Multiple Forms of Alkaline Phosphatase in Plasma of Hemodialysis Patients

L. Tibi, S. C. Chhabra, V. M. Sweeting, R. J. Winney, and A. F. Smith

We used quantitative assays to measure the activity of the bone, liver, and intestinal forms of alkaline phosphatase in plasma in 75 patients with end-stage chronic renal failure undergoing hemodialysis. The results were correlated with radiological and other biochemical indices of bone disease and with biochemical indices of liver disease. The total activity of alkaline phosphatase in plasma increased in 28 patients. In 10 of these patients, nine of whom had increased activity of \( \gamma \)-glutamyltransferase in plasma, the increase in total activity of alkaline phosphatase was from the liver isoenzyme alone (nine patients) or from the liver and bone isoenzymes together (one patient). Intestinal alkaline phosphatase in plasma, although >23 U/L in eight patients, was solely responsible for the increase in total alkaline phosphatase in one patient (who had normal \( \gamma \)-glutamyltransferase). Bone alkaline phosphatase in plasma was increased in 25 patients, seven of whom had normal total alkaline phosphatase, and was closely correlated \( (r = 0.78) \) with osteocalcin concentration in plasma, which was increased in a much greater proportion of patients (99%). Both total and bone alkaline phosphatase were correlated with parathyrin in plasma \( (r = 0.46 \) and 0.50, respectively) and with osteocalcin \( (r = 0.60 \) and 0.78, respectively). Osteocalcin and bone alkaline phosphatase, but not parathyroid, decreased with age, implying that the skeletal response to parathyrin may be age dependent. In patients with increased total alkaline phosphatase undergoing hemodialysis, the concurrent measurement of \( \gamma \)-glutamyltransferase may help identify whether the enzyme increase originates from the liver or bone, but this approach wrongly identified the source of the increase in three of 28 patients. Therefore, we recommend a separate measurement of the bone isoenzyme of alkaline phosphatase.

Additional Keyphrases: isoenzymes · bone disease · osteocalcin · parathyrin · aluminum

Metabolic bone disease is a leading cause of morbidity in patients with chronic renal failure who are maintained with long-term dialysis. The pattern of bone disease is variable; parathyroid bone disease is most common, but osteomalacia and osteosclerosis may also be present (1). The causes are multifactorial and complex and may involve inadequate 1-hydroxylation of 25-hydroxycholecalciferol and 25-hydroxyergocalciferol by the diseased kidneys, phosphate retention, secondary hyperparathyroidism, and chronic acidosis. Bone disease attributable to aluminum toxicity may further complicate the picture.

We measured alkaline phosphatase (ALP; EC 3.1.3.1) and its tissue forms, or isoenzymes, in patients undergoing chronic hemodialysis. In particular, we investigated whether quantitative measurement of the bone form of ALP has advantages over measurement of total ALP activity and assessed the relationship of each to concentrations of osteocalcin and parathyrin (PTH). We also measured biochemical indices of liver disease to determine whether, by taking the indices into consideration in interpreting total ALP, it is possible to obtain a useful assessment of the activity of the bone form of ALP in plasma. In addition, we studied the intestinal form of ALP, taking secretor status into consideration, to determine whether the increases in this isoenzyme limit the diagnostic value of measurement of total ALP. Roentgenograms were scanned for the presence of erosions to determine the relationship of erosions to biochemical disturbances.

Materials and Methods

Patients. We studied 75 patients with endstage chronic renal failure undergoing hemodialysis (Table 1), 34 of whom were treated by hospital hemodialysis and 41 by home hemodialysis. Twenty-one patients had dialysis two times per week and 54 had dialysis three times per week. Hours of dialysis ranged from 2.5 to 5 h with a blood flow and dialysis fluid flow of 200 and 500 mL/min, respectively. The calcium concentration of dialysis fluid was 1.75 mmol/L and the magnesium concentration was 0.75 mmol/L. At the time of our study, 12 patients were being treated with alfalcacidol, 0.25–0.5 \( \mu g \) daily. Nine patients had required parathyroidectomy in the past, seven of whom were treated by total parathyroidectomy with forearm autotransplantation. Until 1979, 17 of the 75 patients had been treated by hemodialysis with softened water; since then, dialysis in all patients has involved reverse osmosis-treated water with regular monitoring of aluminum concentration in dialysis fluid and plasma. All patients were treated with Alu-Cap* (Riker Labs., St. Paul, MN 55144), 1.4–2.8 g/day, to maintain the phosphate in plasma at <2 mmol/L. Two patients had aluminum-related osteomalacia, resulting in fractures before the introduction of reverse osmosis-treated water. However, at the time of our study, no patient had clinical or radiological features suggestive of aluminum-related bone disease, and no patient had current fractures. All patients tested negative for hepatitis B.

