Feasibility of simultaneous measurement of bone formation and bone resorption markers to assess bone turnover rate in postmenopausal women: An EPOLOS study

Jacek Łukaszkiewicz, Elżbieta Karczmarewicz, Paweł Płudowski, Maciej Jaworski, Edward Czerwiński, Andrzej Lewiński, Ewa Marcinowska-Suchowierska, Andrzej Milewicz, Marek Spaczyński, Roman S. Lorenc and the EPOLOS Group

Summary

Background: One of the most important risk factors for osteoporotic fractures in postmenopausal women is elevated bone turnover (EBT), occurring in 25–30% of this population. This study's aim was to find a correlation between bone resorption and bone formation markers to assess bone turnover rate and qualify an individual postmenopausal woman as a possible EBT subject.

Material/Methods: Three hundred twenty postmenopausal women (≥ one year after the last menstruation, ≤ 70 years old) were enrolled at seven clinical sites in this cross-sectional observational study conducted within the EPOLOS. The group was a random sample of the population. The study was performed in a referral center involved in the diagnosis and treatment of osteoporosis. The exclusion criteria included pregnancy, cancer, fracture in the last year, and overweight (> 100 kg). Bone mineral density (BMD) measurements of the lumbar spine, total hip, trochanter, and femoral neck regions were performed. Bone resorption and formation rates were evaluated by serum levels of C-terminal telopeptide of type I collagen (CTX) and osteocalcin (OC), respectively.

Results: Using logistic regression to correlate the concentrations of CTX and OC it was possible not only to distinguish the EBT subgroup, but also to construct a simple nomogram for easy classification of individual postmenopausal women as a possible EBT subject. EBT patients showed generally decreased BMD values and increased bone formation and resorption rates.

Conclusions: Evaluation of both CTX and OC levels enables a more proper indication for EBT. The proposed nomogram may assist in evaluating outcome from the two markers of bone turnover.

key words: biochemical markers of bone turnover • elevated bone turnover • C-terminal cross-linked peptide • osteocalcin • osteoporosis • menopause • population-based • cross-sectional

Full-text PDF: http://www.medscimonit.com/fulltxt.php?ICID=869464

Word count: 2740

Tables: 3

Figures: 2

References: 41

Author’s address: Roman S. Lorenc, Children’s Memorial Health Centre Institute, Biochemistry Dpt., Aleja Dzieci Polskich 20, 04-736, Warsaw, Poland
**Background**

The definition of osteoporosis given by the National Institutes of Health conference in 2000 [1] specifies this condition as “a skeletal disorder characterized by compromised bone strength predisposing a person to an increased fracture risk.” Bone strength is determined by bone mineral density (BMD) and bone quality. BMD is easy to define and to measure with the proper equipment. Bone quality, in contrast, is a more elusive trait which depends mainly on microarchitecture and bone turnover [2, 3]. At present, the only noninvasive method to assess bone quality is to measure the concentration of specific compounds (markers) reflecting the intensity of bone turnover. It is already well known that elevated bone marker levels predict osteoporotic fractures [4], especially when combined with low BMD [4, 5], and that they correlate with the rate of bone loss [5–17]. Using bone turnover markers to specify women for anti-catabolic agents treatment improves cost-effectiveness and has already been approved for the monitoring of alendronate therapy. It is also known that bisphosphonate treatment is most effective in women with elevated bone turnover [18, 19]. However, even modern automated marker assays produce results that seem to be more feasible for population research than for a convincing evaluation of fracture risk in an individual patient [20, 21].

Simultaneous assessment of two markers (resorption and formation) may, at least in theory, improve the reliability of information about bone turnover intensity in each individual. We have already shown that by using two markers it is possible to distinguish a subpopulation characterized by elevated bone turnover among postmenopausal women with the aid of cluster analysis [22]. In the present study, a markedly increased number of subjects enabled us to obtain an even more convenient tool for the detection of individuals with elevated bone turnover (EBT) within the postmenopausal population. Therefore the aim of this study was to confirm our previous finding that cluster analysis can be used to separate postmenopausal women with EBT from the rest of the population. Taking advantage of the sufficient number of subjects, a model presenting a new concept for solving the problem of individual EBT detection was constructed.

