Circulatory osteoprotegerin is related to osteoporosis of the hip in patients with COPD

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Summary

Background: Osteoprotegerin (OPG), a potent inhibitor of osteoclastogenesis, decreases bone resorption and has protective effects on bone mineral density (BMD). Recently we have shown that the adipose-tissue derived OPG relates to BMD in patients with chronic obstructive pulmonary disease (COPD), a condition associated with increased risk of osteoporosis.

Objective: Here we aimed to investigate the potential of circulatory OPG to reflect hip BMD in patients with COPD.

Patients and methods: In 56 subjects with COPD [age, 61.7 ± 6.7 years; forced expiratory volume in 1 s (FEV1), 53.6 ± 19.2% predicted], total femur BMD was assessed by dual energy X-ray absorptiometry, serum OPG and β-crosslaps, a marker of increased bone resorption, by commercially available assays.

Results: From patients with normal hip BMD (n = 32, T-score 0.1 ± 0.8) to those with osteopenia (n = 14, T-score −1.6 ± 0.4) and osteoporosis (n = 10, T-score −3.4 ± 0.7) serum OPG levels significantly increased (6.6 ± 1.8 versus 7.2 ± 2.9 and versus 8.6 ± 1.5 pmol/l, p = 0.036). In addition, hip T-scores were directly related to FEV1, and inversely to β-crosslaps (R = 0.40, p = 0.002; R = 0.38, p = 0.01, respectively). In multivariate analysis, OPG independently predicted hip T-scores after adjustments for age, gender, FEV1, and β-crosslaps (p = 0.011, adjusted R² = 0.354). Area under receiver operator curve for OPG as a discriminator of osteoporosis was 0.787 (95% CI, 0.653–0.921) (p = 0.005).

Conclusions: Present results suggest that osteoporosis of the hip is associated with increased circulatory levels of OPG in patients with COPD. OPG might serve as a biomarker of this COPD-related comorbidity.

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Introduction

Chronic obstructive pulmonary disease (COPD) represents one of the leading causes of mortality worldwide, and moreover, hospitalizations associated with this condition result into a significant burden to health-care resources [1]. Nevertheless, increased morbidity in patients with COPD is not entirely due to pulmonary complications; a substantial proportion of adverse outcomes result from COPD-related systemic effects and comorbidities including coronary heart disease, cachexia, muscle dysfunction, and osteoporosis [2].

Reduced bone mineral density (BMD) is highly prevalent in patients with COPD, affecting 9–69% of subjects in different cohorts [3]. Nevertheless, pathological processes involved in the increased risk of COPD-related osteoporosis are poorly understood. Our recent study suggested that adipokines including the adipose-tissue derived osteoprotegerin (OPG) relate to BMD in patients with COPD, and appear to act as mediators between fat mass and BMD [4]. Nevertheless, there are no literary data on the potential of circulatory OPG to reflect BMD in such patients.

The receptor activator of nuclear factor κB (RANK) pathway has been identified to play a central role in bone remodeling and disorders of mineral metabolism. The interaction of RANK with its ligand (RANKL) was shown to stimulate the formation and activation of osteoclasts in conjunction with various cytokines and calcitropic hormones. OPG, an anti-inflammatory protein derived from osteoblasts inhibits osteoclastogenesis and reduces bone resorption by acting as a decoy receptor to competitively inhibit RANKL [5]. Imbalance of the RANKL and OPG pathway has been shown to play a central role in the pathogenesis of COPD and other lung diseases [6].

The Bergen COPD Cohort Study increased plasma OPG levels were associated with the severity of the disease reflected by the frequency of exacerbations and severity of respiratory impairment, conditions known to correlate with systemic inflammation [7], thus rising an intriguing question whether circulatory OPG levels relate to BMD in patients with COPD. Nevertheless, BMD was not measured in the Bergen Cohort Study, and two further investigations yielded inconclusive results: on one hand a link between lumbar vertebral BMD and circulatory RANKL but not OPG was observed in COPD patients [8], on the other hand OPG was related to hip but not lumbar BMD in mild-to-moderate COPD [9]. The latter study did not include patients with severe and very severe COPD. Importantly, circulatory OPG was previously found to be related to hip but not lumbar BMD in terminal stages of disorders other than COPD such as advanced chronic heart or renal failure [10,11]. Therefore, the purpose of the present study was to investigate serum OPG and RANKL levels in relation to osteoporosis of the hip across all stages of COPD severity.