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2 Nonstandard abbreviations: ALP, alkaline phosphatase; PTH, parathyrin; ALT, alanine aminotransferase; GGT, \( \gamma \)-glutamyltransferase; and 25-OH-D, 25-hydroxyvitamin D.
Table 1. Details of 75 Patients Studied

| Sex      | No. | Mean Age (range), years
|----------|-----|------------------------
| Male     | 48  | 49 (19–74)             |
| Female   | 27  | 47 (18–68)             |
| Duration of Dialysis |       | 6.7 (0.3–24)          |

Samples. The following nonfasting samples were collected on the day preceding dialysis:

- Blood (lithium heparin): We measured in plasma the total ALP, ALP isoenzymes, bilirubin, alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), osteocalcin, albumin, calcium, phosphate, magnesium, and aluminum. Erythrocytes were used to determine ABO and Lewis blood groups.

- Blood (plain glass tube): In serum, separated within 1 h of collection and stored at –20 °C, we measured PTH and 25-hydroxyvitamin D (25-OH-D).

- Saliva: We determined secretor status in saliva of 38 patients.

We also studied a control group of 100 blood donors (sex and age not recorded) to establish reference ranges for total, liver, bone, and intestinal ALP in plasma. Intestinal ALP was related to secretor status, as inferred from the ABO and Lewis blood groups.

Total ALP and isoenzymes. Total ALP was measured with a continuous monitoring method at 37 °C with p-nitrophenyl phosphate as a substrate and 2-aminoo-2-methyl-1-propanol, pH 10.5, as buffer (2). The absorbance change at 405 nm was monitored at 37 °C with a Cobas-Bio centrifugal analyzer (Roche, Welwyn Garden City, U.K.). Intestinal ALP was determined by coating microtiter plates with a monoclonal antibody to intestinal ALP and measuring ALP activity after adding plasma (3). The lower limit of detection for intestinal ALP in serum by this method is 2 U/L. Liver and bone ALP were quantified by scanning densitometry after separation by polyacrylamide gel electrophoresis (4, 5). The "biliary" or high-molecular-mass form of ALP was measured by ion-exchange chromatography (6) in samples in which there was significant ALP staining at the origin of the polyacrylamide gel slab.

Osteocalcin, PTH, and 25-OH-D. Osteocalcin in plasma was measured by a commercial RIA (CIS UK Ltd., High Wycombe, U.K.), which includes antisera against bovine osteocalcin. Serum PTH was measured by using a two-site immunoradiometric assay (Allegro Intact PTH; Nichols Institute, San Juan Capistrano, CA) in which the solid-phase antibody reacts with C-terminal and mid-region PTH and the labeled antibody reacts with N-terminal PTH. Serum 25-OH-D was measured by competitive binding (7). Reference ranges and precision data for these assays and for ALP isoenzymes are given in Table 2.

Blood groups and secretor status. We determined the ABO and Lewis blood groups by agglutination, and the secretor status by hemagglutination-inhibition with saliva samples (8). In patients from whom a sample of saliva was not available, we inferred the secretor status from the Lewis group. Results of these studies were used to divide patients into three blood group/secretor status categories: (a) group B, O secretors—Lewis b; (b) group A secretors—Lewis b; (c) group A, B, and O nonsecretors—Lewis a. Group A secretors were separated from other secretors because, in terms of intestinal ALP activity, they behave as nonsecretors. We excluded group AB secretors and the Lewis group a⁻ b⁻ (from the control group in which saliva was not available).

