Background: Plasma concentrations of procollagen peptides are decreased in osteogenesis imperfecta (OI), whereas other bone formation markers may be increased. We examined the utility of combining these markers in the diagnosis of OI in adults.

Methods: We measured plasma concentrations of procollagen-1 N-peptide (P1NP), osteocalcin, and bone alkaline phosphatase in 24 patients with nondeforming OI, 25 patients with low bone mass due to other causes, and 38 age- and sex-matched controls. The discriminant ability of various test combinations was assessed by the construction of ROC curves.

Results: The median (range) ratio of osteocalcin to P1NP was significantly greater in patients with type I OI [1.75 (0.80–3.86)] than in controls [0.59 (0.34–0.90)] and patients with other causes of low bone mass [0.48 (0.05–1.38); \( P < 0.0001 \)]. This ratio allowed nearly complete differentiation between healthy controls and patients with type I OI, but not patients with type IV OI. With a cutoff of 0.97 for osteocalcin:P1NP, the sensitivity and specificity were maximized at 95% (95% CI 76%–100%) and 88% (69%–97%), respectively, for patients with other causes of low bone mass vs those with type I OI only. For patients with other causes of low bone mass vs all OI patients, sensitivity and specificity were 83% (63%–95%) and 88% (69%–97%), respectively. The addition of bone alkaline phosphatase data did not improve the discriminant ability of the osteocalcin:P1NP ratio.

Conclusions: The osteocalcin:P1NP ratio is a sensitive and specific test for type I OI in adults, but it has less utility in the diagnosis of other types of nondeforming OI.

Osteogenesis imperfecta (OI)³ is often considered a diagnostic possibility when severe osteoporosis presents relatively early in life (1). Mild forms of OI can be difficult to diagnose, however; radiological findings are nonspecific, in some cases sclerae may be of indeterminate hue, there may be no obvious dental abnormalities, and family history is unhelpful (2).

The most commonly encountered forms of OI result from mutations in the \( \text{COL1A1} \)⁴ or \( \text{COL1A2} \) genes, which encode the \( \alpha_1 \) and \( \alpha_2 \) chains of type 1 collagen. These mutations result in the production of reduced amounts of normal type 1 collagen and/or an abnormal type 1 collagen (3). During collagen synthesis, 1 \( \alpha_2 \) chain and 2 \( \alpha_1 \) chains assemble into a triple helix structure, after which the nonhelical propeptides at both the C-terminal and N-terminal ends of the trimer are cleaved. These propeptides—procollagen-1 C-terminal propeptide (P1CP) and procollagen-1 N-terminal propeptide (P1NP)—can be measured in plasma and are considered markers of bone formation (4).

There is no specific, noninvasive, and inexpensive laboratory test for OI; accurate diagnosis requires either sequencing of \( \text{COL1A1} \) and \( \text{COL1A2} \) or electrophoretic studies on collagen secreted by cultured skin fibroblasts (5). Several studies investigating bone turnover markers in OI have reported that, compared with normal individuals, patients with OI have relatively low plasma concentrations of P1CP (6–9), which is believed to reflect the defective synthesis of type 1 collagen in OI. Several studies have suggested that the plasma concentrations of other bone formation markers such as osteocalcin and
bone-specific alkaline phosphatase (bALP) may be increased in OI (7, 9, 10). These observations raise the question that we addressed in this prospective study: could the measurement of bone formation markers, used in combination, be of diagnostic value in determining whether OI is the cause of severe osteoporosis presenting in adulthood?

Materials and Methods

We measured plasma markers of bone formation and bone mineral density in the lumbar spine in 3 groups of adults:

(a) Twenty-four patients with OI (from 15 different families) consecutively referred to our Bone Clinic for evaluation. None had bone deformity and all were of normal stature (≥3rd centile for height). Twenty-one of the 24 had apparently normal teeth and sclerae of varying degrees of blue and thus had type I OI according to the Sillence classification (2). Three patients (2 with obvious dentinogenesis imperfecta) had normal-colored sclerae (type IV). In 18 cases, there was a clear family history of dominant inheritance of the bone disease, but in 6 cases, there was no other affected family member. Six of the 24 patients were being treated with bisphosphonates (oral alendronate of 1 year duration) at the time blood samples were drawn; in all the other cases, the samples were taken before any specific antosteoporotic medication was prescribed. The bone density measures reported here were all made before any treatment was prescribed.

