carbocalcitonin (40 U MRC) i.m. thrice a week; and oral calcium carbonate (1500 mg daily). The treatment was repeated at 2-month intervals following the same protocol for 12 months. This treatment was chosen on the basis of an established protocol for patients with post-menopausal osteoporosis. Treatment was evaluated after 12 months when clinical examination, biochemical tests and dual-photon absorptiometry were repeated.

Biochemical parameters

The following parameters were measured initially and after 6 and 18 months of treatment: (i) Blood samples: Ca, PO_4, Na, K, creatinine, nitrogen, uric acid, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone (PTH) and osteocalcin. (ii) Urine samples: 24-h urinary excretion of: Ca, PO_4, Na, K, Mg, creatinine, uric acid and hydroxyproline.

Serum PTH was evaluated by immunoradiometric assay (IRMA, Allgro TM Intact PTH, Nichols Institute, San Juan Capistrano, U.S.A.). This method detects the biologically intact 84 amino acid chain of PTH.

Serum 25-dihydroxyvitamin D and 1,25-dihydroxyvitamin D were measured by RIA (kit INCSTAR Corporation, Stillwater, MN, U.S.A.) upon extraction from se-

rum by acetonitrile and successive column elution with C_{18}OH column cartridges.

Osteocalcin (GLA protein) measurement was performed by RIA (INCSTAR Corporation, Stillwater, MN, U.S.A.). Quality control of RIA method was obtained by testing a standard serum with known concentration of GLA protein with every batch of assay. The coefficient of variation intra-assay was 3.1% and inter-assay 5.7%.

The other tests were evaluated by conventional methods.

The prognostic index (*PI*) was calculated according to Christensen et al. (14) modified as follows: $PI = 2.52 \times \log_{10}$ bilirubin μmol/l + 0.069 × exp (age yrs −20)/10 − 0.05 × albumin g/l + 0.88 (if cirrhosis present) + 0.52 (if not treated with azathioprine).

Bone density

Bone density was measured by dual-photon absorptiometry of the lumbar spine (L2-L4) (Norland 2600).

Bone mineral content was calculated as the average area density of the three lumbar vertebrae.

The protocol was approved by the local ethical committee.

Statistical analysis

Results were analyzed by Student's *t*-test (for paired and unpaired data) or the linear-regression test where appropriate.

Results

Table 1 shows the biochemical parameters in PBC compared to non-PBC, CLD patients. The mean ± S.D. of the blood and urinary tests was within the normal range in both PBC and non-PBC CLD, except a lower 24-h urinary excretion of Ca in PBC (94.1 ± 57 mg/24 h in PBC vs. 185.6 ± 140 mg/24 h in CLD, $p < 0.01$).

A large proportion of patients showed low serum levels of PTH, 25-hydroxyvitamin D and osteocalcin (Fig. 1). In particular, serum concentration of parathyroid hormone was significantly lower in PBC as compared to CLD patients (12.02 ± 7.39 ng/l vs. 30.12 ± 13 ng/l, $p < 0.001$). All PBC patients had serum parathyroid hormone levels within the normal range, with the exception of six who presented levels below normal. All CLD patients had serum parathyroid hormone levels within the normal range.

The mean ± S.D. of 25-hydroxyvitamin D levels, as well as of osteocalcin, was low, though within the normal range, in both PBC and CLD patients. The mean ± S.D. of 25-hydroxyvitamin D was 11.1 ± 7.9 ng/ml in PBC vs. 10.7 ± 5.3 ng/ml in CLD. The mean ± S.D. of serum osteocalcin concentration in PBC was 1.43 ± 1.5 ng/ml which did not differ significantly from CLD patients (1.30 ± 1.2). Twenty-four PBC patients (63.1%) and nine CLD patients (81.8%) had osteocalcin concentrations lower than normal (Fig. 1).