Other measurements. Concentrations of bilirubin, GGT, ALT, albumin, calcium, and phosphate in plasma were measured with a SMAC II (Technicon Instruments Co., Basingstoke, U.K.). We measured magnesium in plasma by a colorimetric method (9) and aluminum in plasma by atomic absorption spectrometry. Albumin-corrected calcium values were calculated by adding or subtracting 0.02 mmol/L to the plasma calcium for each 1 g/L that the plasma albumin concentration was greater or less than 40 g/L (10).

Radiological assessment. The presence of bone erosion was assessed from roentgenograms of all patients. An arbitrary semiquantitative score of 0 to 3 was given for each patient on the basis of a subjective assessment of severity by one of us (R.J.W.), who was unaware of the biochemical findings at the time (11).

Statistical analysis. For statistical analysis, we used standard parametric and nonparametric methods, as appropriate. The analysis was performed with an IBM PS/2 microcomputer (IBM Corp., Portsmouth, U.K.) by using SPSS/PC + V3.0.

Results

Measurements Relating to Bone Disease

Mean concentrations of total, bone, and liver ALP in plasma, osteocalcin in plasma, and PTH and 25-OH-D in serum are shown in Table 3. This Table also shows the number of patients with less than the optimal concentration for 25-OH-D and results greater than the upper reference value for the remaining variables. The corre-

Table 2. Reference Ranges and Precision Data for ALP isoenzymes, Osteocalcin, PTH, and 25-OH-D

<table>
<thead>
<tr>
<th>ALP Enzyme</th>
<th>Reference range a</th>
<th>Precision b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ALP</td>
<td>50–110 U/L</td>
<td>CV, % n</td>
</tr>
<tr>
<td>Liver ALP</td>
<td>≤55 U/L</td>
<td>1 11</td>
</tr>
<tr>
<td>Bone ALP</td>
<td>≤55 U/L</td>
<td>4 11</td>
</tr>
<tr>
<td>Intestinal ALP</td>
<td></td>
<td>3 11</td>
</tr>
<tr>
<td>Groups B and O secretors</td>
<td>≤23 U/L</td>
<td>6 18</td>
</tr>
<tr>
<td>Nonsecretors and group A</td>
<td>≤4 U/L</td>
<td>6 18</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>≤5 μg/L</td>
<td>9 10</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>≤11 μg/L</td>
<td>7 16</td>
</tr>
<tr>
<td>PTH</td>
<td>10–55 pg/L</td>
<td>7 9</td>
</tr>
<tr>
<td>25-OH-D</td>
<td>&gt;10 μg/L</td>
<td>10 10</td>
</tr>
</tbody>
</table>

a Reference ranges in healthy adults.

b Precision determined from between-batch repeat analysis of specimens from patients for which the values were within or slightly above the reference range (n = no. of batches).
Table 3. Summary Statistics

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Range</th>
<th>No. (%) of abnormal results*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ALP, U/L</td>
<td>114</td>
<td>47-367</td>
<td>28/75 (37)</td>
</tr>
<tr>
<td>Bone ALP, U/L</td>
<td>61</td>
<td>11-349</td>
<td>25/75 (33)</td>
</tr>
<tr>
<td>Liver ALP, U/L</td>
<td>40</td>
<td>6-147</td>
<td>12/75 (16)</td>
</tr>
<tr>
<td>Intestinal ALP, U/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B,0 secretors</td>
<td>19.0</td>
<td>6.9-55.6</td>
<td>6/29 (21)</td>
</tr>
<tr>
<td>A secretors</td>
<td>7.4</td>
<td>2.3-28.9</td>
<td>8/17 (47)</td>
</tr>
<tr>
<td>Nonsecretors</td>
<td>4.2</td>
<td>2.3-8.3</td>
<td>9/24 (38)</td>
</tr>
<tr>
<td>Osteocalcin, µg/L</td>
<td>58</td>
<td>8-246</td>
<td>74/75 (99)</td>
</tr>
<tr>
<td>PTH, ng/L</td>
<td>317</td>
<td>2-3870</td>
<td>61/75 (81)</td>
</tr>
<tr>
<td>25-OH-D, µg/L</td>
<td>15.2</td>
<td>4.2-35.7</td>
<td>27/75 (36)</td>
</tr>
</tbody>
</table>

* Abnormal results are low for 25-OH-D but high for all other variables.