(b) Twenty-five patients (from 22 different families) with low bone mass due to causes other than OI, referred to our Bone Clinic for evaluation. The etiology of low bone mass in these patients was attributed to the following: idiopathic osteoporosis (n = 6); familial osteoporosis (n = 4); glucocorticoid use (n = 3); loss-of-function mutations in the LRP5 (low-density lipoprotein receptor–related protein 5) gene (homozygous, n = 2; heterozygous, n = 1); postmenopausal osteoporosis (n = 2); juvenile Paget disease (n = 2); postpregnancy osteoporosis (n = 2); and anorexia, hypophosphatasia, and depot medroxyprogesterone use (n = 1 each). Three of the 25 patients were being treated with bisphosphonates at the time the blood samples were drawn; in all other cases, the samples were taken before any specific antosteoporotic medication was prescribed. The bone density measures reported here were all made before any treatment was prescribed.

(c) Thirty-eight normal individuals, selected without knowledge of their results for bone formation markers or bone density, to provide an age- and sex-matched comparator group. These individuals were recruited as controls for other studies in our unit, and their baseline data were used in this study.

In 6 patients from groups a and b, because of uncertainty about the diagnosis, we undertook in vitro studies of type 1 collagen on fibroblasts grown from skin. We also undertook these studies on patients with type IV OI and dentinogenesis imperfecta. In brief, we cultured skin fibroblasts and assessed the synthesis and secretion of type 1 procollagen relative to type 3 procollagen by protein gel electrophoresis. In type 1 OI, normal procollagen is synthesized and secreted, but in reduced quantity. In other types of OI, 2 populations of type 1 procollagen are found, 1 normal and the other with abnormal electrophoretic mobility (11).

Biochemical Markers

We measured the plasma concentrations of 3 bone formation markers. In midafternoon, we drew blood samples into lithium-heparin bottles. We did not take account of the stage of menstrual cycle or contraceptive use in participating young women. The plasma was separated and stored at −70 °C until analysis. The tests were carried out in fully accredited hospital laboratories, and the analysts were masked from clinical information about the participants.

We measured bALP by automated sandwich-type immunoassay (Ostase) on a Beckman Access analyzer (Beckman Coulter). Interbatch imprecision (CV) was 8.4% at 11.3 µg/L and 5.2% at 80.1 µg/L. The reference interval quoted by the manufacturer is 4–22 µg/L for men and premenopausal women. We measured osteocalcin by sandwich immunoassay with electrochemiluminescence detection on a Roche E170 analyzer (Roche Diagnostics). This assay measures both the intact (1–49) osteocalcin molecule and the N-Mid (1–43) fragment. Interbatch imprecision was 1.6% at 7 µg/L and 1.5% at 78 µg/L. The normal interval quoted by the manufacturer is 11–46 µg/L for both men and premenopausal women. We measured P1NP by immunoassay on a Roche E170 analyzer. Interbatch imprecision was 6.7% at 79 µg/L and 2.9% at 795 µg/L. The normal interval quoted by the manufacturer is 20–80 µg/L for men and premenopausal women.

We measured bone density by dual-energy x-ray absorptiometry (Lunar) and expressed the results as a standard deviation score relative to age- and sex-matched normal individuals (z score).

Statistics

We compared median values for the various biochemical markers and the ratios between them by the Kruskal–Wallis test (for 3-way comparisons) and the Mann–Whitney U-test (for 2-way comparisons), as the values were not gaussian distributed. We calculated the specificity and sensitivity of the various markers and their ratios for the diagnosis of OI for various cutoff points and plotted them as ROC curves (12) calculated using Prism 4.03 for Windows software (GraphPad Software). We calculated the area under the curve (AUC) by a trapezoidal method (with 95% CIs) and compared it against the area that would be expected by chance (0.5). We compared the
AUCs for various models by use of a nonparametric approach (13).

