Comparison of total and bone-specific alkaline phosphatase in patients with nonskeletal disorders or metabolic bone diseases

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To evaluate the diagnostic validity of new assays for bone-specific alkaline phosphatase (BAP), we compared measurements of total alkaline phosphatase (TAP) in serum with results for three different assays of serum BAP in healthy adults (n = 119), patients with chronic nonskeletal disorders (n = 123), and patients with metabolic bone diseases (n = 113). Serum TAP was determined by a standard colorimetric assay, BAP by the methods of lectin precipitation (L-BAP), enzyme immunoassay (E-BAP), and immunoradiometric assay (I-BAP). Impairment of liver function resulted in significant increases of all alkaline phosphatase (AP) measurements, with the smallest changes being exhibited by E-BAP. Compared with the results by TAP, diagnostic sensitivity (i.e., of values exceeding the reference interval) was not improved by BAP, but receiver-operating characteristic (ROC) curve analyses revealed improved discrimination for primary hyperparathyroidism by E-BAP. These results indicate that, in the presence of liver disease, the specificity of AP measurements is improved by measuring BAP. In most other clinical situations, serum TAP appears to provide sufficient clinical information; however, the cross-sectional study design used here allows no statement about the usefulness of BAP in serial measurements.

INDEXING TERMS: isoenzymes • ROC curve analysis • liver disease • hyperparathyroidism

Total alkaline phosphatase (EC 3.1.3.1; TAP) is a biochemical marker routinely used in the diagnosis and follow-up of liver and metabolic bone disease. In subjects with normal liver function, serum TAP has been shown to be a useful index of bone formation. In the presence of liver disease, however, the diagnostic usefulness of this analyte as a marker of osteoblast activity is greatly impaired because of a significant contribution of the liver-derived isoenzyme to the TAP serum pool. Consequently, several studies indicate that measurements of the bone-specific isoenzyme of alkaline phosphatase (BAP) in serum may provide better indices of bone formation than does TAP [1–3].

The liver and bone isoenzymes of alkaline phosphatase (AP) are derived from the same gene locus and differ only with respect to their posttranslational glycosylation and sialylation [4–7]. Various techniques developed to specifically measure BAP include heat inactivation [8], wheat-germ lectin precipitation [9, 10], and immune electrophoresis [11]. None of these methods, however, seems to provide the sensitivity, specificity, cost effectiveness, and reliability required for routine clinical application [12]. More recently, immunoassays using specific antibodies against human BAP have been developed, and thus far, the clinical data seem to point towards an improved diagnostic accuracy of these new assays with regard to bone diseases and their therapeutic monitoring [3, 13, 14].

To our knowledge, no direct and comprehensive comparison between the various BAP assays has been attempted. Here we report our comparisons of the specificity and sensitivity of serum TAP for bone disease with those of three assays for serum BAP in a group of healthy adults and in patients with either skeletal or nonskeletal diseases.

Subjects and Methods

We studied 355 subjects, stratified into three major groups. Healthy subjects: The 119 healthy adults (50 men, 28 premenopausal women, and 41 postmenopausal women, ages 20–81 years) were recruited as consecutive members of an ambulatory population. All subjects had a thorough physical examination as well as a radiological evaluation of bone status. Age, weight, and height were documented, and fasting serum samples were
obtained from each volunteer. None of the apparently healthy participants had a history of metabolic bone disease or of any other medical condition known to affect bone metabolism. Subjects on medications known to interfere with bone turnover were excluded from the study.

Patients with nonskeletal diseases. A consecutive series of 123 hospitalized patients with various nonskeletal disorders was included in the study as a reference population. Chronic hepatic failure (n = 47) was diagnosed on the basis of clinical and biochemical evidence and histopathological findings obtained from liver biopsies. Among these patients, 21 were diagnosed with alcohol-induced cirrhosis, 7 with posthepatic cirrhosis, 2 with primary biliary cirrhosis, and 8 with liver cirrhosis of unknown etiology; 2 further patients had parenchymal liver fibrosis; and 7 were diagnosed with liver metastases of primary gut-related tumors. None of these 47 patients had radiological or metabolic evidence of skeletal involvement.

