Sandwich Enzyme Immunoassay of Osteocalcin in Serum with Use of an Antibody against Human Osteocalcin

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We have developed and thoroughly validated a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) on microtiter plates for osteocalcin in human serum with use of an antibody raised against human osteocalcin. We used a monoclonal antibody against bovine osteocalcin as the capture antibody; the second antibody was a polyclonal antibody against human osteocalcin. The amount of bound second antibody was determined with use of swine antirabbit antibody labeled with horseradish peroxidase. We demonstrated independence of volume and determined the recovery of added standard and within- and between-assay precision. The minimal detection limit for osteocalcin was between 1.0 and 1.5 μg/L and the midpoint of the standard curve ranged from 14 to 17 μg/L. The intra assay CV was ≤8% in the range 2.7–52 μg/L; the inter assay CV was usually ≤15% in the same range. Analytical recovery of human osteocalcin standard added to serum samples was consistently >90%. Values for osteocalcin measured in serum from 44 normal subjects were similar to those obtained with a competitive enzyme immunoassay (EIA) that used a monoclonal antibody against bovine osteocalcin. There was a good correlation between the two assays ($r^2 = 0.877$, slope and intercept (±SE) = 0.88(±0.051) and 0.316(±0.523), respectively). The range and mean (±SD) for the sandwich ELISA and the competitive EIA were 1.7–18.1 μg/L [8.7(±4.4) μg/L] and 1.9–22.8 μg/L [9.1(±4.4) μg/L], respectively.

Indexing Terms: enzyme-linked immunosorbent assay · bone Gla protein · calcium-binding protein · osteocalcin peptides · epitope binding site

Osteocalcin (bone Gla-protein) is a noncollagenous bone-specific protein (M, 5800), containing three residues of γ-carboxyglutamic acid (1–3). Its biosynthesis by osteoblasts (4, 5) is vitamin K-dependent (6) and is stimulated by 1,25-dihydroxyvitamin D3 (7, 8). Newly synthesized osteocalcin becomes incorporated into the bone matrix and, although its precise function is not known, it has a strong ability to bind to hydroxyapatite (9, 10). A small percentage of the newly synthesized osteocalcin that is not adsorbed into the bone matrix is released into the circulation (11), where its concentration is indicative of the rate of bone formation (12). Serum osteocalcin measurements are used in the management of patients with metabolic bone disorders, including osteoporosis, Paget disease, hyperparathyroidism, and diseases related to excess glucocorticoids (13).

Several immunoassays of osteocalcin have been developed, including radio- and enzyme immunoassays (EIAs) and involving the use of both monoclonal and polyclonal antibodies (14–19). Most use bovine osteocalcin as immunogen, tracer, and standard, because of its availability and because 90% of its amino acid sequence is homologous with that of human osteocalcin (19). Antiseras raised against bovine or ovine osteocalcin cross-react with osteocalcin in human serum (20, 21). However, a relatively low degree of cross-reactivity was reported for an antisera raised against ovine osteocalcin; that assay was also unable to measure osteocalcin in human serum independently of the volume of sample assayed (22). Despite extensive homology between human osteocalcin and bovine or ovine osteocalcin, there are sequence differences in the N-terminal regions. Antisera raised against bovine osteocalcin that recognize antigenic sites only in this latter region may react differently with human osteocalcin in comparison with antisera that recognize other regions of the molecule.

A sandwich immunoassay has been developed with use of antisera raised against synthetic peptides with sequences homologous with human osteocalcin at the C- and N-terminal regions (23); however, insufficient validation data were given on dependence on volume or on cross-reactivity with purified human osteocalcin or osteocalcin in human serum. Here we describe a sandwich ELISA of osteocalcin, performed on microtiter plates with use of a polyclonal antisera raised against human osteocalcin. We compare osteocalcin values measured in human serum with this assay with those of a competitive EIA involving antisera raised against bovine osteocalcin.

Materials and Methods

Subjects

The 44 women studied, ages 49–54 years, were in good health and were taking no drugs known to affect bone metabolism. Serum was separated from blood specimens and stored in small aliquots at –70 °C until analyzed for osteocalcin. Ethical Committee approval was obtained and the subjects gave informed consent.