**Results**

**Bone formation markers**

The patient groups were recruited over the 4-year period of 2003–2006. The 3 groups were similar in mean age, age range, and sex distribution. Summary data on the 3 bone formation markers and the bone density measurements are given in Table 1. In both the OI and other low bone mass groups, there was considerable variability in the results of individual markers compared with the relatively narrow range in controls. The median value for P1NP was significantly lower in the OI group than in normal individuals and the group with other causes of low bone mass \((P < 0.01)\). The median value for osteocalcin was significantly higher in the OI patients than in controls \((P < 0.05)\). Median values for bALP were significantly higher in both the OI and other low bone mass groups than in controls \((P < 0.005); Table 1\).

We then compared the ratios of osteocalcin to P1NP and bALP to P1NP in the 3 groups. There was a clear difference among the groups \((P < 0.0001, \text{Kruskal–Wallis test})\), with a much clearer distinction between the OI patients and either of the other 2 groups. The ratio of osteocalcin to P1NP was significantly greater in patients with type I OI (median, 1.75; range, 0.80–3.86) than in controls (median, 0.95; range, 0.34–0.90) and patients with other causes of low bone mass (median, 0.48; range, 0.05–1.38). The median values were significantly greater for the type I OI patients than both the other groups \((P < 0.0001)\). However, the results from the 3 patients with type IV OI lay below the range of the type I OI patients (0.21, 0.31, and 0.67, respectively; Fig. 1). A similar pattern was seen when the ratios bALP:P1NP and (osteocalcin + bALP):P1NP were compared between the groups (Fig. 2 and see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol53/issue6).

**ROC curves**

The ROC curves for the individual bone formation markers and the various ratios are summarized in Table 2. The

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**Table 1. Bone densitometry and biochemical findings.**

<table>
<thead>
<tr>
<th></th>
<th>OI</th>
<th>Other causes of low bone mass</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>24</td>
<td>25</td>
<td>38</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>11/13</td>
<td>7/18</td>
<td>13/25</td>
</tr>
<tr>
<td>Age, years</td>
<td>37 (15)</td>
<td>37 (14)</td>
<td>38 (11)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>38 (19–64)</td>
<td>35 (17–76)</td>
<td>36 (21–61)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>37 (19–64)</td>
<td>35 (17–76)</td>
<td>36 (21–61)</td>
</tr>
<tr>
<td>Lumbar spine BMD, z score, mean (SD)</td>
<td>-2.5 (1.3)</td>
<td>-3.1 (0.6)</td>
<td>0.5 (1.3)</td>
</tr>
<tr>
<td>P1NP, μg/L</td>
<td>44.1 (64.5)</td>
<td>181.8 (419.7)</td>
<td>41.2 (13.3)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>23.7 (2.9–290.1)</td>
<td>39.3 (4.5–1972)</td>
<td>38.9 (18.5–82.7)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>39.8 (31.9)</td>
<td>40.3 (45.4)</td>
<td>22.9 (7.2)</td>
</tr>
<tr>
<td>Osteocalcin, μg/L</td>
<td>35.2 (6.7–141.9)</td>
<td>23.5 (6.2–178.2)</td>
<td>22.4 (9.2–39.5)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>23.0 (22.2)</td>
<td>29.8 (56.2)</td>
<td>9.2 (3.1)</td>
</tr>
<tr>
<td>bALP, μg/L</td>
<td>16.0 (6.0–102.8)</td>
<td>13.5 (5.5–273.0)</td>
<td>8.5 (4.4–20.9)</td>
</tr>
</tbody>
</table>

* Statistically significant difference compared with controls: \(P < 0.0001\) (Student \(t\)-test).

* Statistically significant difference compared with controls: \(P < 0.01\).

* Statistically significant difference between OI patients and patients with low bone mass due to other causes: \(P < 0.01\) (Mann–Whitney \(U\)-test).

* Statistically significant difference compared with controls: \(P < 0.05\).

* Statistically significant difference compared with controls: \(P < 0.005\) (Mann–Whitney \(U\)-test).
ROC curves illustrating the ability of P1NP and the osteocalcin:P1NP, bALP:P1NP, and (osteocalcin + bALP):P1NP ratios to distinguish OI from other causes of low bone mass are shown in Fig. 3. Fig. 2 in the online Data Supplement illustrates the ability of P1NP and the osteocalcin:P1NP, bALP:P1NP, and (osteocalcin + bALP):P1NP ratios to distinguish OI from normal individuals, as well as those with other causes of low bone mass. Although the AUCs for P1NP and bALP alone were significantly different from the curve that would be produced by chance, the osteocalcin:P1NP, bALP:P1NP, and (osteocalcin + bALP):P1NP ratios all yielded CIs that span 0.8 to 1.0; values >0.8 are usually taken to indicate good diagnostic utility (14). The AUCs for the osteocalcin:P1NP, bALP:P1NP, and (osteocalcin + bALP):P1NP ratios were all significantly better than that for P1NP alone in distinguishing type I OI (P < 0.05).