Fig. 2 shows bone mineral content at lumbar spine ex-

TABLE 1

Basal values of the biochemical parameters

	Normal values		PBC	CLD	*p*
Blood tests					
Ca	8.4–10.4	mg/dl	8.9 ± 1.58	9.4 ± 0.48	N.S.
P	2.7–4.5	mg/dl	3.5 ± 0.43	3.3 ± 0.44	N.S.
Na	136–145	mm/l	140.8 ± 2.49	141.3 ± 2.06	N.S.
K	3.5–5	mm/l	4.2 ± 0.29	4.3 ± 0.29	N.S.
Cl	96–108	mm/l	105.7 ± 2.46	106.8 ± 3.1	N.S.
Nitrogen	15–45	mg/dl	31.7 ± 6.3	33.5 ± 6.2	N.S.
Uric acid	3.6–6.5	mg/dl	4.5 ± 1.29	4.8 ± 0.92	N.S.
Creatinine	0.7–1.4	mg/dl	0.8 ± 0.2	0.8 ± 0.12	N.S.
PTH	10–55	ng/l	12 ± 7.39	30.1 ± 13	<0.001
25 OH vit D	9–40	ng/ml	11.1 ± 7.9	10.7 ± 5.3	N.S.
1,25(OH)2 vit D	15–49	pg/ml	25.3 ± 4.6	22.3 ± 3.9	N.S.
Osteocalcin	1.4–6.2	ng/ml	1.4 ± 1.5	1.3 ± 1.4	N.S.
Urine tests					
Ca	108–300	mg/24 h	94.1 ± 57	185.6 ± 140	<0.01
P	400–1302	mg/24 h	347.6 ± 242	307.4 ± 209	N.S.
Na	80–215	mEq/24 h	157.5 ± 91	157.3 ± 133	N.S.
K	50–120	mEq/24 h	58.2 ± 25	52.7 ± 54	N.S.
Mg	75–125	mg/24 h	30.5 ± 25	49.7 ± 27	N.S.
Creatinine	598–1796	mg/24 h	960 ± 188	1075 ± 189	N.S.
OH-proline	6–22	mg/24 h	19.1 ± 8.9	11.4 ± 4.1	N.S.
Uric acid	248–744	mg/24 h	396 ± 251	281 ± 196	N.S.

N.S., not significant.