Patients with chronic renal failure (n = 47) were defined by serum creatinine concentrations >110 μmol/L and by impaired glomerular function, as determined by endogenous creatinine clearance <90 mL/min. Of these patients, 17 were diagnosed with glomerulonephritis, 6 with glomerulosclerosis, and 9 with renal malfunction of hereditary origin; 8 patients had inflammatory autoimmune disease with renal involvement (Wegener granulomatosis, lupus erythematosus); and in 7 patients the cause of renal dysfunction could not be established. None of these patients showed evidence of secondary hyperparathyroidism or a significant impairment in calcium balance.

Patients with chronic obstructive pulmonary disease (COPD; n = 29) had clinical signs of impaired respiratory function and pathological results during spirometry. None of these patients had clinical, biochemical, or radiological evidence of metabolic bone diseases, and none was terminally ill. Subjects taking medications known to affect bone turnover were excluded from the study.

Patients with skeletal diseases. The third group studied comprised a consecutive series of 113 hospitalized patients with metabolic bone diseases. Diagnosis of Paget disease of bone (n = 26) was based on characteristic radiographic, scintigraphic, and laboratory (serum TAP) findings. Patients with primary hyperparathyroidism (PHPT; n = 31) were defined by high serum concentrations of intact parathyroid hormone (>65 μg/L) and total calcium (>2.65 mmol/L), associated with significant calciuria (>10 mmol/day). Secondary hyperparathyroidism (SHPT; n = 35) was diagnosed in patients with chronic renal failure and high serum concentrations of intact parathyroid hormone (>65 μg/L). Finally, a group of 21 women with breast cancer and bone metastases was included. In these subjects, skeletal involvement was diagnosed by positive bone scan and radiographic evaluation, and in 8 of these patients, additional evidence of liver metastases was documented. None of the patients with metabolic bone diseases had clinical or biochemical evidence of significant renal or hepatic impairment, including the 8 patients with breast cancer and liver metastases.

Samples
Serum samples were collected between 0800 and 1000 with subjects in the fasting state. Specimens were centrifuged within 2 h of sample collection and stored in 0.5-mL portions at −80 °C until assayed. Samples were analyzed in multiple runs (n = 10) during a 1-month period. The study was approved by the Ethics Committee of the University of Heidelberg, and written consent was obtained from all subjects before phlebotomy.

Assays
Serum TAP. The serum activity of TAP was measured by an automated colorimetric assay with a BM/Hitachi System 704 analyzer (Boehringer Mannheim, Mannheim, Germany) at 37 °C; p-nitrophenyl phosphate was the substrate, as recommended in the optimized standard method of the Deutsche Gesellschaft für Klinische Chemie [13]. All values were expressed as U/L and recalculated to a temperature of 25 °C. Intra- and interassay CVs were <5%, and the limit of detection was 33 U/L.

Serum BAP measured by lectin precipitation (L-BAP). BAP activity in serum was determined by lectin precipitation as described earlier [10], with use of a commercial test kit ("Test-combination of isoenzymes of alkaline phosphatase"; Boehringer Mannheim). Serum BAP activity was calculated from the difference between serum TAP activity before and after precipitation with wheat-germ lectin at 25 °C. Wheat-germ lectin (≥2 g/L) was dissolved in 5 mmol/L acetate buffer (pH 4.5), containing 20 mL/L Triton X-100 to prevent coprecipitation of the liver isoenzyme of AP (precipitation reagent). To perform the assay, we mixed 100 μL of serum with 100 μL of precipitation reagent and incubated for 30 min at room temperature. After centrifugation at 10 000g for 2.5 min, we measured the supernatant activity with a BM/Hitachi System 704 analyzer. Serum L-BAP activity, expressed as U/L, was calculated by using the following correcting function [10]:

L-BAP = (1.18 × TAP activity) − (2.35 × supernatant activity).