**Material and Methods**

**Subjects**

The EPOLOS (European Polish Osteoporosis Study) is a multi-site population-based study of osteoporosis and its determinants in Poland. The study was supported by the Ministry of Health and the Committee of Scientific Research. Participation proposals together with an explanation of the study’s aim were sent to a random sampling of men and women aged 20–80 years whose addresses were obtained from the registry of the Ministry of Home Affairs and Administration, Department of the National Registry. Informed written consent was obtained from each participant. The participants were enrolled at seven clinical sites in the geographical areas of Warszawa, Poznań, Wrocław, Kraków, Łódź, and Bydgoszcz. The general response rate for the EPOLOS was 18%. The exclusion criteria for the EPOLOS included pregnancy, cancer, fracture in the last year, and overweight (>100 kg). The study was approved by the Ethics Committee of the Children’s Memorial Health Institute, Warsaw, Poland. Women were classified as postmenopausal at least one year after the last menstruation. Measurements of bone mineral density (BMD, g/cm²) and the levels of bone turnover markers of resorption (CTX, collagen type I cross-linked N-telopeptide) and formation (OC, osteocalcin, a bone GLA protein) were performed in 320 postmenopausal women (at least one year after the last menstruation and not older than 70 years). Body weight and height in the studied group were measured at the time of BMD assessment. Table 1 shows the baseline characteristics of the studied population.

**BMD measurements**

BMD was measured with a pencil-beam dual-energy X-ray absorptiometry (Lunar DPD-X, GE Lunar, USA). Daily calibration and quality control procedures were performed regularly according to the manufacturer’s recommendations. Hologic Anthropomorphic Spine Phantom as well as the European Spine Phantom measurements were performed in each of the seven study centers for the standardization of all BMD measurements. Bone mineral density at the anteroposterior lumbar spine (BMD-L2L4) and right hip were measured and analyzed in all subjects. At the hip area, three regions of interest were evaluated: the femoral neck (BMD-NECK), trochanter (BMD-TRCH), and total hip (BMD-TOTL). The coefficients of variation (CV) in assessing the precision of DXA measures of the lumbar spine and femoral neck were 1.2–1.5% and 1.6–1.8%, respectively, for all the study centers.

**Biochemical measurements**

Fasting serum samples were collected between 8 and 10 a.m. and stored at −80°C until assayed. β-CrossLaps (CTX) and N-MID-osteocalcin (OC) levels were measured with reagents which included calibrations and control sera (Roche Diagnostic, Mannheim, Germany) using the electrochemiluminescence immunoassay (ECLIA) in an Elecsys 2010 automated analyzer. The Elecsys CTX serum/EDTA plasma assay is specific to isomerized type I collagen fragments. The assay specificity is guaranteed through the use of two monoclonal antibodies each recognizing linear β-8 amino acid

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>59.3</td>
<td>6.3</td>
</tr>
<tr>
<td>BMI</td>
<td>27.8</td>
<td>4.5</td>
</tr>
<tr>
<td>OC (ng/ml)</td>
<td>27.8</td>
<td>12.2</td>
</tr>
<tr>
<td>CTX (ng/ml)</td>
<td>0.43</td>
<td>0.22</td>
</tr>
<tr>
<td>BMD-NECK (g/cm²)</td>
<td>0.886</td>
<td>0.131</td>
</tr>
<tr>
<td>BMD-TRCH (g/cm²)</td>
<td>0.777</td>
<td>0.137</td>
</tr>
<tr>
<td>BMD-TOTL (g/cm²)</td>
<td>0.950</td>
<td>0.145</td>
</tr>
<tr>
<td>BMD-L2L4 (g/cm²)</td>
<td>1.046</td>
<td>0.184</td>
</tr>
</tbody>
</table>