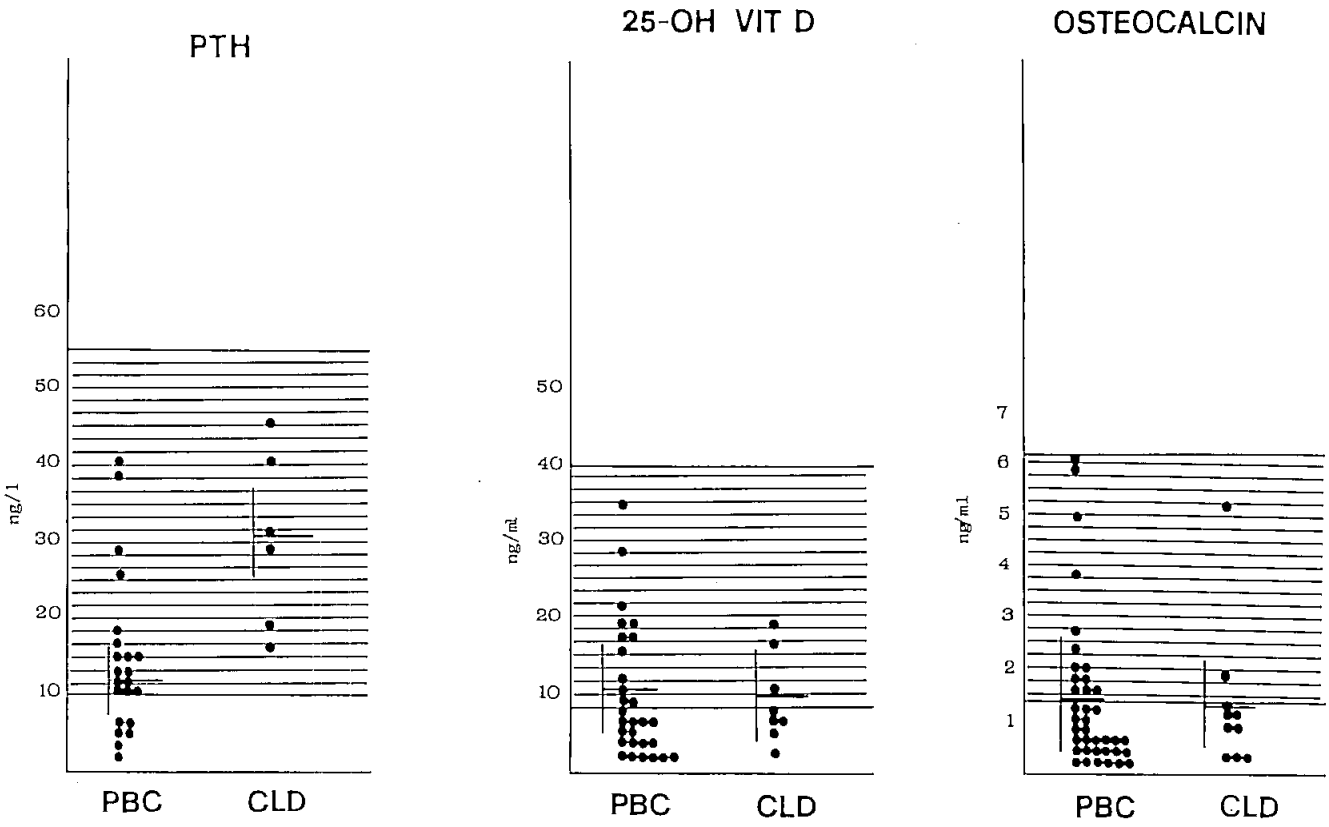

Fig. 1. Serum levels of PTH, 25-hydroxyvitamin D and osteocalcin in PBC and CLD patients.

pressed as AAD in PBC, in CLD and in sex- and age-matched controls. The mean ± S.D. was 0.82 ± 0.16 g/cm^2 in PBC, 0.80 ± 0.14 in CLD and 0.93 ± 0.06 g/cm^2 in the healthy subjects. AAD was significantly lower in both PBC and CLD patients than in sex- and age-matched controls (PBC vs. controls $t = -3.03$, $p < 0.01$, CLD vs. controls $t = -3.04$, $p < 0.01$).

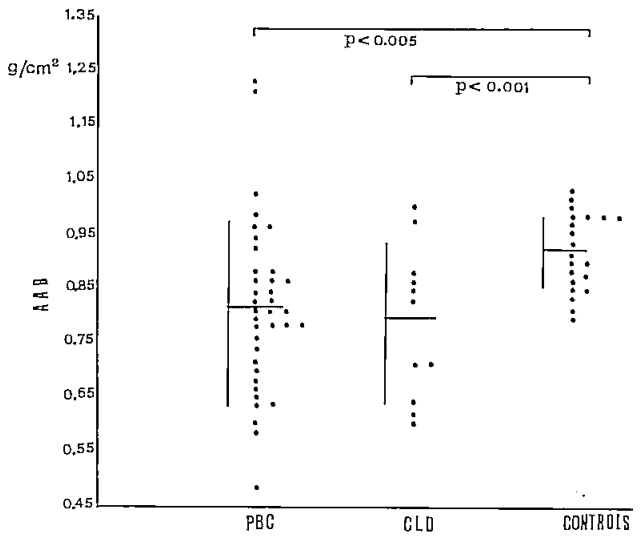

Fig. 2. Bone mineral content (expressed as average areal density) in PBC, CLD and controls.

Among the PBC patients no correlation was found between AAD and parameters of cholestasis and/or disease activity (alkaline phosphatase, bilirubin, GGT, AST, cholesterol and prognostic index), cholestyramine treatment, parameters of bone turnover (such as urinary hydroxyproline, PTH and osteocalcin) and the duration of the disease as well as the duration of menopause.

Bone content was negatively correlated with the age of the patients ($r = -0.43$, $p < 0.01$) and was lower in post-menopausal than pre-menopausal patients ($t = -3.68$, $p < 0.0005$).

At a 6-month interval a significant reduction in AAD was observed in PBC ($\Delta\% -6.2 \pm 6.4$, $p < 0.005$). There was no trend observed towards a reduction in CLD patients or in the controls (Fig. 3).