CVs ranged between 2.8% and 3.5% for intraassay variance and between 3.2% and 3.8% for interassay variance.

Serum BAP measured by IRMA (I-BAP). We also used the Tandem-R Ostase (Hybritech, San Diego, CA) solid-phase, two-site IRMA to quantify serum BAP. Two monoclonal mouse-anti-BAP antibodies directed towards different sites of the BAP molecule were used in a solid-phase sandwich format as described previously [16]. In brief, 100 μL of standard (SAOS-2 human osteosarcoma cell extracts, a bovine protein matrix containing human skeletal AP; provided with the assay), control, or serum sample was mixed with 100 μL of tracer antibody (mouse monoclonal IgG against BAP labeled with 125I, activity <222 kBq) and added to each test tube. After we introduced the solid phase into the tubes (i.e., added beads coated with mouse monoclonal IgG directed against human BAP), the samples were incubated for 19 ± 2 h at 2–8 °C. Thereafter, the solid phase was washed three times with detergent solution containing 3 g/L
sodium azide, and the radioactivity was quantified with a gamma counter. Results were calculated by interpolating the signals for the unknown samples with the point-to-point curve-fitting equation describing the signals for the standards and were expressed as BAP μg/L. Intra- and interassay CVs ranged between 3.7% and 6.7% and between 7.0% and 8.1%, respectively, and the detection limit was 2.0 μg/L.

**Serum BAP activity measured by enzyme immunoassay (E-BAP).** Serum activity of BAP was further determined in a commercially available enzyme immunoassay (Alphaphase-B™, Metra Biosystems, Mountain View, CA). This microtiter plate format immunoassay utilizes a plate-coated monoclonal anti-BAP capture antibody, and the activity of the captured enzyme is detected with p-nitrophenol phosphate as substrate. In brief, 125 μL of buffer (100 mmol/L sodium phosphate and 150 mmol/L sodium chloride, pH 7.0) and 20 μL of standard, control, or serum sample were added to each well. After incubation for 3 h (±10 min) at room temperature, we washed the microtiter plate four times with detergent solution (10 mmol/L sodium phosphate, 150 mmol/L sodium chloride, and 0.5 mL/L Tween 20). To start the reaction, we added substrate solution (2 g/L p-nitrophenyl phosphate in 0.4 mol/L 2-amino-2-methyl-1-propanol buffer, pH 10.4, containing 2 mmol/L HEDTA, 1 mmol/L zinc sulfate, and 2 mmol/L magnesium acetate) to each well and incubated for 30 min at room temperature. The reaction was stopped with 100 μL of 1 mol/L NaOH, and the absorbance was read at 405 nm with a microtiter plate reader. To calculate the results, we used a quadratic curve-fitting equation and expressed the results in U/L. CVs were 3.2–3.5% for intra- and 6.2–7.9% for interassay variation, and the detection limit was 0.7 U/L.

**STATISTICAL ANALYSIS**

The SAS software package (SAS Institute, Cary, NC) was used for statistical analysis. Descriptive values are presented as mean ± SD unless stated otherwise. Z-scores are expressed as the SD of the mean for healthy adults according to the equation: z = (x – μ)/SD. Linear regression analysis was performed to assess correlations between markers. Group differences were determined by using the Wilcoxon rank-sum test for nonparametric data. The Kruskal–Wallis test was used as a global test for equality. Receiver-operating characteristic (ROC) analysis, modified according to Holle [17], was performed to evaluate the diagnostic validity of the different assays. All statistical tests were two-tailed, and P < 0.05 was considered statistically significant. To correct for multiple testing, we adjusted the P-value according to the Bonferroni inequality (i.e., P/number of tests).

