New Automated Multiplex Assay for Bone Turnover Markers in Osteoporosis

Aurélie Claudon,1 Philippe Vergnaud,1 Cécile Valverde,1 Anita Mayr,2 Ursula Klause,2 and Patrick Garnero1,3*

BACKGROUND: Serum C-terminal cross-linked telopeptide of type I collagen (CTX-I), N-terminal propeptide of type I collagen (PINP), and osteocalcin (OC) are among the most sensitive bone turnover markers for evaluating osteoporosis. Each marker is currently measured individually by manual or automated immunoassays that are time consuming and require substantial sample volume. We evaluated the performance characteristics of a novel, fully automated, protein-array chip system that allows the simultaneous measurement of CTX-I, PINP, OC, and intact parathyroid hormone (PTH) in 20 μL of serum.

METHODS: We measured CTX-I, PINP, OC, and PTH using multiplex and corresponding automated single assays in 157 healthy premenopausal women, 74 healthy men, and 56 postmenopausal osteoporotic women before and 6 months after treatment with oral ibandronate (150 mg/month).

RESULTS: Within- and between-run CVs of the multiplex assay were similar to those of single measurement assays (<10% for all markers), whereas the limit of quantification was lower, except for OC. Multiplex values highly correlated (r > 0.93, P < 0.0001 for all markers) with the corresponding single assays, and measured concentrations were comparable. After 6 months of ibandronate, CTX-I, PINP, and OC decreased by a median of 48%, 63%, and 52%, respectively (P < 0.0001 for all 3 markers), magnitudes similar to those of the corresponding single assays.

CONCLUSIONS: The automated protein-array chip demonstrated similar analytical precision, improved analytical sensitivity, and comparable measured concentrations to those of single assays. The multiplex assay should be useful for assessing bone metabolism in large clinical studies, particularly when sample volume is limited.

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Previously published online at DOI: 10.1373/clinchem.2008.105866

Nonstandard abbreviations: OC, serum osteocalcin; bone ALP, bone alkaline phosphatase; PINP, type I N-terminal propeptide; PTH, parathyroid hormone; LLOD, lower limit of detection; LLOQ, lower limit of quantification.
tion of multiple analytes, potentially higher sensitivity, miniaturization of assay procedures, and reductions in sample and reagent volumes. To date, the most commonly arrayed proteins are antibodies (6), since they are robust molecules that can be easily handled and immobilized by standard procedures on flat surfaces such as glass slides (chips) or membranes. The most widely adopted strategy consists of a multiplex adaptation of the classical antibody sandwich assay, where a pair of antibodies binds 2 discrete recognition surfaces on each protein (7, 8).

Here, we applied protein array chip technology currently under development for routine diagnostics applications. The aim of this study was to evaluate the performance of this fully automated multiplex assay allowing simultaneous measurements of serum CTX-I, PINP, OC, and PTH in 20 μL of serum sample. We compared results to those generated with corresponding automated assays for single markers.

Materials and Methods

MULTIPLEX AUTOMATED ASSAY

Roche Professional Diagnostics (Roche Diagnostics GmbH) is developing a multimarker platform under the working name IMPACT (Immunological Multi-Parameter Chip Technology).

The technology is based on a small polystyrene chip manufactured by proprietary and tested procedures. Chip manufacturing employs streptavidin–biotin interactions. The chip surface is coated with a streptavidin layer, onto which the biotinylated antibodies are then spotted for every assay. For each marker, spots of antibodies are loaded in a vertical line onto the chip. During the assay, the array is probed with specimen samples containing the specific analytes. The serum volume required per specimen for measuring the 4 bone markers is 20 μL, and 20 μL of a second digoxigenylated monoclonal antibody is incubated at the same time to capture analytes. The second antibody is finally detected with 40 μL of an antibody antidigoxigenin coupled with fluorescent latex conjugate. Using this label, <10 individual binding events in a single spot can be detected, resulting in very high sensitivity down to the fmol/L concentration. Chips are transported into the detection unit, and a charge coupled device (CCD) camera generates an image that is transformed into signal intensities using dedicated software. Individual spots are automatically located at predefined positions and quantified by image analysis. For each marker, lines of 10–12 spots are loaded on the chips, and a minimum of 5 spots is required to determine the mean concentration of samples. The advantages of the technology are the ability of multiplexing up to 20 parameters in a sandwich or competitive format, low consumption of sample (4–20 μL), and high sensitivity. The throughput of the prototype is 41 patient determinations in duplicate per hour. One run is designed to contain a total of 100 single determinations, including 6 standards and 3 controls. Limitations relate mainly to difficulties in combining some specific parameters, especially when they are in very different concentrations in serum (g/L vs ng/L).