The AUC values for distinguishing patients with OI from those with other causes of low bone mass were the greatest for the osteocalcin:P1NP ratio. With a cutoff of 0.75 for osteocalcin:P1NP, the sensitivity and specificity were maximized at 100% (95% CI 84%–100%) and 97% (86%–100%), respectively, for controls vs type I OI only; for controls vs all OI patients, the sensitivity and specificity were 91% (72%–99%) and 97% (86%–100%), respectively. With a cutoff of 0.97 for osteocalcin:P1NP, the sensitivity and specificity were maximized at 95% (76%–100%) and 88% (6%–97%), respectively, for patients with other causes of low bone mass vs type I OI only; and vs all OI patients, 83% (63%–95%) and 88% (69%–97%), respectively.

Five patients with type I OI were taking bisphosphonates when the biochemical tests were done. The mean (SD) osteocalcin:P1NP ratio in this group [1.94 (0.67)] was not significantly different (P = 0.48, Student’s t-test) from that in the 16 type I OI patients not taking bisphosphonates [1.77 (0.73)].

Six individuals had skin collagen studies for diagnostic purposes. In 4, normal type 1 collagen was produced in normal quantities by cultured fibroblasts; these individuals were included in the “low bone mass due to other causes” group. In these patients, the osteocalcin:P1NP ratio were 0.40–0.84, with a mean value of 0.58 (results in controls were 0.34–0.90; mean, 0.57). The collagen studies indicated OI type IV in 1 case and type I in the other. The latter patient had no family history of OI, only slightly blue sclerae, and delayed puberty. He sustained his first fractures after considerable trauma at the age of 14. When assessed at the age of 19, his osteocalcin:P1NP ratio was 0.80 (results in the other type I OI patients were 1.00–3.86; mean, 1.86).

### Table 2. ROC curve analyses for individual bone markers and their ratios in the diagnosis of OI.

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Control vs Type I OI</th>
<th>All OI</th>
<th>Other causes of low bone mass vs All OI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1NP, µg/L</td>
<td>0.78 (0.63–0.93)</td>
<td>0.71 (0.55–0.87)</td>
<td>0.75 (0.60–0.89)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0005</td>
<td>&lt;0.01</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Osteocalcin, µg/L</td>
<td>0.66 (0.48–0.84)</td>
<td>0.66 (0.49–0.83)</td>
<td>0.56 (0.38–0.52)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>0.52</td>
</tr>
<tr>
<td>bALP, µg/L</td>
<td>0.84 (0.72–0.96)</td>
<td>0.83 (0.71–0.95)</td>
<td>0.76 (0.62–0.90)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>bALP:P1NP</td>
<td>0.99 (0.99–1.00)</td>
<td>0.98 (0.95–1.00)</td>
<td>0.91 (0.82–1.00)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Osteocalcin:P1NP</td>
<td>0.99 (0.99–1.00)</td>
<td>0.92 (0.80–1.00)</td>
<td>0.97 (0.93–1.00)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(Osteocalcin + bALP):P1NP</td>
<td>1.00 (1.00–1.00)</td>
<td>0.91 (0.80–1.00)</td>
<td>0.96 (0.93–1.00)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Data are AUC (95% CI) and statistical significance for ROC curve analyses of patients with OI compared with controls and patients with other causes of low bone mass.
Discussion

Although there was considerable overlap in the concentrations of the 3 plasma bone formation markers in the 3 diagnostic groups, the osteocalcin:P1NP and bALP:P1NP ratios both provided excellent differentiation of type I OI patients from normal individuals. These ratios did not discriminate so well patients with OI from a heterogeneous group of patients with other causes of low bone mass, but they still performed well, with areas under the ROC curve ranging from 0.88 to 0.97. Incorporating both osteocalcin and bALP data in the ratio [(osteocalcin + bALP):P1NP] did not add to the discriminant value, and of course added the imprecision of having 3 analytes rather than 2.