**Results**

**CHARACTERISTICS OF THE STUDY POPULATION AND NORMAL REFERENCE VALUES**

General and clinical characteristics of the study population are summarized in Table 1. Anthropometric data were similar in all groups, except for significant differences in weight and height between sexes. Compared with all other groups, patients with renal failure and patients with SHPT had significantly greater concentrations of serum creatinine and serum phosphate. By
definition, patients with SHPT showed increased values of intact parathyroid hormone in serum. Hepatic failure was associated with low concentrations of serum albumin, whereas in all other groups, mean serum albumin values did not differ significantly from the normal reference range values. Compared with healthy adults, patients with PHPT had significant hypercalcemia.

Table 2 shows the mean values in healthy volunteers. Serum L-BAP and E-BAP were significantly higher in men than in premenopausal women (P < 0.001), but no such difference was seen between men and postmenopausal women. Mean serum concentrations of all AP measurements tended to be higher in postmenopausal than in premenopausal women. These differences were statistically significant for serum TAP, L-BAP, and E-BAP (P < 0.01, < 0.05, and < 0.05, respectively). Because of the sex-related differences in most AP measurements, we performed statistical analyses on results for both the total group and for the subgroups of male and premenopausal and postmenopausal female subjects.

AP ISOENZYMES IN NONSKELETAL DISEASE
TAP and all measurements of BAP except E-BAP were significantly greater in patients with chronic hepatic failure (P < 0.001 vs controls), independent of age, sex, or menopausal status (Fig. 1A).

In patients with COPD, mean AP values differed only slightly from the control group. However, mean serum TAP was marginally but significantly higher than in the healthy subjects (P < 0.05). When the COPD patients were stratified according to sex and menopausal status, the TAP activities were higher in men only (Table 3).

Chronic renal failure (without SHPT) was associated with significantly higher values of serum TAP (P < 0.001), whereas the BAP assays showed no significant differences from values in healthy controls.

AP ISOENZYMES IN METABOLIC BONE DISEASES
Serum TAP and BAP activities were markedly increased in patients with Paget disease of bone (P < 0.001 vs healthy adults, all assays). Numerical differences among BAP assays were only marginal, the greatest changes being seen with L-BAP (Fig. 1B).

In patients with breast cancer and overt metastatic bone involvement but no clinical evidence of liver metastases, only serum TAP and I-BAP concentrations were significantly higher than in healthy women (P < 0.001 and P < 0.05, respectively). After we applied the Bonferroni correction, no changes seen for

Table 2. Reference values for serum AP in healthy adults.

<table>
<thead>
<tr>
<th>AP</th>
<th>Total</th>
<th>Men</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAP, U/L</td>
<td>103.1 ± 34.3</td>
<td>103.7 ± 38.9</td>
<td>89.4 ± 26.1</td>
<td>111.6 ± 29.9*</td>
</tr>
<tr>
<td>L-BAP, U/L</td>
<td>44.0 ± 16.8</td>
<td>47.5 ± 16.0*</td>
<td>37.0 ± 14.6</td>
<td>44.5 ± 17.6*</td>
</tr>
<tr>
<td>I-BAP, µg/L</td>
<td>10.8 ± 3.9</td>
<td>11.0 ± 3.5</td>
<td>10.1 ± 3.2</td>
<td>11.0 ± 4.6</td>
</tr>
<tr>
<td>E-BAP, U/L</td>
<td>20.1 ± 8.3</td>
<td>23.2 ± 7.2*</td>
<td>14.8 ± 6.2</td>
<td>20.7 ± 9.0*</td>
</tr>
</tbody>
</table>

* P < 0.05, # P < 0.01, † P < 0.001 vs premenopausal women. ‡ P < 0.05, after Bonferroni adjustment.
any of the BAP measurements were statistically significant (Fig. 1B and Table 3). Even including the eight patients with evidence of liver metastases in the statistical analysis did not affect the P-values for serum TAP and BAP.