In this study, we evaluated the bone-panel chip comprising CTX-I, PINP, OC, and intact PTH. It uses the same monoclonal antibodies as the single automated Elecsys assays (see below). Calibration curves use 6 different concentrations of standards (excluding 0). The ranges of standards are 0.013–4.650 μg/L, 12.8–573.1 μg/L, 9.8–164.9 μg/L, and 14.9–1240.4 ng/L for CTX-I, PINP, OC, and PTH, respectively.

SINGLE AUTOMATED ASSAYS

We also individually measured serum CTX-I, PINP, OC, and PTH on the Elecsys 2010 analyzer (Roche Diagnostics GmbH) using the S-Crosslaps™, S-total PINP™, S-N-Mid Osteocalcin™, and S-Intact PTH™ reagents, respectively (9, 10). In these assays, the biotinylated antibody is incubated with serum. The second antibody labeled with a ruthenium complex is added together with streptavidin-coated microparticles. These microparticles are then magnetically captured onto the surface of an electrode. Application of the voltage on this electrode induces a chemiluminescent emission. The serum volume required for single determination is 20 μL for PINP and OC and 50 μL for CTX-I and PTH. Thus a minimal total volume of 140 μL is required for the 4 markers, with an additional dead volume of 150 μL. Calibration curves are performed using 2 standards with values of 0.05 and 2.00 μg/L for CTX-I, 25 and 850 ng/L for PINP, 0.5 and 280 ng/L for OC, and 0.05 and 2500 ng/L for PTH.

TESTS OF ANALYTICAL PRECISION, SENSITIVITY, AND LINEARITY

Three pools of serum were generated by mixing samples of 30 healthy postmenopausal women selected to obtain values of analytes in the low, medium, and high concentration range. These pools were used for all analytical performance tests.

SUBJECTS

We measured serum CTX-I, PINP, OC, and PTH using both the new multiplex automated system and the reference corresponding single automated assays in the following populations. All measurements were performed on fasting serum samples collected before 10 a.m. and stored at a temperature below −70 °C before analyses.
We investigated samples from 157 healthy premenopausal women [mean (SD) age 40 (4) years, from 30–46] and 74 men [age 38 (12) years, from 20–59] participating in a blood donor program in Lyon, France. All subjects were healthy without any disease or treatment that could interfere with bone metabolism, including hormone replacement therapy.

We investigated 56 postmenopausal women with osteoporosis [age of 65 (9) years; range 48–80 years]. These patients were randomly selected from a larger population of 360 patients who were participating in a 6-month clinical trial of the bisphosphonate ibandronate. Women were newly diagnosed with postmenopausal osteoporosis as determined by a bone mineral density (BMD) T score of $\leq -2.5$ or less at the hip by dual x-ray absorptiometry (DXA) and have never been exposed to bisphosphonate therapy. Patients were excluded if they had inability to stand or sit in the upright position for at least 60 min, inability to swallow a tablet whole, hypersensitivity to any component of ibandronate, administration of any investigational drug within 30 days preceding the first dose of study drug, been on hormone (estrogen) replacement therapy within the last 3 months, vitamin D deficiency (serum 25-hydroxy vitamin D $\leq 25$ nmol/L), renal impairment (serum creatinine $\geq 2.4$ mg/dL, equivalent to 216 µmol/L), or a history of major upper gastrointestinal disease.

After fasting overnight (6 h or more) all patients received monthly oral ibandronate (150 mg taken in the morning) in an upright position (sitting or standing) with a glass of plain water. All patients also took a combination of a dietary supplement containing vitamin D 200 IU and elemental calcium 500 mg, twice a day with meals, for the full duration of the study.

We collected fasting serum samples in all subjects before initiating therapy and after 1, 2, 3, and 6 months of treatment. At all time points, serum was obtained before drug intake.

The studies were approved by the local ethics committee, and written informed consent was obtained from each participant.

### Statistical Analysis
Comparison of concentrations measured by the multiplex and single assays were analyzed using linear regression analyses and the Bland-Altman plot (11). Differences in concentrations between pre- and postmenopausal women were assessed using the Mann-Whitney test. Changes of marker concentrations in women treated with ibandronate were expressed as median and 95% CI, and significance was assessed by Wilcoxon paired test. All analyses were performed using Statistical Analysis Software (SAS).