(n=320)
(AA) octapeptides (EKAHD-\(b\)-GGR). This CTX assay therefore quantifies all type I collagen degradation fragments that contain the isomerized octapeptide \(b\)-8AA twice (\(b\)-CTX). The intra- and inter-assay precision (CVs) was 2.6% (n=10) and 4.1% (n=10), respectively. N-MID-osteocalcin (OC) was measured using a two-site immunoluminometric technique. One monoclonal antibody (Mab) reacts with a mid-sequence epitope and the other with an N-terminal epitope (AA10–17 and 21–29); thus both intact osteocalcin and the most important cleavage product, N-MID-osteocalcin, can be detected. The assay is independent of the labile C-terminal part of the molecule (AA 43–49). Both the inter- and intra-assay variations were <7%.

**Study design**

The study population (n=320) was randomly divided into two equal groups (A and B) with the aid of the STATISTICA random number generator. Then cluster analysis (K-means) with OC and CTX concentrations as “dimensions” was applied to obtain two subgroups within group A: 1) those with EBT and 2) the rest of group A. Then, using logistic regression, a model equation with OC and CTX concentrations was formed to obtain a single parameter, \(P\) (probability). This parameter enables classification of a particular subject to one of the turnover modes (EBT or the rest). The model obtained for group A was then transposed to find the P values for each participant in group B. Women with a P value exceeding 0.5 were qualified to the EBT subgroup. Individuals with a probability greater than 50% (\(P>0.5\), n=68) were considered to be EBT (elevated bone turnover) and the rest of group A patients (cluster 2) was found with respect to age and body mass index (BMI). On the other hand, the EBT individuals had significantly decreased BMD values (Table 2). Therefore, logistic regression was applied to obtain a mathematical model based on OC and CTX concentrations able to distinguish the EBT subgroup. The logistic regression equation obtained for group A was:

\[ P = \frac{\exp(-29.7782 + 0.4643 \times OC + 31.1743 \times CTX)}{1 + \exp(-29.7782 + 0.4643 \times OC + 31.1743 \times CTX)} \]

where \(OC\) is the osteocalcin concentration (ng/ml), \(CTX\) the CTX concentration (ng/ml), and \(P\) the probability value.

**RESULTS**

The baseline characteristics of the study population (n=320) are provided in Table 1. To verify the group A division made by cluster analysis, the crucial parameters were compared within the EBT (elevated bone turnover) and the rest of group A (Table 2). No significant difference between the EBT (cluster 1) and the rest of the group A patients (cluster 2) was found with respect to age and body mass index (BMI). The EBTs and the rest of the group did not differ with respect to age and BMI (Table 3). Although the division into subgroups was achieved by different means for groups A and B, the proportions of EBT subjects in each group did not differ significantly (\(p=0.2518\), Fisher’s exact test).

To build a nomogram allowing a rapid classification of an individual patient as EBT or “low bone turnover” case, a 0.5 probability curve was drawn on the scatterplot of the OC and CTX values for group B. The 0.9 and 0.99 probability lines were also drawn. To draw the 0.5, 0.9, and 0.99 lines, the logistic regression model obtained for group A was used. The final nomogram is shown in Figure 1.

**DISCUSSION**

Osteoporosis-related fractures, especially in postmenopausal women, pose a serious problem not only for the patient, but...
Results of prospective studies suggest that the levels of bone turnover markers (both in serum and urine) are in positive correlation with the incidence of fractures and that women with elevated bone turnover show increased fracture risk [6–11, 24–26]. Therefore, evaluation of bone turnover markers appeared to be an important tool in terms of screening for postmenopausal women with elevated bone turnover and increased risk for fracture [27]. At present, markers of bone turnover may provide information concerning the bone loss rate of a given subject and predict it in the future [28–30]. It has been shown that the velocity of bone loss in postmenopausal women does not follow a uniform distribution pattern. Women with a bone loss rate exceeding 3% per year, the so-called “fast bone losers” (FBL), constitute approximately 1/4 to 1/3 of the population [31–34]. Therefore, FBL women can lose a significant percentage of their bone mineral over two years, which in turn is a typical time span for consecutive BMD measurements using the DXA method.