Materials

Buffers. The buffers were similar to those previously used in a competitive EIA of osteocalcin (16). General assay buffer consisted of, per liter, 122.5 mmol of sodium chloride, 25 mmol of EDTA, 1 mL of Tween 20, 1 g of bovine serum albumin, and 10 mmol of Na2HPO4/NaH2PO4, pH 7.4. Borate-buffered saline (pH 8.4–8.5) contained, per liter, 75 mmol of sodium chloride, 100 mmol of boric acid, and 40 mmol of sodium bicarbonate.
Coating buffer, pH 9.6, was NaHCO₃/Na₂CO₃, 50 mmol/L. Buffered substrate, pH 5.0, consisted of 0.05 mol of citric acid and 0.13 mol of Na₂HPO₄ per liter, to which were added just before assay, 5.9 mmol of H₂O₂ and 16 mmol of o-phenylenediamine per liter. All buffers contained thimerosal (100 mg/L) as preservative and were stored at 4 °C. The plate-washing solution was a 20-fold dilution of a concentrate consisting of 1.5 mol of NaCl and 5 mL of Tween 20 per liter. Plate-stabilizing solution consisted of phosphate-buffered saline, 0.01 mol/L, 1 volume of which was mixed with 5 volumes of glycerol, 4 volumes of distilled water, and enough bovine serum albumin to give a final concentration of 20 g/L. All buffer constituents were analytical grade (Riedel De Haen, Hanover, Germany). Flat-bottomed microtiter strips were from Nunc AS, Kamstrup, Denmark.

Osteocalcin. Human osteocalcin was purified by a modification of a previous method (24). The final yield of pure osteocalcin was 1.5 mg per 100 g of dried bone powder. Homogeneity of the purified osteocalcin was confirmed by electrophoresis on polyacrylamide gel (25) and by high-pressure liquid chromatography on a 250 × 4.6 mm Ultra Tech 5 ODS column (Labquip Ireland Ltd., Dublin, Ireland) with use of an acetonitrile gradient (from 200 to 800 mL/L) containing trifluoroacetic acid, 1 g/L, and an on-line integrator (C-R6A computing integrator; Shimadzu Corp., Kyoto, Japan). Osteocalcin eluted as a single peak at 500 mL/L acetonitrile, which is a higher acetonitrile content than that described by others (24). The N-terminal amino acid sequence of the first 20 amino acids also confirmed the purity of the osteocalcin. Osteocalcin was quantified by means of the Lowry dye-binding assay, combined with use of the absorbivity (ε₂₈₀) = 13.2 L·mol⁻¹·cm⁻¹ (26). Although this is the absorbivity for bovine osteocalcin, we used it to quantify human osteocalcin because there is 90% sequence homology between the two proteins (which differ from each other by only five amino acids). This absorbivity was also recently used by Bouillon et al. (27) to quantify purified human osteocalcin. Osteocalcin stock standards were freeze-dried and stored in 4-, 20-, and 100-μg aliquots. Working standard solutions (1.6–100 μg/L) were stored at −70 °C in 0.5-mL aliquots and were used within 1 week of thawing.

Osteocalcin peptides. Human osteocalcin peptide 14–28 was a gift from Caren Gundberg. Human osteocalcin peptides 38–49 and 45–49 and bovine osteocalcin peptide 14–28 were gifts from Michael diMuzzio (Abbott Labs., N. Chicago, IL). Human osteocalcin peptide 7–19 was from Sigma Chemical Co., Dorset, UK.

Antisera. Immunogens were prepared by conjugation of purified human osteocalcin to bovine serum albumin with use of water-soluble carbodiimide. Antisera were raised in New Zealand White rabbits as described previously (15). Antiserum R-188, the antiserum with the highest titer, was stored at −70 °C in 1-mL aliquots, subsequently diluted 10-fold with borate-buffered saline, and stored at 4 °C before use. The monoclonal antibody 72C8C8, raised against bovine osteocalcin (16), was produced in ascites fluid and stored at 4 °C diluted 10-fold with borate-buffered saline.

Swine anti-rabbit antibody labeled with horseradish peroxidase (Dakopatts, Glostrup, Denmark) was used at a suitable dilution.

Osteocalcin-free serum. We removed osteocalcin from serum by mixing with anti-osteocalcin antibody bound to CNBr-activated Sepharose 4B (Pharmacia, Bromma, Sweden). The resulting serum contained <1.5 μg/L osteocalcin as measured by EIA. This was divided into 250- and 500-μL aliquots and stored at −70 °C. Purified human osteocalcin was added to batches of osteocalcin-free serum to give controls containing 0, 10, and 25 μg/L. These quality-control samples were stored in 50-μL aliquots at −70 °C. Ten serum samples containing a range of osteocalcin concentrations as determined by EIA were also divided into 100-μL aliquots and stored at −70 °C to act as supplementary quality-control samples for validation studies.