Table 4. Correlation Matrix for Total Liver, Bone, and Intestinal ALP; GGT; Osteocalcin; PTH; Presence of Bone Erosions; and Age

<table>
<thead>
<tr>
<th></th>
<th>Liver ALP</th>
<th>Bone ALP</th>
<th>Intestinal ALP</th>
<th>GGT</th>
<th>Osteocalcin</th>
<th>PTH</th>
<th>Bone erosions</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ALP</td>
<td>0.35a</td>
<td>0.83b</td>
<td>0.01</td>
<td>0.25</td>
<td>0.60b</td>
<td>0.46b</td>
<td>0.43b</td>
<td>-0.20</td>
</tr>
<tr>
<td>Liver ALP</td>
<td>-0.20</td>
<td>-0.11</td>
<td>-0.15</td>
<td>0.62b</td>
<td>-0.28</td>
<td>-0.04</td>
<td>-0.19</td>
<td>0.39b</td>
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<tr>
<td>Bone ALP</td>
<td></td>
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<tr>
<td>Intestinal ALP</td>
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<td>GGT</td>
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<tr>
<td>Osteocalcin</td>
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<td>PTH</td>
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<tr>
<td>Bone erosions</td>
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</tbody>
</table>

n = 75. * P < 0.01. ** P < 0.001.

Fig. 1. Correlation between total ALP activity and osteocalcin in plasma
O, patients with increased plasma GGT activity (>55 U/L in males or >35 U/L in females); #, patients with normal GGT activity

The measurement of total ALP with GGT correctly identified the source of increased total ALP (where an increased GGT was thought to indicate a liver source, and a normal GGT a bone source) in 25 of 28 patients. This approach correctly identified the source of the increased total ALP in three patients: one patient had increased liver ALP with a normal GGT; a second patient had increased liver ALP and GGT with an additional increase in bone ALP; and a third patient had increased intestinal ALP with a normal liver ALP, bone ALP, and GGT.

Bone ALP was increased in 25 patients (33%) and was closely correlated with osteocalcin concentration (Fig.

None of the other variables measured (calcium or albumin-corrected calcium, phosphate, magnesium, and aluminum in plasma and 25-OH-D in serum) showed any statistically significant correlations with biochemical, clinical, or radiological indices of the presence of bone disease. The concentration of aluminum in plasma strongly correlated only with the duration of dialysis (r = 0.47; P < 0.001). Serum 25-OH-D was low in 36% of patients (Table 3) but showed no strong relation to any other indices measured.

Bone erosions were present in only 11 (15%) of the patients. All had increased plasma osteocalcin and serum PTH; in general, erosions occurred in the younger patients (Table 4). Two patients with grade 1 erosions had total and bone ALP activities within the reference range, four patients had normal total ALP with increased bone ALP activity, and one patient had in-

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creased total ALP (caused by an increased liver ALP) with normal activity of bone ALP. The four other patients with erosions had both total and bone ALP increased. Patients with erosions had greater values for biochemical indices of bone metabolism than did other patients (nine of 11 in the upper quartile of the osteocalcin distribution, and seven of 11 and six of 11, respectively, in the upper quartile of the PTH and bone ALP distributions). There was no correlation between the duration of dialysis and the presence of erosions.

In addition to the relationship between age and erosions, both osteocalcin and bone ALP decreased with age in this group of patients, but PTH did not show any notable variation with age (Table 4). Figure 3 shows the relationship between osteocalcin and PTH in patients (a) younger than 40 years and (b) 40 years and older: plasma osteocalcin is greater in younger patients at any given concentration of PTH. We confirmed our results by multiple regression analysis and found that the effect of age is additional to any effect of PTH on osteocalcin (P < 0.0001). Similarly, age has an effect on bone ALP independent of any effect of PTH on bone ALP (P < 0.0001).