Bone turnover markers are a potential tool for the early identification of postmenopausal women who could benefit from antiresorptive treatment, leading to a marked decrease in fracture risk [24]. Nevertheless, it has still not been elucidated which markers should be used for this clinically important purpose and to what extent [24]. It is known that the inherent variability in marker assays still renders their use difficult [35, 36].

Significant progress in this area was made by the introduction of automated bone turnover marker assay systems, such as ELECSYS (Roche), which solved some of the variability problems. Application of this analytical system for the assessment of CTX concentrations in a large population of postmenopausal women resulted in obtaining a certain predictive power. Nevertheless, these results are insufficient to implement this new method in individual subjects [19]. However, even though the sensitivity and specificity of a single-marker assay is not sufficient, the application of two markers, reflecting both formation and resorption rates, would very likely bring a more accurate outcome than a single measurement [38].

We have already shown that the evaluation of two marker concentrations, namely urinary type I collagen N-telopeptide (NTx, resorption) and serum bone alkaline phosphatase (b-ALP, formation), enabled a proper separation of the subgroup with elevated bone turnover and significantly re-

Figure 1. Scatterplot of the patients in group A (n=160) with OC and CTX levels marked on the X and Y axes, respectively. Filled circles are patients belonging to the elevated bone turnover (EBT) subgroup as determined by cluster analysis. The division line was obtained by logistic regression according to the model described in the Results section. The line corresponds to the probability P=0.5 (50%).

Figure 2. Scatterplot of the patients of group B (n=160) with OC and CTX levels marked on the X and Y axes, respectively. The superimposed 0.5 (50%), 0.9 (90%), and 0.99 (99%) probability lines are derived from the logistic regression model “trained” on group A. Broken lines represent the upper limits (above the 95th percentile) established for the premenopausal women for CTX (0.49 ng/ml) and OC (34.0 ng/ml).

Table 3. Characteristics of the group “B” divided by logistic regression model established for group “A” (Results). 1 – with elevated bone turnover, 2 – rest of the group.

<table>
<thead>
<tr>
<th></th>
<th>Subgroup 2 (n=92)</th>
<th>Standard deviation</th>
<th>Subgroup 1 (n=68)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>59.2</td>
<td>7.1</td>
<td>60.1</td>
<td>5.7</td>
</tr>
<tr>
<td>BMI</td>
<td>28.0</td>
<td>4.2</td>
<td>26.8</td>
<td>4.5</td>
</tr>
<tr>
<td>BMD-NECK (g/cm²)</td>
<td>0.9018</td>
<td>0.127</td>
<td>0.856</td>
<td>0.142</td>
</tr>
<tr>
<td>BMD-TRCH (g/cm²)</td>
<td>0.806</td>
<td>0.136</td>
<td>0.742</td>
<td>0.142</td>
</tr>
<tr>
<td>BMD-TOTL (g/cm²)</td>
<td>0.979</td>
<td>0.141</td>
<td>0.909</td>
<td>0.157</td>
</tr>
<tr>
<td>BMD-L2L4 (g/cm²)</td>
<td>1.072</td>
<td>0.186</td>
<td>1.001</td>
<td>0.187</td>
</tr>
</tbody>
</table>