### Results
All biomarker tests were performed simultaneously using the multiplex bone panel chips, but results for each analyte were assessed separately. Analytical performance evaluation studies were also performed in parallel, using the same samples for both the chip and the single assays.

### Precision, Sensitivity, and Linearity
Studies of intraassay variation as assessed by 20 measurements of 3 serum samples with low, medium, and high concentrations gave CVs <5% for CTX-I, OC, and PTH and <7% for PINP (Table 1). CVs for the interassay variation determined using 5 different serum samples were <6% for all markers except PINP, for which the CVs were <10%. The lower limit of detection (LLOD), calculated as the concentration 3 SD higher than that of the lowest standard, was 0.002 µg/L, 0.26 µg/L, 0.51 µg/L, and 0.39 ng/L for serum CTX-I, PINP, OC, and PTH, respectively (Table 1). The lower limit of quantification (LLOQ), defined as the lowest
concentration in human serum that can be measured with an imprecision of <20%, was 0.023 μg/L, 5.25 μg/L, 1.76 μg/L, and 3.32 ng/L for CTX-I, PINP, OC, and PTH. As shown in Table 1, analytical intra- and interassay CVs obtained with the multiplex assay were similar to those observed with the single assay systems, although a slightly higher variability was observed in the multiplex assay for serum PINP. The LLOD and LLOQ were lower with the multiplex assay in comparison with the corresponding single assays for all markers except OC, for which the values were virtually identical. For CTX-I, the LLOD and LLOQ were respectively 5-fold and 3.8-fold lower with the multiplex than with the single assay.

Fig. 1. Comparison of multiplex and single automated assay for serum CTX-I (A), PINP (B), osteocalcin (C), and PTH (D) in 157 healthy premenopausal women, 56 untreated postmenopausal women, and 74 healthy men. For each marker, both the linear regression (left panel) and the Bland-Altman (right panel) graphs are shown. For the Bland-Altman plots, the graph shows the individual differences between values obtained with the multiplex assays and the corresponding single determination (y axis) in relation to the average values obtained with the 2 methods (x axis). The horizontal plain line represents the average difference between the 2 assays and the dotted lines the 95% CI.
Table 2. Mean concentrations and reference intervals of biochemical markers by multiplex assay by sex and age groups in healthy women and men.