In the patients with nondeforming type IV OI, we observed the opposite pattern to that seen in type I OI—their P1NP concentrations were relatively high in relation to osteocalcin. The likely explanation is that in type I OI the production of normal type 1 collagen is approximately halved (hence the low P1NP and P1CP) but no abnormal collagen is produced. In contrast, in type IV OI abnormal collagen is produced (often associated with obvious dentinogenesis imperfecta) but the total quantity (normal + abnormal) is not necessarily decreased (3, 11). Our findings are consistent with those of both Lund et al. (6) and Brenner et al. (7), who found that plasma P1CP was proportionately lower in type I OI than in types III or IV. If our findings of differing ratios in type IV OI are confirmed in larger numbers of patients, then the diagnostic utility of the osteocalcin:P1NP ratio in differentiating type IV OI from other causes of low bone mass is going to be limited, but the ratio could be of value in distinguishing type I from type IV OI. Nor is the osteocalcin:P1NP ratio likely to be useful in the diagnosis of other rare forms of OI not associated with abnormalities of type 1 collagen (15); however, type I OI is significantly more prevalent than all the other types of OI combined (1, 2). We have not included in this report data on adult patients with deforming forms of OI (type III and the more severe forms of type IV), because these rarely pose a diagnostic dilemma.

We have not included data on children. It is quite probable that during growth the ratios between the various bone formation markers will differ at various ages and between children and adults (16). The limited information we have suggests that, compared with adults, osteocalcin:P1NP ratios are substantially lower in children with type I OI [that is, the procollagen peptide is increased relative to osteocalcin (data not shown)]. This may explain why the osteocalcin/PICP ratio did not clearly distinguish the 19-year-old with late-presenting type I OI, who was not at skeletal maturity when assessed for this study. The possibility that the osteocalcin:P1NP ratio could be of value in diagnosing OI in childhood is clearly worthy of further investigation, because OI usually presents in childhood and can be mistaken for non-accidental injury (17). Patients with osteoporosis of any cause are often prescribed bisphosphonates or other antiresorptive agents that increase bone density predominantly by lowering bone turnover. In patients with type I OI, we found no difference in the mean osteocalcin/PICP ratio between the small number taking bisphosphonates and those not taking bisphosphonates. If this observation is confirmed, then the clinical utility of the test would be enhanced, because it would not be necessary to stop antiresorptive therapy before testing.

Most of the earlier studies of procollagen peptides have focused on the peptide derived from the C-terminal end (PICP) rather than P1NP (6–9), and there have been relatively few data published concerning P1NP in OI. Lund et al. (6) reported that in adults with OI, plasma P1NP concentrations were low in children but normal in adults. We chose to study P1NP because it has proven to
be a robust and sensitive marker of bone formation (18). Our results, that plasma concentrations of P1NP are low in adults with type I OI, contrast with the findings of Lund et al. (6) but are more in keeping with what we understand of the abnormality in collagen metabolism in this disorder. It is not clear why osteocalcin and bALP concentrations are relatively increased in OI, but osteoblast numbers are increased in OI bone (19), and given the putative role of osteocalcin and bALP in mineralization it is interesting to note that in patients with OI the bone tends to be hypermineralized (20), and in diaphyseal bone, volumetric bone density may be increased (21). Osteocalcin is incorporated into bone matrix and released during resorption, so the increased plasma concentrations may reflect increased bone turnover in OI.

Bone markers are generally thought to have little use in the specific diagnosis of particular metabolic bone diseases, because resorption and formation markers tend to change in parallel. There are a few rare circumstances (such as Ehlers–Danlos syndrome type VIA) in which a distinctive pattern of bone markers is of diagnostic value (22). The accurate diagnosis of OI requires either sequencing of COL1A1 and COL1A2 or electrophoretic studies on collagen secreted by cultured skin fibroblasts, but these tests are not always readily available. Our data suggest that the ratio of the plasma concentrations of osteocalcin or bALP to P1NP could be helpful in the diagnosis of OI when definitive tests on type I collagen are not possible. As with all preliminary case-control studies, the findings in this report need to be confirmed in other studies in independent patient populations.

References