Procedures

Coating microtiter plates. Each well was filled with 200 μL of 72C8C8 anti-bovine osteocalcin ascites at a final dilution of 10 000-fold in coating buffer. Plates were covered with plastic film and incubated overnight at room temperature (20 °C). Plates were washed and covered with clamping film; they could be stored at 4 °C with 200 μL of plate-stabilizing solution per well for 1 month.

Osteocalcin immunoassay. Pipet 10 μL of standard solution (0–100 μg/L) into coated microtiter wells in duplicate. At the same time, add 10 μL of assay buffer to the sample and control wells. Add 10 μL of osteocalcin-free serum to the standard wells and 10 μL of control or sample to the sample wells. Finally add 100 μL of assay buffer to all wells, mix gently, cover, and incubate at 37 °C for 60 min. Add R-188 diluted 5000-fold in assay buffer, 100 μL per well. This yields a final antibody dilution of 10 000-fold in each well. Mix again and incubate at 37 °C for a further 60 min. Wash the strips thoroughly and tap to remove excess moisture. Add to each well 200 μL of swine anti-rabbit horseradish peroxidase-labeled antibody, diluted in assay buffer. An appropriate dilution for batch 030 was 25 000-fold. Cover the plate again and incubate overnight at 4 °C. Wash thoroughly, tap dry, and add 150 μL of the buffered enzyme substrate solution per well. After 30 min, stop the reaction with 50 μL of 4 mol/L H₂SO₄ and, after gentle mixing, read the absorbance at 492 nm with a microtiter plate reader [we used a Dynatech (West Sussex, UK) MR 5000 microplate reader].

Results

Calibration curve and precision profile. The calibration curve (Figure 1) represents the mean from six successive assays where standards were assayed in duplicate during 1 month. Nonspecific binding ranged from 0.02 to 0.04 A and the midpoint of the calibration curve for osteocalcin was 14 to 17 μg/L. The detection limit, defined as the concentration corresponding to the
Fig. 1. Composite calibration curve (D) calculated from the results of six successive ELISAs for osteocalcin carried out in duplicate, and the precision profile (B), which represents the within-assay CVs (n = 15) for five serum samples containing a range of osteocalcin concentrations.

Fig. 2. Determination of the relationship between dilution factor and the amount of osteocalcin detected for five serum samples containing different osteocalcin concentrations.

mean absorbance for the zero calibration plus twice the SD of multiple determinations of nonspecific binding, ranged from 1.0 to 1.5 μg/L for this set of calibration curves. Figure 1 also shows the precision profile (within-assay CV) values for five serum samples over the range of the calibration curve.

Analytical recovery. We assessed the ability of the assay to measure accurately human osteocalcin standard added to serum samples from four different subjects having a range of endogenous osteocalcin concentrations. The total osteocalcin was measured and the recovery of added osteocalcin determined after subtracting the value for endogenous osteocalcin (Table 1). The overall mean percentage recovery was 103% (SD 13.8%, n = 32).

Independence of volume. We examined the ability of the assay to measure endogenous osteocalcin in serum independently of the volume of sample used. We measured osteocalcin in five serum samples from different subjects, containing a range of osteocalcin concentrations, which were serially diluted with osteocalcin-free serum. Results for the five samples were independent of the volume assayed over the range tested and over the range of the calibration curve (Figure 2). Results for three of the samples containing lower endogenous concentrations were reliable only up to an eightfold dilution; beyond this, the osteocalcin concentration became too low for accurate estimation. The low degree of variation in the dilution curves (<15%) indicates minimal immunological interference by serum components.

Assay variation. Interassay CVs for 11 samples containing 1.7 to 40.2 μg/L osteocalcin were estimated by assays of five to eight calibration curves. The variation was 6.35% to 19% (mean 12%, SEM 0.31%) (Table 2). Only one sample yielded a CV of 19%; in the majority of cases, values of <15% are readily attainable. Within-assay variation was calculated by replicate determinations of 13 serum samples from different subjects in a single assay. The mean intraassay CV was 7.0% (SD 1.2%) over the range 2.7 to 52.2 μg/L.