<table>
<thead>
<tr>
<th>Sex and age group</th>
<th>n</th>
<th>CTX-I, µg/L</th>
<th>PINP, µg/L</th>
<th>OC, µg/L</th>
<th>PTH, ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.5th to 97.5th percentile</td>
<td>Mean (SD)</td>
<td>2.5th to 97.5th percentile</td>
<td>Mean (SD)</td>
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<tr>
<td>Premenopausal women</td>
<td></td>
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<tr>
<td>30–34 years</td>
<td>25</td>
<td>0.353 (0.216)</td>
<td>44.9 (27.4)</td>
<td>28.0 (14.4)</td>
<td>34.2 (10.0)</td>
</tr>
<tr>
<td>35–39 years</td>
<td>52</td>
<td>0.274 (0.126)</td>
<td>32.8 (12.0)</td>
<td>17.8 (8.1)</td>
<td>34.8 (14.9)</td>
</tr>
<tr>
<td>40–44 years</td>
<td>56</td>
<td>0.260 (0.106)</td>
<td>32.9 (11.8)</td>
<td>18.8 (7.2)</td>
<td>35.5 (14.2)</td>
</tr>
<tr>
<td>45–49 years</td>
<td>22</td>
<td>0.280 (0.108)</td>
<td>34.2 (10.1)</td>
<td>20.4 (7.8)</td>
<td>32.7 (10.9)</td>
</tr>
<tr>
<td>35–49 years</td>
<td>130</td>
<td>0.269 (0.114)</td>
<td>33.1 (11.5)</td>
<td>19.1 (7.6)</td>
<td>34.7 (13.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.105–0.589</td>
<td>17.9–60.4a</td>
<td>11.0–33.1a</td>
</tr>
<tr>
<td>All</td>
<td>155</td>
<td>0.282 (0.139)</td>
<td>35.0 (15.7)</td>
<td>20.5 (9.6)</td>
<td>34.7 (13.3)</td>
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<td></td>
<td></td>
<td></td>
<td>0.103–0.632a</td>
<td>15.7–67.2a</td>
<td>11.2–41.7a</td>
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<tr>
<td>Postmenopausal women</td>
<td></td>
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<tr>
<td>48–80 years</td>
<td>56</td>
<td>0.559 (0.276)</td>
<td>63.3 (40.3)</td>
<td>37.5 (19.0)</td>
<td>35.7 (15.5)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.154–1.140</td>
<td>20.2–162.0</td>
<td>8.4–70.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.116–1.249a</td>
<td>17.6–149.1a</td>
<td>8.8–63.3a</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
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<tr>
<td>20–29 years</td>
<td>20</td>
<td>0.466 (0.128)</td>
<td>87.2 (66.1)</td>
<td>33.4 (6.8)</td>
<td>24.1 (10.2)</td>
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<tr>
<td>30–39 years</td>
<td>21</td>
<td>0.278 (0.131)</td>
<td>61.8 (66.0)</td>
<td>26.2 (11.3)</td>
<td>32.5 (9.8)</td>
</tr>
<tr>
<td>40–49 years</td>
<td>19</td>
<td>0.237 (0.085)</td>
<td>49.0 (18.5)</td>
<td>20.2 (8.1)</td>
<td>33.1 (8.8)</td>
</tr>
<tr>
<td>50–59 years</td>
<td>14</td>
<td>0.229 (0.051)</td>
<td>44.8 (10.4)</td>
<td>19.7 (7.3)</td>
<td>36.8 (16.7)</td>
</tr>
<tr>
<td>40–59 years</td>
<td>33</td>
<td>0.234 (0.072)</td>
<td>47.2 (15.5)</td>
<td>27.9–79.6</td>
<td>11.3–36.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.093–0.338a</td>
<td>27.0–80.6a</td>
<td>11.2–30.6a</td>
</tr>
<tr>
<td>All</td>
<td>74</td>
<td>0.309 (0.342)</td>
<td>62.2 (30.6)</td>
<td>28.3–121.7</td>
<td>11.7–44.6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.145–6.565</td>
<td>25.4 (23.7)</td>
<td>10.2–57.2</td>
</tr>
</tbody>
</table>

Because there was no significant change in marker concentrations between age 35 and 49 in premenopausal women and between age 40 and 59 in men, values in these groups (bold) were used to establish reference intervals. a Reference intervals of the corresponding automated single assays for comparison.
Three pools of serum samples were diluted 1:2, 1:4, 1:8, and 1:10 in duplicate with normal saline solution (0.9% wt/vol) at pH 7.4. Dilution recoveries ranged from 98% to 122% (mean 106%) for CTX-I, 81% to 122% (mean 94%) for PINP, 112% to 130% (mean 121%) for OC, and 84% to 103% (mean 96%) for PTH.

CORRELATION BETWEEN MULTIPLEX AND SINGLE AUTOMATED ASSAYS
Concentrations of CTX-I, PINP, OC, and PTH were measured with both the multiplex bone panel chip and the corresponding single assays in 157 healthy premenopausal women, 56 untreated postmenopausal women, and 74 healthy men. As shown in Fig. 1, for all 4 biomarkers there was a very high correlation between the multiplex chip assay results and those for the single assays, with correlation coefficients ranging from 0.93 to 0.97 (P < 0.0001).

Fig. 1 also shows for each marker the Bland-Altman bias plot of the comparison between multiplex and single assays. Absolute concentrations measured by the multiplex assays were very similar to those observed with the corresponding single assays for serum CTX-I (average difference 0.037 µg/L), PINP (average difference 6.8 µg/L), and PTH (average difference 3.6 ng/L). In addition, for these 3 assays, the mean difference remained relatively constant across the whole range of values. For OC, on average, values obtained with the multiplex assay were 3.7 µg/L higher than with the single-assay measurements, and there was a trend of increased observed differences between the 2 methods with increasing average OC concentrations.

AGE-RELATED CHANGES OF BIOCHEMICAL MARKERS IN HEALTHY WOMEN AND MEN AND REFERENCE INTERVALS
In premenopausal women, there was a significant age-related decrease of serum PINP and OC (P < 0.05) but not CTX-I and PTH (Table 2). However, when the analysis was restricted to women age 35 years and older, values of both PINP and OC remained stable. Consequently, the reference interval defined as the 2.5th to 97.5th percentiles was based on concentrations in premenopausal women between the ages of 35 and 49 (Table 2). In postmenopausal women, no significant age-related changes were observed for any of the 4 biochemical markers. Serum CTX-I, PINP, and OC were significantly higher (by 108%, 91%, and 96%, respec-
respectively, \( P < 0.0001 \) for all 3 markers) in postmenopausal
women than in premenopausal controls age 35 and
older (Table 2 and Fig. 2). When concentrations were
measured by the corresponding single automated as-
says, data were virtually identical, with increases of
95% \( (P < 0.0001) \), 108% \( (P < 0.0001) \), and 63% \( (P <
0.0001) \), respectively, in postmenopausal women com-
pared to premenopausal women age 35–49 years (data
not shown). Serum PTH concentrations were similar
in postmenopausal and premenopausal women.