PHPT was associated with significantly greater serum concentrations of all AP measurements, with z-values slightly below those seen in patients with Paget disease of bone (Fig. 1C). In contrast, values for TAP and BAP were much lower in patients with renal SHPT, whose absolute serum concentrations were only marginally above the normal reference range. Nonetheless, the changes in serum TAP and I-BAP (in SHPT patients vs healthy controls) were statistically significant (Fig. 1C).

CORRELATIONS BETWEEN ASSAYS
For the total group (n = 355), correlations between serum TAP and BAP were \( r = 0.86 \) for TAP vs L-BAP, \( r = 0.83 \) for TAP vs I-BAP, and \( r = 0.77 \) for TAP vs E-BAP (P < 0.001 for all assays). Serum BAP values were also all highly correlated with each other (\( r > 0.90, P < 0.001 \)).

In patients with nonskeletal disorders, including those with chronic hepatic disease, correlations between measurements of TAP and BAP were somewhat lower than in the total group: TAP vs L-BAP, \( r = 0.81 \); TAP vs I-BAP, \( r = 0.69 \); TAP vs E-BAP, \( r = 0.60 \) (P < 0.001 for all assays). When the reference group (nonskeletal disorders) was reanalyzed after excluding individuals with impaired hepatic function, the respective correlations were as follows: \( r = 0.75, 0.72, \) and 0.79 (P < 0.001 for all assays). In the group with metabolic bone disease, correlations between serum TAP and BAP were slightly higher than in the group with nonskeletal disorders: TAP vs L-BAP, \( r = 0.93 \); TAP vs I-BAP, \( r = 0.91 \); TAP vs E-BAP, \( r = 0.86 \) (P < 0.001 for all assays). Group-specific scatter plots between individual values for serum TAP and BAP are shown in Fig. 2.

DIAGNOSTIC SPECIFICITY, SENSITIVITY, AND ACCURACY OF AP MEASUREMENTS FOR METABOLIC BONE DISEASES
To estimate the diagnostic specificity of the different techniques used in this study, we determined the upper limit of normal (ULN) for each assay, defined as the mean for the healthy control group + 2 SD. Using this ULN as a cutoff, we calculated as a measure of specificity the percentage of patients with nonskeletal diseases who had serum concentrations below the ULN (i.e., the number of true negatives). As shown in Table 4, serum TAP was associated with the fewest true-negative results, indicating a low degree of specificity regarding bone metabolism. In contrast, the greatest number of true-negative results and therefore the highest specificity was seen with E-BAP, where impairment of hepatic function had only a moderate effect on the results. However, when we performed the same analyses using the manufacturers’ recommended ULN, the highest specificity was calculated for L-BAP and I-BAP (data not shown). Excluding patients with chronic impairment of hepatic function from the analyses yielded a specificity of >70% for serum TAP and all three assays of serum BAP (Table 4).
Sensitivity was assessed by calculating the percentage of patients with metabolic bone diseases whose results were above the ULN of the healthy control group (i.e., true positives). Overall sensitivity varied between 41% for E-BAP and 57% for TAP. However, when subgroups were analyzed individually, the diagnostic sensitivity of the different assays varied considerably (Table 4). Thus, in patients with Paget disease of bone all assays had a sensitivity of almost 100%, whereas in patients with breast cancer and bone metastases, serum TAP measurements yielded the most true-positive results. When the same analyses were performed with the ULNs provided by the manufacturers, the sensitivity of the E-BAP measurement was markedly improved; no such changes were observed for the other three assays (data not shown).