Measurements Relating to Liver Disease

Only 16 of the 75 patients (21%) had any biochemical evidence of liver abnormality. Liver ALP was increased in 12 patients (Table 3) and showed significant correlations with GGT and age (Table 4). Ten of these 12 patients had increased total ALP in plasma. Plasma GGT was increased in 12 patients; in nine patients this was associated with increases in total and liver ALP. One patient with increased liver ALP, total ALP, and GGT also had increased bone ALP; another had increased ALT and bilirubin. Only one other patient had increased bilirubin, and this was associated with increased GGT, total ALP, and liver ALP. Four patients had increased ALT but normal liver ALP. Plasma GGT was increased in two of these patients; in the other two patients, ALT was the sole abnormal liver-function test result, but their total ALP was increased because of an increase in the activity of the bone isoenzyme. In five patients with increased liver ALP, the electrophoretoograms also stained for biliary ALP at the origin; the mean biliary ALP in these patients was 22 U/L (range 6–60 U/L).

Intestinal ALP, and Blood Group and Secretor Status

Table 3 shows the mean intestinal ALP activities for each blood group and secretor-status category. Intestinal ALP, when considered for each blood group and secretor-status category alone or for all patients together, did not correlate with other biochemical indices or with age. Activities of intestinal ALP in patients with renal failure and in the control group are shown in Figure 4. Within each category of blood group and secretor status, renal-failure patients had significantly higher intestinal ALP than did the controls (P < 0.001 in all cases). Renal-failure patients who were B, O secretors had greater intestinal ALP activity than did patients who were A secretors or ABO nonsecretors (P < 0.01). Patients who were A secretors did not have intestinal ALP activities that were significantly different from nonsecretors (P > 0.05), although two patients had activities considerably greater than the rest of the A secretor group (Figure 4). We noted no variation of liver or bone ALP with blood group or secretor status.

Eight patients had intestinal ALP activity >23 U/L
(seven B,O secretors and one A secretor). None of these patients had abnormal liver ALP or GGT activities; intestinal ALP accounted for a large proportion of total ALP activity (mean percentage 30%, range 16%–42%). Three of these patients had increased total ALP: in one, increased intestinal ALP was the only reason for the increase in total ALP; in the other two patients, both bone and intestinal ALP were increased.

Discussion

Early detection of parathyroid bone disease is important to reduce the need for parathyroidectomy. It is recognized that erosions on skeletal roentgenograms are late manifestations of parathyroid bone disease. Our results, showing that 15% of the patients had erosions, confirm this and suggest that measurement of bone ALP, which was increased in 33% of the patients, may provide a more sensitive index of bone involvement. However, bone ALP measurements are not widely available, whereas measurements of total ALP activity in plasma are available in nearly all hospitals. Total ALP was increased in nearly 40% of our patients on hemodialysis and correlated well with osteocalcin and PTH, but the increases in activity were sometimes from an increase in liver ALP. Because this limits the diagnostic value of total ALP in detecting bone disease, it is not surprising that the correlation of bone ALP with PTH and osteocalcin was better than that of total ALP.

Assays of bone ALP, PTH, and osteocalcin are much less readily available and are more difficult, time consuming, and expensive than that of total ALP activity. Although one can improve the specificity of total ALP for bone by taking an concurrent measurement of GGT into account, the question arises whether this combination can satisfactorily substitute for less widely available assays. At present, when medical costs are scrutinized and questioned (12), it is important to use more expensive tests and procedures only if they can be shown to benefit the patient. This is especially so in patients with chronic disorders who must be monitored at regular intervals, such as those on hemodialysis. However, a few patients will have increased total ALP—from greater concentrations of intestinal ALP, immunoglobulin-bound ALP, or even liver ALP—in whom GGT is normal, causing the increase to be incorrectly ascribed to bone disease. In addition, some patients will have a slight increase in bone ALP but normal concentrations of total ALP. Therefore, in using biochemical markers of bone disease, we consider it necessary to quantify the bone isoenzyme of ALP for maximum diagnostic accuracy.