For healthy men, concentrations of serum CTX-I
were higher in individuals younger than 30 years and
then remained stable up to age 59. For the markers of
bone formation PINP and OC, concentrations de-
creased up to age 39 years and then remained stable.
PTH concentrations were slightly lower in men \( \leq 29 
\) years old and then remained stable up to age 59. Con-
sequently, the reference interval for men has been es-
tracted in men between the ages of 40 and 59 years
(Table 2).

**EFFECTS OF BISPHOSPHONATES ON BIOCHEMICAL MARKERS**

As shown in Fig. 3, monthly oral ibandronate induced
a rapid decrease of serum CTX-I, with a median fall of
\(-30\% \) (mean \(-28\% \)) at 1 month and reaching a max-
imum decrease of \(-48\% \) (mean \(-46\% \)) at 6 months.
The decrease for PINP and OC was delayed compared
with serum CTX-I. For all 3 markers, the magnitude
and pattern of changes were very similar when con-
centrations were measured by either the multiplex or
the single assay technology. As shown in Table 3, after
treatment with ibandronate no measured concentra-
tions were lower than LLOQ when CTX-I was assessed
by the multiplex assay (23 ng/L) at all time points,
but 28% were lower than the corresponding LLOQ
(87 ng/L) with the single-assay technology at 6 months.
No CTX-I values were lower than the LLOD with either
the multiplex or single assays (data not shown). After 6
months of ibandronate therapy, 18% and 4% of values
were lower than LLOQ for PINP and OC, respectively,
with the single assay but none with the multiplex ana-
lyzer (data not shown).

**Discussion**

In this study, we evaluated the performance character-
istics of a new fully automated multiplex assay that al-
lows the simultaneous determinations of 4 biochemical
markers of bone metabolism, including serum CTX-I,
PINP, and OC, which are currently among the most
Fig. 3. Median (95% CI) percentage change from baseline in biochemical markers of bone turnover over 6 months
in postmenopausal women with osteoporosis receiving oral monthly ibandronate 150 mg.

Only values higher than LLOQ were considered to plot the graphs. (A). CTX-I. (B). PINP. (C). OC.
sensitive biochemical markers of bone resorption and formation (1). We showed that the analytical precision achieved with this multiplex technology was similar to that observed with the reference single automated assays, and the sensitivity was better than the single assays. The magnitudes of change induced by menopause and bisphosphonate therapy were also similar to those seen with the reference single assays. Because of the very low sample volume required for the multiplex assay and the rapid turnaround time provided by this assay system, the new bone marker panel chip array may be particularly useful for analysis of a large number of samples in epidemiological and interventional studies of osteoporosis and other metabolic bone diseases.

The multiplex bone marker panel assay demonstrated intra- and interassay CVs <6% for all analytes except PINP, whose values were still acceptable (<10%). These precision figures are similar to those obtained with the corresponding single assays, which themselves have been shown to be more precise than conventional manual immunoassays (9, 10). The analytical and functional sensitivities were very high for all 4 parameters and compared favorably with those achieved using single reference assays. The improved analytical sensitivity of the multiplex assay is related to the unique property of the fluorescent dye incorporated in the latex particle combined with a very low background signal. The LLOQ was found to be low and lower than the concentration of the lowest standard for PINP, OC, and PTH. Thus, adding a standard with values close to the estimated LLOQ should be considered in the next generation of the assays to ensure accuracy of measurements in the low range. Improved LLOQ is of particular importance when assays are used to document effects of antiresorptive therapies in osteoporosis. Indeed, because of the profound effect of these drugs on bone turnover, values reached after a few months of treatment may be very low, reaching the sensitivity limits of the test. For serum CTX-I, we found that the LLOQ was 3.8-fold lower with the multiplex than with the single assays. This allowed the accurate determination of bone resorption in all patients receiving ibandronate, whereas when using the single assays a substantial number of patients had values below the limit. Thus this multiplex assay may be particularly useful to document effects of antiresorptive therapies including bisphosphonates and other potent antiresorptive therapies such as the anti–RANK-L antibody (12). The LLOQ was also lower for the other biochemical markers, including intact PTH, which may be useful when assessing patients with hypoparathyroidism, although additional studies in this population would have to be performed.