ROC curve analyses were performed to compare the subgroups of patients with skeletal disease with the remaining study population (i.e., healthy subjects plus patients with nonskeletal disease). Because sex-specific ROC curve analyses revealed no significant differences from unstratified data, results are shown for entire subgroups (Fig. 3). In patients with breast cancer and metastatic bone involvement, none of the individual AP measurements sufficiently distinguished between patients with and without skeletal disease. In this population, however, serum TAP showed the largest area under the curve (AUC; a measure of the mean sensitivity across the range of possible specificities) (Table 5). When patients with chronic hepatic failure were excluded from the analyses, the AUC value for serum TAP rose to 0.909. In Paget disease of bone, all assays exhibited a high AUC, ranging from 0.945 to 0.986, whereas in patients with PHPT the best results were obtained for serum I-BAP and E-BAP (AUC of 0.803 and 0.822, respectively). Notably, none of these assays sufficiently discriminated between the PHPT group and subjects without skeletal disorders (no significant difference from AUC = 0.5). Moreover, eliminating the patients with liver disease from the analysis improved results only for

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**Fig. 2.** Correlations between serum TAP and the various serum BAP measurements.

Patients with nonskeletal disease had chronic hepatic failure, chronic obstructive pulmonary disease, or chronic renal failure. Patients with skeletal disease had Paget disease of bone, PHPT, SHPT, or metastatic bone disease.

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**Table 4. Specificity and sensitivity of AP measurements according to sex and menopausal status.**

<table>
<thead>
<tr>
<th></th>
<th>Cutoff</th>
<th>NSD</th>
<th>HF</th>
<th>COPD, RF</th>
<th>SD</th>
<th>PHPT</th>
<th>SHPT</th>
<th>BM</th>
<th>Paget</th>
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<tr>
<td><strong>Premenopausal women</strong></td>
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<tr>
<td>TAP</td>
<td>141.6 U/L</td>
<td>54</td>
<td>25</td>
<td>67</td>
<td>56</td>
<td>67</td>
<td>50</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>L-BAP</td>
<td>66.2 U/L</td>
<td>65</td>
<td>50</td>
<td>72</td>
<td>44</td>
<td>67</td>
<td>38</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>I-BAP</td>
<td>16.5 µg/L</td>
<td>81</td>
<td>63</td>
<td>89</td>
<td>50</td>
<td>83</td>
<td>25</td>
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</tr>
<tr>
<td>E-BAP</td>
<td>27.2 U/L</td>
<td>88</td>
<td>75</td>
<td>94</td>
<td>44</td>
<td>67</td>
<td>25</td>
<td>—</td>
<td>—</td>
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<tr>
<td><strong>Postmenopausal women</strong></td>
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<tr>
<td>TAP</td>
<td>171.4 U/L</td>
<td>73</td>
<td>38</td>
<td>100</td>
<td>52</td>
<td>50</td>
<td>22</td>
<td>64</td>
<td>83</td>
</tr>
<tr>
<td>L-BAP</td>
<td>79.7 U/L</td>
<td>77</td>
<td>46</td>
<td>100</td>
<td>39</td>
<td>50</td>
<td>11</td>
<td>27</td>
<td>83</td>
</tr>
<tr>
<td>I-BAP</td>
<td>20.2 µg/L</td>
<td>87</td>
<td>69</td>
<td>100</td>
<td>39</td>
<td>42</td>
<td>22</td>
<td>27</td>
<td>83</td>
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<tr>
<td>E-BAP</td>
<td>38.7 U/L</td>
<td>90</td>
<td>77</td>
<td>100</td>
<td>37</td>
<td>42</td>
<td>11</td>
<td>27</td>
<td>83</td>
</tr>
<tr>
<td><strong>Men</strong></td>
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<tr>
<td>TAB</td>
<td>181.5 U/L</td>
<td>61</td>
<td>42</td>
<td>73</td>
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<td>100</td>
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<tr>
<td>L-BAP</td>
<td>79.5 U/L</td>
<td>66</td>
<td>31</td>
<td>88</td>
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<td>0</td>
<td>11</td>
<td>—</td>
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<tr>
<td>I-BAP</td>
<td>18.0 µg/L</td>
<td>76</td>
<td>65</td>
<td>87</td>
<td>53</td>
<td>50</td>
<td>17</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>E-BAP</td>
<td>37.6 U/L</td>
<td>91</td>
<td>81</td>
<td>98</td>
<td>44</td>
<td>25</td>
<td>0</td>
<td>—</td>
<td>95</td>
</tr>
</tbody>
</table>

NSD, nonskeletal disease; HF, hepatic failure; RF, renal failure; SD, skeletal disease; BM, bone metastases.
serum TAP (AUC = 0.847). None of the assays could distinguish SHPT patients from the nonskeletal disease populations.