Calcium dependence. We previously showed that the monoclonal antibody 72C8C8 binds osteocalcin independently of the presence of calcium (16). The ELISA assay buffer contains EDTA, which suggests that antibody R-188 also interacts with osteocalcin independently of calcium. Therefore we propose that the ELISA is probably calcium independent.

Specificity. To establish that the material detected in serum corresponded to osteocalcin and not to other serum proteins of different molecular mass, we fractionated 2 mL of a serum sample containing a high endogenous osteocalcin concentration by passage through a Sephadex G-100 column (1.5 × 65 cm). The column was calibrated with molecular mass standards of 66–3.5 kDa to ensure that it could resolve peptides <6 kDa. The resulting fractions were analyzed by a competitive EIA with use of anti-bovine osteocalcin monoclonal antibody (72C8C8), and by the sandwich ELISA with use of

Table 1. Analytical Recovery of Osteocalcin Added to Eight Different Serum Samples, as Measured by Sandwich ELISA

<table>
<thead>
<tr>
<th>Added</th>
<th>Range measured</th>
<th>Mean recovered (and % of added)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.4–2.9</td>
<td>3.7–13.2</td>
</tr>
<tr>
<td>3.1</td>
<td>3.7–6.0</td>
<td>6.9–16.3</td>
</tr>
<tr>
<td>6.25</td>
<td>7.2–8.9</td>
<td>10.2–20.0</td>
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<td>12.5</td>
<td>13.8–15.4</td>
<td>17.4–27.6</td>
</tr>
<tr>
<td>25</td>
<td>23.3–28.5</td>
<td>28.7–42.0</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Added</th>
<th>Range measured</th>
<th>Mean recovered (and % of added)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.4–2.9</td>
<td>3.7–13.2</td>
</tr>
<tr>
<td>3.1</td>
<td>3.7–6.0</td>
<td>6.9–16.3</td>
</tr>
<tr>
<td>6.25</td>
<td>7.2–8.9</td>
<td>10.2–20.0</td>
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<tr>
<td>12.5</td>
<td>13.8–15.4</td>
<td>17.4–27.6</td>
</tr>
<tr>
<td>25</td>
<td>23.3–28.5</td>
<td>28.7–42.0</td>
</tr>
</tbody>
</table>

n = 4 each.

Table 2. Interassay Variation in Seven Quality-Control Samples

<table>
<thead>
<tr>
<th>Osteocalcin, μg/L</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>2.3</td>
<td>0.27</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6.5</td>
<td>0.4</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>14.6</td>
<td>1.2</td>
<td>8.0</td>
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<td>8</td>
<td>18.8</td>
<td>1.8</td>
<td>9.4</td>
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<td>7</td>
<td>27.5</td>
<td>0.38</td>
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<td>7</td>
<td>27.8</td>
<td>5.3</td>
<td>19.0</td>
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<td></td>
<td>8</td>
<td>32.4</td>
<td>4.5</td>
<td>13.7</td>
</tr>
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</table>
antibody R-188 and polyclonal antibody R-187, also raised against human osteocalcin. All three antisera detected a major immunoreactive peak with an elution volume corresponding to the molecular mass of osteocalcin (Mr, 6000) (Figure 3). Antiserum 72C8C8 and R-188 overestimated osteocalcin by 31–38%, compared with the value obtained in whole, unfractionated serum.

Antiserum R-187 detected an immunoreactive peak in the void volume. A similar result was reported with antisera raised against bovine osteocalcin (15, 17). The void volume peak contained about 20% of the total immunoreactive material detected in all the fractions from the column. With antiserum R-187, however, total recovery was only 89% of that measured in the original serum sample (Table 3). None of the three antisera detected any minor immunoreactive peaks eluting after intact osteocalcin.

The sandwich ELISA appears to be specific for human osteocalcin. This conclusion is based on the fact that purified bovine osteocalcin and dilutions of fetal calf serum, which was previously reported to contain high concentrations of endogenous bovine osteocalcin (20), were not detected by the assay, whereas the dilution curve for human serum completely paralleled the standard curve for human osteocalcin (data not presented).

Cross-reactivity studies. To determine whether antibody R-188 detects human osteocalcin peptides, we tested cross-reactivity of various synthetic peptides. The assay did not detect human osteocalcin peptides 7–19, 38–49, or 45–49 or bovine osteocalcin peptide 14–28.