Methods

Subjects

This is a prospective cross-sectional study in patients with diagnosis of COPD according to the Global initiative for chronic Obstructive Lung Disease (GOLD) recommendations [1], free from exacerbation for ≥8 weeks who were consecutively recruited from two out-patient clinics affiliated with the university hospital setting. The study inclusion criteria required the patients to be clinically stable, performing regular mild physical activity, and receiving an adequate and balanced diet. Exclusion criteria were: use of systemic corticosteroids, treatment with long-term home oxygen therapy, respiratory disorders other than COPD, known malignancy, and any concomitant endocrine, rheumatoid, kidney, liver or gastrointestinal tract disease that was capable to interfere with bone metabolism and mineral density. None of the patients had previous diagnosis of osteoporosis nor had been treated with bone-targeted medications. Dyspnea severity was evaluated using the Modified Medical Research Council (MMRC) scale [12].

On the investigation day, each subject completed a fasting blood sample collection, pre-bronchodilator and post-bronchodilator spirometry and bodyplethysmography, and measurements of BMD. Information regarding COPD exacerbations in the preceding year, comorbidities and medication use was retrieved from patients’ charts of the referring physicians. The study had approval of the Ethics Committee of the L. Pasteur University Hospital, Kosice, Slovakia, and all subjects gave written consent to the study.

Pulmonary function tests

Pulmonary function tests were assessed with the use of bodyplethysmography (Ganshorn, Germany) in accordance with European Respiratory Society standards [13]. Tests were performed with patients in a sitting position by the same technician in order to ensure consistency of the technique. Three technically acceptable measurements were performed in each patient, and the highest value was included in the analyses. COPD diagnosis and airflow obstruction severity was evaluated on the basis of the GOLD recommendations [1].

Bone mineral density and body composition

BMD and body composition were measured by dual energy X-ray absorptiometry (DEXA) with fan-beam technology using a total body scanner (Lunar Prodigy, GE Healthcare, United Kingdom). Individual measurements of the left hip (total femur), antero-posterior lumbar spine (L1 to L4), and total body BMD were expressed in absolute values in grams of mineral per unit area scanned (g/cm²) and relative T-scores. This score represents the difference in SDs from mean reference BMD values that have been matched for race and sex from peak bone mass. Calibration was performed routinely every morning using the standard provided by the manufacturer. Coefficient of variation during measurement of standard phantom was less than 1%. The diagnosis of hip osteoporosis was established according to the recommendations of the World Health Organization [14] for a specific measurement site. Body weight and fat free mass was adjusted for height squared to calculate body mass index (BMI) and fat free mass index (FFMI).

Biochemical analyses

In all patients, peripheral venous blood samples from the antecubital vein were collected between 7.00 and 8.00 a.m.
after 10 h fast. Routine biochemical and hematological assessments were performed at the day of collection. At the time of venous blood samples collection, arterial blood sample was obtained by puncture of radial artery to determine arterial oxygen tension (PaO2) and arterial carbon dioxide tension (PaCO2). Samples used to assess the serum levels of C-reactive protein (CRP), inflammatory cytokines, bone turnover markers, OPG and RANKL, were stored at -80 °C until the day of the analyses. High-sensitivity serum CRP levels were assessed by immunoturbidimetric method (Randox, United Kingdom). The analytical sensitivity of this CRP assay is 0.1 mg/l. Serum tumor necrosis factor-α and interleukin-6 levels were measured using commercially available enzyme-linked immunosorbent assay kits (Beckmann-Coulter Immunootech). Bone turnover markers osteocalcin and total type I collagen (β-crosslaps) reflecting bone resorption [15] were assessed using the electrochemiluminescence immunoassays (Roche, Germany). Serum OPG and total RANKL levels were determined by enzyme-linked immunosorbent assays using commercially available kits for OPG (Biomedica, Austria) and total RANKL (Immundiagnostik, Germany). Assays were performed according to manufacturers’ protocols.