**Discussion**

Serum TAP is a widely used marker of bone formation, but its clinical usefulness in regard to bone disease is greatly impaired in the presence of hepatobiliary disorders. With the recent advent of more-specific immunoassays for measurement of BAP, this isoenzyme has increasingly regained clinical interest, and several studies have indicated that in certain situations BAP might provide a better index of bone formation than measurements of TAP. The present comparative study demonstrates that quantifying serum BAP leads to a substantial gain in diagnostic specificity, although the various BAP assays display major differences. In contrast, in patients with metabolic bone disease and no evidence of hepatic dysfunction, serum BAP does not appear to be a more sensitive index of bone formation than is serum TAP. Furthermore, with regard to diagnostic validity assessed by ROC curve analysis, differences between the various assays seem marginal. However, the cross-sectional design of the present study allows no statement about the clinical usefulness of serum BAP in serial measurements.

The clinical sensitivity and specificity of a marker are highly dependent on the definition of the cutoff point or of the normal reference interval. For TAP, reference values are well established, and the serum TAP activities of healthy controls in the present study were within this range. Also, control values obtained by the lectin precipitation assay (L-BAP) and by the IRMA (I-BAP) were in good keeping with the ranges published by others and by the manufacturers. In contrast, serum E-BAP activities of healthy controls (both sexes and all age groups) differed from the reference interval provided by the manufacturer of this assay. Similar discrepancies were recently reported by Gomez et al. [14], although the reasons for this remain unclear. Careful evaluation of reference intervals seems essential for the clinical use of these new assays.

With all assays, mean serum AP values were higher in postmenopausal than in premenopausal women, and the differences were statistically significant for serum TAP and E-BAP. The menopause-related increase in serum AP activity is considered to reflect accelerated bone turnover after menopause [3, 13, 18], and our results support previous reports [19, 20] suggesting that this change is mainly due to an increase of the bone isoenzyme rather than the liver-derived fraction. Interestingly, healthy men also showed significantly higher TAP, L-BAP, and E-BAP concentrations than premenopausal women.

![Fig. 3. ROC curve analyses for the various AP measurements: I, patients with metastatic bone disease vs remaining group (i.e., healthy subjects and patients with nonskeletal disease); II, patients with Paget disease of bone vs remaining group; III, patients with PHPT vs remaining group; IV, patients with SHPT vs remaining group.](image)

<table>
<thead>
<tr>
<th>Patient group compared*</th>
<th>TAP (AUC)</th>
<th>L-BAP (AUC)</th>
<th>I-BAP (AUC)</th>
<th>E-BAP (AUC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Metastatic bone disease</td>
<td>0.663-0.877</td>
<td>0.406-0.692</td>
<td>0.569-0.803</td>
<td>0.503-0.785</td>
</tr>
<tr>
<td>II. Paget disease</td>
<td>0.915-0.975</td>
<td>0.972-1</td>
<td>0.957-1</td>
<td>0.929-1</td>
</tr>
<tr>
<td>III. PHPT</td>
<td>0.576-0.786</td>
<td>0.671-0.855</td>
<td>0.707-0.899</td>
<td>0.733-0.911</td>
</tr>
<tr>
<td>IV. SHPT</td>
<td>0.517-0.709</td>
<td>0.414-0.622</td>
<td>0.499-0.703</td>
<td>0.378-0.596</td>
</tr>
</tbody>
</table>

*Vs remaining groups; I-IV and ROC curves as shown in Fig. 3.

b. 1 = complete correspondence between assay value and disease diagnosis.
but no difference in I-BAP results. These observations are consistent with previous reports involving assays of serum I-BAP [13] and E-BAP [14]. Given that I-BAP is a measure of AP mass rather than activity, these data suggest that sex-specific differences in the serum activity of AP may reflect differences in enzyme activation rather than in molecular mass. Because of the pronounced variation in normal values, reference ranges for serum TAP and BAP should be corrected for sex and menopausal status.