| Table 3. Mean (SD) concentrations of CTX-I and percentage of patients with values below the LLOQ before and after treatment with monthly oral ibandronate 150 mg in the multiplex and single automated assays. |
|---|---|---|---|---|---|
| | Baseline | Month 1 | Month 2 | Month 3 | Month 6 |
| Mean (SD) of values higher than LLOQ | 0.556 (0.283) | 0.556 (0.314) | 0.360 (0.258) | 0.398 (0.250) | 0.305 (0.214) |
| Median of values higher than LLOQ | 0.50 | 0.49 | 0.34 | 0.31 | 0.23 |
| Values lower than LLOQ, % | 0 | 0 | 0 | 16 | 0 |

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In healthy premenopausal women, untreated postmenopausal women, and men, we found that biochemical marker concentrations obtained by the multiplex assay were highly correlated with those for the single assays, a finding that was expected, as the 2 methodologies use the exact same antibodies although in a different assay format. The Bland-Altman bias plot showed that the measured concentrations were similar for both techniques, except for serum OC, suggesting that values obtained using both technologies could be compared. For OC, however, values generated with the multiplex assay were slightly higher, and the difference between the 2 assays seemed to increase with higher concentrations for reasons that were unclear. It is possible that the recognition of the various circulating immunoreactive OC forms—which include the intact 1–49 molecule and the large N-Mid collagen fragment (13)—whose relative proportion may vary with bone turnover rate—could be slightly different in the 2 assays. Further studies will be required to address this question.

Biochemical markers of bone turnover in osteoporosis have been suggested to be useful to assess fracture risk together with BMD and clinical risk factors, although currently one of the most promising roles is to assess efficacy of antiresorptive therapies. Indeed, markers of bone turnover decrease rapidly within a few months after initiation therapy, and the magnitudes of observed changes are associated with the reduction in risk of fracture (14, 15). In our study using the multiplex technology, we found that oral monthly ibandronate produced rapid and large decreases in serum CTX-I, with a magnitude similar to that recently reported using the same regimen in another population of women with osteoporosis (16). There was also a decrease in serum PINP and OC that was delayed compared to CTX-I, as expected owing to the coupling mechanism between bone resorption and bone formation. The times and magnitudes of changes in serum CTX-I, PINP, and OC were very similar with the multiplex assay technology and the corresponding single measurements.

The current bone panel chip comprises the 3 biochemical markers of bone turnover and intact PTH that are among the most useful in the assessment of postmenopausal osteoporosis. However, it may be interesting to expand this panel in the future with additional markers of bone metabolism. These may include 25-hydroxyvitamin D, which is the key parameter to document vitamin D insufficiency (17), and bone alkaline phosphatase, which has been suggested to be particularly useful in assessing bone turnover abnormalities in patients with renal failure (18). Serum CTX-I reflects the degradation of β-isomerized type I collagen which has limited sensitivity in patients presenting with Paget’s disease (19) or bone metastases (20). The addition of a marker reflecting the degradation of both β-isomerized and nonisomerized type I collagen, such as the N-terminal cross-linked telopeptide of type I collagen (NTX-I) or the nonisomerized αCTX-I, which appear to be very sensitive in these 2 conditions (19, 20), would also be of value.

In summary, we have evaluated the first automated multiplex assay which allows the simultaneous determination of 4 biochemical markers of bone metabolism in only 20 μL of serum. This assay system demonstrated analytical performance characteristics similar to those of the corresponding individual measurements, with increased analytical sensitivity in the low range for CTX-I, PINP, and OC. This new technology should be particularly useful for assessing bone turnover profile in clinical studies involving large numbers of patients and when sample volume is limited.

Grant/Funding Support: Roche Diagnostics provided the bone chip assay kits used in this study.

Financial Disclosures: P. Garnero, A. Claudon, P. Vergnaud, and C. Valverde are employees of Synarc. U. Klause and A. Mayr are employees of Roche Diagnostics.

Acknowledgments: We thank Hans-Jürgen Müller, Hubert Paul, and Hans Hornauer for expert technical assistance.

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