Statistical analyses

Statistical analyses were performed using SPSS software version 14.0 (SPSS Inc., USA). The Kolmogorov–Smirnov test of normality was applied. Differences between groups in normally distributed variables were tested by one-way analysis of variance (ANOVA) with all-pairwise comparisons using the Holm-Sidak method. Differences between groups in non-normally distributed variables were tested by Kruskal–Wallis ANOVA on ranks and Dunn’s test was used for all-pairwise comparisons. Fisher exact test was used to compare the proportion of categorical variables between groups. Least-squares linear regression analysis was used to assess the unadjusted relationships between hip T-score as the dependent variable, and pulmonary functions, body composition parameters, and bone serum markers. Pearson product-moment correlation coefficient (R) is reported to show the degree of linear relationship between variables. In the multivariate analysis, a multiple linear regression model was used with hip T-score as the dependent variable, and age, gender, forced expiratory volume in 1 s (FEV1), and bone serum markers as independent variables. Coefficient of determination (R²) is reported to indicate the model fit, and percentage of variance of the dependent variable explained by the predictors included in the final model. Non-normally distributed variables were log-transformed before entering the regression analyses. Receiver operator characteristic (ROC) analysis was used to examine the discriminator performance of OPG in predicting hip T-score equal to or lower than a cut-off value for osteoporosis. The area under ROC curve and associated 95% confidence intervals (CI) were used as indicators of the overall accuracy of identifying positive cases of osteoporosis.

A p value of <0.05 was considered statistically significant. Continuous variables are shown as means ± SD, non-normally distributed variables as median (interquartile range).

Results

Patient characteristics

Fifty six patients with COPD (52 men and 4 women) were enrolled. They were generally late middle-aged (mean age 61.7 ± 6.7 years) with a mean 36.6 ± 20.7 pack years history of smoking. Patients were divided into three groups according to the presence of the hip osteoporosis: the first one was formed by 32 (57.1%) patients with normal hip (total femur) BMD (T-score ≥−1), the second by 14 (25.0%) patients with osteopenia (T-score <−1 and >=−2.5), and the third by 10 (17.9%) patients with osteoporosis (T-score ≤−2.5). Table 1 displays demographic data, respiratory symptoms, comorbidities and body composition parameters in the three respective groups. No differences were observed in age, gender proportion, smoking history, COPD duration or dyspnea severity scores between the groups. No differences were observed between the groups in the proportion of patients with coronary artery disease or type 2 diabetes. Two patients had previous myocardial infarction; one with normal hip BMD and one with osteopenia. The proportion of patients with arterial hypertension was the lowest among patients with osteoporosis (p < 0.05). Patients with osteoporosis had significantly reduced BMI, FFMI, and fat mass percentage compared to patients with normal BMD or osteopenia (p < 0.05 for both comparisons). Table 2 displays parameters of bone density, and Table 3 pulmonary function tests and arterial blood gases in the three groups of patients. Patients with osteoporosis had significantly lower FEV1, FEV1/forced vital capacity (FVC) ratio, and higher residual volume (RV)/total lung capacity (TLC) ratio compared to those with normal BMD or osteopenia (p = 0.002; p = 0.002; p = 0.007; respectively).

Serum levels of β-crosslaps and osteocalcin significantly increased from patients with normal BMD to those with osteopenia and osteoporosis (p = 0.025; p = 0.017; respectively) (Table 4). In contrast, no differences were observed between the groups in the neutrophil count, serum CRP, inflammatory cytokines, or thyroid and parathyroid hormone levels. In univariate analyses, hip T-score was directly related to FEV1, and both BMI and FFMI (R = 0.40, p = 0.002; R = 0.61, p < 0.001; R = 0.57, p < 0.001; respectively), and inversely to RV and RV/TLC ratio, and to log transformed serum β-crosslaps (R = 0.38, p = 0.004; R = 0.35, p = 0.007; R = 0.35, p = 0.01; respectively).