To test for clinical specificity, we applied the AP assays to serum samples from patients with nonskeletal diseases. Chronic hepatic failure in all assays resulted in significantly increased values, with the greatest increases seen for serum TAP and the smallest changes for E-BAP. These results indicate that interference by, or cross-reactivity with, the liver isoenzyme should be expected in all systems. This is in good keeping with recent data that show a cross-reactivity with the liver isoform of 8% for L-BAP [3], 10–16% for I-BAP [13], and 3–10% for E-BAP [14].

In men with COPD, only serum TAP was increased significantly over the normal reference range. The reasons for this are unclear, but the normal BAP values in this group suggest that the increase in TAP activities may be attributed to congestive liver disease. Chronic renal dysfunction appeared to have only small effects on serum AP concentrations, although statistically significant changes were seen for serum TAP and L-BAP. In contrast, previous studies have found that serum BAP concentrations in patients on hemodialysis were as much as three times greater than those in healthy adults [13]. This discrepancy is most probably attributable to differences in patient population characteristics, because only a small number of our patients were on hemodialysis. Notably, the group of patients diagnosed with SHPT (indicating a more advanced stage of renal failure) showed more pronounced changes in serum AP measurements. These results support the notion of increased bone turnover in patients with chronic renal failure [13, 19, 21, 22], and detection of active renal osteodystrophy may well be an important clinical application of BAP measurements.

Regarding metabolic bone diseases, none of the BAP assays showed significantly greater clinical sensitivity than the measurement of serum TAP. This was confirmed by group-specific ROC curve analyses. Interestingly, in the patients with breast cancer and bone metastases, determination of serum TAP resulted in the greatest number of true-positive results, even when patients with overt liver metastases were excluded. However, the high serum TAP activity in this group could still be due to impairment of hepatic function, e.g., as a result of chemotherapy. Also, the mainly osteolytic character of bone metastases in patients with breast cancer may not lead to significant increases of serum BAP. This seems to be of particular relevance during early stages of metastatic bone involvement, as reported by Cooper et al. [23].

In Paget disease of bone and in PHPT, no improvement of clinical sensitivity was achieved by measuring serum BAP. Serum TAP activities are markedly increased in active Paget disease of bone [3, 13, 21, 22, 24], so it is not surprising that the use of serum BAP yields no advantage over the TAP assay in this major perturbation of bone metabolism. However, given that some studies have shown that serum BAP may be a more sensitive indicator of changes in bone turnover during bisphosphonate treatment [3, 13, 25], therapeutic monitoring may be an application of BAP measurements in Paget disease of bone.

Consistent with previous reports [3, 13], we found PHPT to be associated with increases in the mean concentrations of both serum TAP and BAP. These changes seemed to be somewhat more pronounced for serum BAP and, according to ROC curve analyses, the use of serum BAP may at least theoretically provide a diagnostic advantage over measurements of serum TAP (Fig. 3, III).

Group-specific correlations between the various assays demonstrated improved specificity for serum BAP measures, as shown by a steeper linear regression in the group of patients with skeletal disorders than in the patients with nonskeletal diseases (Fig. 2). Again, significant differences were noted between the BAP assays, and steepest regression slopes were observed for E-BAP. Even for the enzyme immunoassay, however, the correlation between serum TAP and E-BAP values remained significant in patients with hepatic failure.

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