Mean values of each parameter were compared by t – test for each cluster. Significance value for p was 0.05.
duced bone mineral density among postmenopausal women [22]. In the present study, a different pair of markers: serum CTX and OC, were used, which was a consequence of the adopted assay technology, i.e. the ELECSYS (Roche) automated analyzer. In the current study, postmenopausal women were characterized as those who had had their last menstruation more than one year before the study [38] and were no older than 70 years of age. These features seem to be the optimum upper threshold value for the possible application of bone turnover markers as indicators for the antiresorptive treatment [24,40,41]. As in our previous paper, cluster analysis was used to separate the EBT subgroup from the rest of the subjects. The correctness of this division was evidenced by comparison of their crucial BMD values (Table 2), which were significantly reduced in the subjects with elevated bone turnover. The values of possible confounders, such as age and BMI, were not significantly different in both groups (Table 2). Furthermore, a markedly increased number of the studied postmenopausal women allowed us to apply the Boyd idea [42]. According to Boyd, utilizing logistic regression “enables integration of information from several tests (CTX and BGP in our case) in a single function that produces probability estimates of outcome” [42]. With Boyd’s ideas in mind, we used the pattern of a two-dimensional contour plot of the function given in the Results section. Such a plot can facilitate the stratification of outcome (elevated bone turnover) with respect to its probability (Figure 2). Positioning a given individual subject with known CTX and OC concentration values with respect to the probability lines of the nomogram only allows stating that the likelihood of the subject’s belonging to the EBT group is not less than, for example, 50%, 90%, or 99% (Figure 2). This figure also shows a pair of additional (broken) lines corresponding to the upper premenopausal limits for each marker, as found during the EPOLOS project. It is worth mentioning that their crossing point coincides with the P=0.9 probability line. We think this supports the view that these limits are relevant for postmenopausal women. It appeared that evaluation of a patient’s bone turnover based only on the measurement of one marker is limited and may lead to false conclusions. In contrast, by utilizing both bone formation and bone resorption markers it seems possible to elucidate EBT individuals properly, which would otherwise be hidden based on one marker.

The validity of the obtained model was further confirmed by group B, which was not involved in setting up the model. The OC and CTX values for each subject were used to find the subject’s relationship to the P=0.5 value (line). Again, postmenopausal women with diagnosed EBT, irrespective of the skeletal region, had significantly reduced BMD compared with the cases with relatively normal bone turnover rates. This indicates the proper operation of our model. This was also supported by the fact that the proportions of EBT subjects found in groups A and B were not significantly different.

The study has some limitations related to the lack of possibility to follow up the evolution of BMD values, fracture incidence, and the levels of bone turnover markers. Nevertheless, the aim of the study was not to construct a ready-to-use tool, but rather a certain concept for further verification. We believe that the general target was achieved. On the other hand, the model’s verification in relation to fractures requires a prospective large-cohort study.

**Conclusions**

We have shown that application of the simultaneous measurement of a pair of bone turnover markers (formation and resorption) can provide an unambiguous answer regarding a subject’s bone turnover rate. This information could be of some practical value. Nonetheless, future prospective studies focused on EBT and its impact on fracture incidence are strongly needed to take advantage of the presented model’s potential.

**Acknowledgement**

Jacek Łukaszewicz, present affiliation: Pharmacy Faculty, Biochemistry Department, Medical University of Warsaw.


1) Dept. of Bioch. and Exp. Medicine, Children’s Memorial Health Institute, Warsaw; 2) Orthopedic and Traumatology Clinic, The Ludwik Rydygier Medical University in Bydgoszcz, Bydgoszcz; 3) Dept. of Orthopedics, Jagiellonian University, Cracow; 4) Regional Center of Menopause and Osteoporosis, Dept. of Thyroidology, Clinical Hospital No. 3, Łódź; 5) Dept. of Internal Medicine, Postgraduate Medical Education Center, Orlowski Hospital, Warsaw; 6) Dept. and Clinic of Endocrinology and Diabetology, Wrocław University of Medicine, Wrocław; 7) Dept. of Gynecology and Obstetrics, Division of Gynecological Oncology, Karol Marcinkowski University of Medical Sciences, Poznań, Poland.

**References:**

1. NIH: Consensus Development Panel on Osteoporosis Prevention, Diagnosis and Therapy. JAMA, 2001; 285: 783–805