Twenty-four out of 38 PBC patients (10 pre- and 14 post-menopausal) completed the 18-month study. Of the 14 who did not complete the study, three died from hepatic failure, one underwent liver transplantation, and 10 did not complete the protocol for reasons unrelated to the treatment (change of residence, family problems, concomitant extra-hepatic diseases, etc.). They were allocated into two groups according to the degree of bone loss

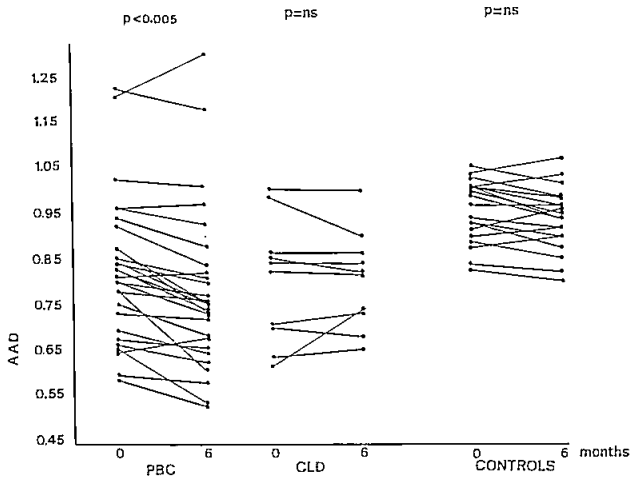

Fig. 3. Variation of bone mineral content in PBC, CLD and controls at a 6-month interval.

(group A: AAD >0.800 g/cm^2, no treatment and group B: AAD <0.800 g/cm^2, treatment with carbocalcitonin + vitamin D and calcium supplementation as protocol). Eleven patients were allocated into the group A and 13 into the group B. The *PI* was comparable in groups A and B (3.6 ± 1.1 and 4 ± 1.3, respectively). No significant changes in *PI* were observed during the following 12 months.

At the end of the study (after 12 months) we found a significantly greater decrease in AAD in untreated than in treated patients ($\Delta = -4.93 \pm 8$ in the untreated group vs. -0.91 ± 7 in the treated group, $p < 0.05$).

Hydroxyproline excretion was significantly reduced (from 22.18 ± 8.9 to 7.9 ± 6 mg/24 h, $t = -6.16$, $p < 0.001$) in treated patients (group B), while in untreated patients (group A) the reduction of hydroxyproline excretion was not statistically significant (from 16.88 ± 13 to 7.7 ± 4 mg/24 h, $t = 1.8$, p = N.S.). No significant difference was observed in serum 25 OH vitamin D levels in treated patients compared to untreated patients.

Discussion

In order to evaluate the clinical pattern of hepatic osteodystrophy we carried out a longitudinal study with biochemical parameters of bone metabolism and dual-photon absorptiometry which is considered a reliable method in assessing the variation of bone mineral content.

We selected only female subjects to evaluate the risk factors related to bone metabolism. In the first part of the study, we considered patients suffering from PBC who were not receiving Ca or vitamin D supplementation. The results of this group were compared to those of a high-risk group for osteodystrophy (CLD patients, most of whom complained of bone pain) and a group of healthy age-matched controls.

The bone metabolism parameters in PBC show a slight reduction in the 24-h urinary excretion of calcium and low serum levels of 25-hydroxyvitamin D, PTH and osteocalcin.

Low levels of 25-hydroxyvitamin D were also found in CLD patients. Although in accordance with other authors (10,14), the reason to still to be clarified. In fact reduced serum levels of 25-hydroxyvitamin D may be due to: (i) Impaired 25-hydroxylation of vitamin D: this point is not sufficiently supported (15) because an impaired enzyme activity should mostly be present in advanced disease. (ii) Malabsorption of vitamin D: this has been documented in jaundiced patients with significant steatorrhea (16,17). Vitamin D malabsorption may also be aggravated by treatment with cholestyramine which binds vitamin D and by reduced excretion of bile acids. (iii) Increased excretion of water-soluble conjugates in the urine of jaundiced patients (16,18,19).

Low serum levels of 25-hydroxyvitamin D may probably have a multifactorial cause and most likely play an important role in the pathogenesis of bone disease.

Serum parathyroid hormone is also decreased in PBC. We found significant lower levels of PTH in PBC compared to non-PBC CLD. This result does not confirm a secondary hyperparathyroidsm to explain osteodystrophy (20,21), while Herlong et al. (3) reported normal serum levels of PTH.

Osteocalcin, a vitamin-K dependent glycoprotein synthesized by osteoblasts, was reduced in 62% of our patients as well as in non-PBC CLD. Decreased serum levels of osteocalcin have also been found by Hodgson et al. (22) and by Diamond et al. (23) not only in PBC, but also in non-PBC CLD. Serum osteocalcin levels are due to osteoblastic activity and are correlated to the rate of bone apposition. Thus, an impaired osteoblastic function could be of some importance in this kind of bone disease. The triggering factors are not completely known. Hogdson et al. (22) suggested that impaired osteoblastic function may be due to toxic substances produced during cholestasis. But it is possible that other factors may play a role in decreasing osteoblastic function, such as oestrogen deficiency occurring in menopause and/or vitamin D deficiency.