The activity of bone ALP paralleled the concentration of plasma osteocalcin quite closely, as found previously (13); both measurements reflect osteoblastic activity. However, bone ALP was abnormal in 25 of the 75 patients, whereas osteocalcin was abnormal in nearly all the patients. Osteocalcin ALP was also found to be abnormal in virtually all patients with chronic renal failure (13) and to increase sharply when the glomerular filtration rate is <20 mL/min (14). This is probably attributable to a failure to excrete osteocalcin (which has a molecular mass of ~5800 Da) rather than to osteocalcin’s being a more sensitive index of bone turnover than is bone ALP (which is not normally excreted in urine). This problem in defining an appropriate reference range for plasma osteocalcin in patients with renal failure may cause some difficulty in interpreting single results and may limit its role to monitoring trends. On the other hand, a study of osteocalcin in 30 patients with renal osteodystrophy, each of whom had a bone biopsy, showed that osteocalcin concentration correlated better with histological findings than did total ALP activity (15).

Although serum PTH was significantly correlated with bone ALP and osteocalcin, there was marked variation in response at any given concentration of PTH. In particular, 36 patients had increased concentrations of PTH and normal bone ALP activity. This suggests that bone ALP lacks sensitivity in detecting PTH-related disturbances of bone metabolism. However, other factors may affect the skeletal effects of PTH. Aluminum is known to inhibit bone formation, but we found no evidence that variation in plasma aluminum affected the plasma bone ALP response to a given PTH concentration. However, our results suggest that the patient’s age may be an important factor in modulating bone response to PTH. Bone erosions were much more common in the younger age groups and, for a given PTH concentration, amounts of osteocalcin and bone ALP in plasma tended to be greater in young patients.

Although the main abnormality in metabolism of vitamin D in chronic renal failure is the impaired 1-hydroxylation of 25-OH-D, nutritional status as assessed by 25-OH-D concentrations has been demonstrated to contribute to disturbed bone metabolism in chronic renal failure (16). In this study, although 25-OH-D was less than the optimal concentration (10 μg/L) in one-third of the patients, these values correlated neither with other biochemical indices of bone metabolism nor with clinical or radiological variables.

We found that increased intestinal ALP was responsible for increased activity of total ALP in only a small number of our patients with chronic renal failure. One patient had increased activity of total ALP solely attributable to the intestinal isoenzyme and two other patients had increased bone and intestinal isoenzymes. Intestinal ALP was, however, much higher in patients with renal failure than in controls, whether the group was considered as a whole or subdivided into blood group and secretor-status categories (Figure 4). As reported in other diseases, the B,O secretors had greater activities for intestinal ALP within both the renal-failure and control groups than did the A secretors or the nonsecretors (5, 17). The reason for the increase in intestinal ALP activities (up to 41% of total ALP activity) in the serum of a few patients is not apparent. Increased concentrations of intestinal ALP are found most often in patients with chronic liver disease and are thought to be due to an inability of the liver to clear the
enzyme from circulation (18). This situation is unlikely in our patients because no patient had any evidence of severe liver disease. However, perhaps an abnormal composition of plasma could affect the intestinal ALP molecule in some minor way, causing an abnormal rate of clearance by the liver. Other workers have found an inverse relationship between intestinal ALP and plasma calcium and have suggested that the plasma calcium concentration affects hepatic binding of intestinal ALP (19). However, in our study, we found no relationship between intestinal ALP and plasma calcium. An intestinal-type ALP has been described in kidneys removed during transplantation or post mortem; some suggest that the kidney itself is the source of the intestinal isoenzyme in patients with chronic renal failure (20). The location of intestinal ALP in the brush border of the S3-segment of the kidney makes the release of intestinal ALP from this site into the circulation unlikely (21).

We have demonstrated the various sources of increased ALP in plasma of patients treated with hemodialysis and note that measurement of total ALP activity, combined with GGT activity, identifies many of the patients with renal bone disease. In our study, 28 patients had increased total ALP; in nine patients, the increase would have been attributed to liver disease because of the GGT results. However, in three of the 28 patients (11%; 95% confidence interval 3%–28%), an incorrect conclusion about bone involvement would have been reached: one patient with increased liver and bone ALP with increased GGT, one patient with increased intestinal ALP and normal GGT, and one patient with increased total and liver ALP but normal GGT. Thus, for maximum diagnostic accuracy, one should carry out specific measurements of the bone isoenzyme.

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References