Human osteocalcin peptide 14–28 displayed ~44% of the reactivity of intact human osteocalcin. The only difference between the bovine and the human osteocalcin peptide 14–28 is at amino acid 19, which is Lys in the former and Arg in the latter.

Clinical samples. Osteocalcin concentrations in serum from 44 women were measured with this ELISA and with a competitive EIA that uses an anti-bovine osteocalcin monoclonal antibody (72C8C8). Absolute serum osteocalcin values measured with the two assays were similar and there was a good correlation between them (Figure 4): \( r^2 = 0.877; \) the intercept (±SE) was 0.316 (± 0.523) and the slope (±SE) was 0.88 (± 0.051). The mean (±SD) and range of values for the sandwich ELISA and the competitive EIA were 8.7 (±4.4) µg/L, range = 1.7–18.1 µg/L, and 9.1 (±4.4) µg/L, range = 1.9–22.8 µg/L, respectively.

Discussion

This ELISA has several advantages over previously published RIAs of osteocalcin (15, 20, 22, 28, 29), including the relative stability of the peroxidase-labeled antibody compared with \(^{125}\)I-labeled osteocalcin and the lack of any requirement for special handling and disposal facilities.

Most previously published immunoassays for osteocalcin, both RIAs and EIAs, have used bovine osteocalcin as standard and immunogen (15–18, 30). A competitive EIA involving biotinylated IgG antibodies to bovine osteocalcin was also recently published (31). The

![Fig. 3. Elution profile for the fractionation of a serum sample on a Sephadex G-100 column](image-url)

Each fraction was analyzed for osteocalcin (Oc) by ELISA with use of antiserum R-187. Results obtained with antibodies R-188 and 72C8C8 are shown in Table 3.

![Fig. 4. Correlation of osteocalcin concentrations obtained in the assay of the same 44 serum samples by sandwich ELISA with polyclonal antiserum R-188 and an in-house competitive EIA with monoclonal antibody 72C8C8](image-url)

Table 3. Fractionation of a Serum Sample on a G-100 Column and Analysis of the Fractions for Osteocalcin by EIA and ELISA with Three Different Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>In void volume</th>
<th>In main peak (and % total)</th>
<th>Total</th>
<th>In whole serum</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>72C8C8</td>
<td>40</td>
<td>174 (81)</td>
<td>214</td>
<td>240</td>
<td>89</td>
</tr>
<tr>
<td>R-188</td>
<td>40</td>
<td>100 (100)</td>
<td>100</td>
<td>76</td>
<td>131</td>
</tr>
<tr>
<td>R-187</td>
<td>40</td>
<td>152 (100)</td>
<td>152</td>
<td>110</td>
<td>138</td>
</tr>
</tbody>
</table>

CLINICAL CHEMISTRY, Vol. 39, No. 6, 1993 945
present ELISA, in contrast, uses human osteocalcin as standard and immunogen. Furthermore, this antiserum (R-188) appears to be specific for intact human osteocalcin. Unlike other antisera raised against either intact human osteocalcin (27, 29) or human osteocalcin peptides (23, 32), antiserum R-188 displays no cross-reactivity with bovine osteocalcin and, except for one, does not recognize small peptides with amino acid sequences similar to human osteocalcin. The latter feature is an important advantage because osteocalcin peptides are probably formed by proteases in the serum of normal individuals (28) and have been detected in serum of patients with metabolic bone diseases (26, 33, 34). Also, osteocalcin peptides in serum may reflect the rate of bone resorption rather than bone formation (13, 33). 

Immunoassays that measure such peptides in addition to intact osteocalcin may give distorted values for osteocalcin concentrations and thus for bone formation rates. Because a sandwich immunoassay requires that the analyte has at least two nonoverlapping antigenic sites, recognition of such peptides, although feasible, is less probable.