Low serum levels of both serum parathyroid hormone and osteocalcin associated with low urinary excretion of calcium and normal OH-proline excretion, suggest a low turnover osteoporosis in PBC. This is in agreement with Stellon et al. (4) who completed the data by bone biopsy. In our study we could not distinguish osteoporosis from osteomalacia because our patients did not undergo bone

biopsy during the longitudinal study. Nevertheless, from the clinical point of view it is not important to distinguish osteoporosis from osteomalacia because the response to therapy was good. Thus we can define osteodystrophy in terms of a reduced mineral content whose rate can be assessed in the follow-up.

Mineral bone content appears to be more greatly decreased in asymptomatic PBC patients than in a group of chronic liver disease patients selected for bone pain.

Osteodystrophy is not correlated with biochemical parameters of bone metabolism (25-hydroxyvitamin D, PTH, osteocalcin, OH-proline excretion), severity of the disease (cholesterol, ALT, GGT, alkaline phosphatase, bilirubin, prognostic index, duration of the disease) or cholestyramine treatment. We only found a negative correlation with the age of the patients and the state of menopause. These results suggest an early process of bone loss in PBC. In fact osteodystrophy may have a multifactorial origin. From this point of view, oestrogen deficiency may have a significant role in accelerating bone turnover.

There is in fact a similarity between post-menopausal osteoporosis and bone loss. Many studies have demonstrated an alteration of oestrogen metabolism in PBC. Goudie et al. (24) reported a significantly higher incidence of breast cancer in women with PBC than in an age- and sex-matched population. Stellon and Williams (25) reported a significantly higher incidence of previous hysterectomy and dilatation and currettage among women with PBC. Although these contributions suggest an alteration of oestrogen metabolism in PBC, this peculiar aspect is underestimated. Further studies are therefore needed to clarify the role of oestrogens in osteodystrophy associated with PBC or the other primary hepatocellular diseases.

In our study we have demonstrated that loss of bone mineral content occurs very rapidly in PBC because only in this group of patients (compared to AMA-negative chronic liver disease and healthy controls) was a significant decrease in AAD observed in a time period of 6 months.

In the third part of the study, we evaluated the effect of a combined therapy (Ca, 1,25-dihydroxyvitamin D and calcitonin) on bone resorption. We treated only PBC patients with severe osteodystrophy on the basis of an arbitrary cut-off point of AAD.

Vitamin D supplements alone do not always increase the plasmatic levels of vitamin D metabolites and/or correct osteodystrophy. Probably oral 25-hydroxyvitamin D is effective only in the case of osteomalacia (1), but is not helpful in reversing osteoporosis (2).

Calcium supplements alone also do not reverse bone loss. A recent study from Denmark (26) which examined the effect of oral Ca (2000 mg daily) vs. oestrogen therapy in the early post-menopausal period has demonstrated that Ca is not as effective as oestrogen in the prevention of bone loss.

In PBC a controlled trial in 64 post-menopausal PBC women receving parenteral vitamin D_2 showed that the addition of calcium gluconate and microcrystalline hydroxyapatite compounds prevented bone thinning and promoted positive cortical bone balance (27). Nevertheless, long-term treatment with this combination is not known to be effective in osteodystrophy.

Finally, there are no reports dealing with calcitonin in PBC. Although we did not perform a controlled study this is the only contribution concerning PBC and calcitonin. Recently a 2-year randomized pilot study performed in 70 patients with post-menopausal bone loss demonstrated that human calcitonin (50 IU daily) was as effective as oestradiol in the prevention of bone loss (9).

We did not observe side-effects caused by calcitonin therapy. Our data suggest that Ca, vitamin D and calcitonin supplements are effective in controlling bone resorption in PBC patients with severe osteodystrophy. Nevertheless, controlled trials with long-term combined vitamin D, calcium and calcitonin therapy are needed in PBC. In addition, second generation diphosphonate, 3-amino-1-hydroxypropildene, useful in the prevention of osteoporosis induced by steroids (28), may be used in controlled trials for the treatment of PBC bone disease.

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