The nature of the epitope recognized by this antiserum (R-188) may be in the midmolecular region of osteocalcin near amino acids 19–20. A similar epitope was suggested for another antiserum against human osteocalcin (29). In human and ovine osteocalcin, residue 19 is Arg (3, 21); it is Lys in bovine osteocalcin (19). Antisera raised against ovine osteocalcin cross-react to a greater extent with human osteocalcin than with the bovine molecule (22). We found in this study that a synthetic peptide with an amino acid sequence homologous with human osteocalcin between amino acids 14–28 cross-reacted with intact human osteocalcin when measured with antiserum R-188. Peptide 14–28 would not occur in human serum because the tryptic cleavage of human osteocalcin would release peptides 1–19 and 20–49; it could, however, arise from hydrolysis by other proteases. Because antiserum R-188 did not cross-react with bovine osteocalcin, this suggests that R-188 recognizes a midmolecule epitope of human osteocalcin. Moreover, human osteocalcin peptides 7–19 and 38–49 were not detected by the assay; therefore, R-188 probably recognizes an epitope that includes amino acid 19 in combination with other amino acids at the α-helical region of osteocalcin (13).

Antisera R-187 and R-188 and a monoclonal antibody raised against bovine osteocalcin (72C8C8) detected a protein with the same molecular mass (6 kDa) as osteocalcin, as shown when serum containing high osteocalcin concentration was fractionated by passage through a Sephadex G-100 column. Similar findings were reported previously by us (15) and others (22, 26, 33). Antiserum R-188 and 72C8C8 overestimated osteocalcin in the fractions from the column, which confirms previous findings and may be due to some serum component influencing the binding of the antibody to osteocalcin (15). Neither antisera R-187 or R-188 detected any minor peaks that might elute after the major immuno-reactive osteocalcin peak. This confirms that these antisera do not cross-react with the small peptides that could arise from proteolytic cleavage of intact osteocalcin during the process of bone resorption (34).

A good correlation was obtained between this assay and a competitive EIA involving anti-bovine osteocalcin monoclonal antibody, 72C8C8 (Figure 3). There was a striking similarity between the two assays in the osteocalcin concentrations measured in serum samples from 44 women, and the results agreed well with published values (16, 35). We and others previously found that osteocalcin concentrations measured with different immunoassays are antibody dependent (15, 26, 36). For example, serum osteocalcin concentrations measured by a sandwich ELISA with use of antibody 72C8C8 and an anti-bovine osteocalcin polyclonal antiserum R-26D were significantly lower than values obtained with the aforementioned competitive EIA (37). Similar results were reported by others when serum osteocalcin was measured by a two-site immunoradiometric assay and a conventional RIA (32). Another study reported similar serum osteocalcin concentrations when monoclonal and polyclonal antibody-based RIAs were compared (14).

The similarity between serum osteocalcin concentrations measured with this sandwich ELISA and the competitive EIA may arise because the specificity of a sandwich immunoassay is determined by the combined selectivity of the two antibodies (38). The monoclonal antibody (72C8C8) is common to both immunoassays, which may contribute to the similarity in the osteocalcin measurements. Another reason may be the similarity between the epitopes recognized by the antibodies. We found that the human osteocalcin peptide 14–28 also showed reactivity in the competitive EIA (data not presented), which provides additional evidence that both have epitope-binding sites in the midmolecule region of osteocalcin. Tracy et al. (26) reported that serum osteocalcin values measured with a monoclonal antibody-based RIA were 50% higher than when measured with a polyclonal antibody-based immunoassay. This was attributed to the ability of the monoclonal antibody to cross-react with other immunoreactive forms of osteocalcin, including peptides. In our case, neither antibody 72C8C8 or R-188 recognized small synthetic peptides of osteocalcin and may therefore not recognize osteocalcin peptides in human serum.

In contrast with the similarity between osteocalcin values measured with the competitive EIA and the sandwich ELISA with use of antiserum R-188, there were striking differences in values when antiserum R-187 was used in the latter assay (Table 3). Serum with high concentrations of osteocalcin, such as one from a patient with primary hyperparathyroidism measured in this study, may give different osteocalcin values when measured with the competitive EIA and the sandwich ELISA with use of R-188: e.g., osteocalcin values were 78 μg/L with the sandwich assay and 110 μg/L with the competitive EIA. This difference may be due to the hook effect, which results in underestimation of antigen when high concentrations are present. This could be overcome by dilution of sera to give osteocalcin values <50 μg/L (32).
It has been recommended recently that measurement of bone mass with techniques such as dual energy x-ray absorptiometry should be combined with assays of specific biochemical markers of bone metabolism such as osteocalcin. This combination may predict the risk of fracture better than bone densitometry alone (39, 40). It is important that fully validated osteocalcin immunoassays be used in which, if possible, the epitope recognized by the antibody has been determined.

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References