Serum OPG and RANKL concentrations and hip BMD

Serum levels of OPG significantly increased from patients with normal hip BMD to those with osteopenia and osteoporosis (6.6 ± 1.8 versus 7.2 ± 2.9 and versus 8.6 ± 1.5 pmol/l; respectively, P for trend = 0.036). In multiple pair-wise comparisons, serum OPG levels were significantly higher in patients with osteoporosis compared to patients with normal hip BMD (Fig. 1). No differences were observed in RANKL levels between the three groups [0.82 (0.43, 1.23) versus 0.91 (0.37, 3.63) and versus 1.56 (0.46, 3.93) μg/l; respectively, p = 0.534]. A significant inverse relationship was seen between plasma OPG, and both BMI and FFMI (R = 0.32, p = 0.016;
log-transformed dependent variables, only FEV1, OPG independently predicted hip T-score (adjusted Zp = 0.008; respectively). In contrast, no such relationships were observed between RANKL levels and BMI or FFMI. Also there were no relationships between plasma OPG and RANKL levels or between OPG or RANKL levels and pulmonary function parameters or bone turnover markers (data not shown).

In multivariate linear analysis with age, gender, FEV1, log-transformed β-crosslaps, and serum OPG levels as independent variables, only FEV1, β-crosslaps, and plasma OPG independently predicted hip T-score (adjusted R² = 0.354, Table 5). Area under the ROC curve for OPG as a discriminator of hip T-score ≤−2.5 was 0.787 (95% CI, 0.653–0.921) (p = 0.005).

**Discussion**

The present study provides a novel observation on the increased circulatory OPG in COPD-related osteoporosis, and its potential to serve as a biomarker of reduced hip BMD.

### Table 1  Patient characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Entire cohort</th>
<th>Normal BMD</th>
<th>Osteopenia</th>
<th>Osteoporosis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, No (%)</td>
<td>56</td>
<td>32 (57.1)</td>
<td>14 (25.0)</td>
<td>10 (17.9)</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>61.7 ± 6.7</td>
<td>61.2 ± 6.7</td>
<td>60.8 ± 6.7</td>
<td>64.7 ± 6.3</td>
<td>0.289</td>
</tr>
<tr>
<td>Male gender, No. (%)</td>
<td>52 (91.2)</td>
<td>31 (96.9)</td>
<td>13 (92.9)</td>
<td>8 (80.0)</td>
<td>0.195</td>
</tr>
<tr>
<td>Packyears</td>
<td>36.6 ± 20.7</td>
<td>36.4 ± 23.1</td>
<td>39.7 ± 18.8</td>
<td>32.4 ± 15.5</td>
<td>0.492</td>
</tr>
<tr>
<td>Duration of COPD, yr.</td>
<td>6.3 ± 7.4</td>
<td>5.9 ± 5.8</td>
<td>4.6 ± 6.4</td>
<td>10.3 ± 12.0</td>
<td>0.285</td>
</tr>
<tr>
<td>MMRC grade</td>
<td>1.4 ± 0.7</td>
<td>1.4 ± 0.7</td>
<td>1.2 ± 0.7</td>
<td>1.8 ± 0.6</td>
<td>0.158</td>
</tr>
<tr>
<td>Borg score</td>
<td>2.8 ± 2.0</td>
<td>2.6 ± 1.7</td>
<td>2.5 ± 2.2</td>
<td>3.7 ± 2.5</td>
<td>0.277</td>
</tr>
<tr>
<td>Arterial hypertension, No. (%)</td>
<td>32 (57.1)</td>
<td>22 (68.8)</td>
<td>8 (57.1)</td>
<td>2 (20.0)</td>
<td>0.025</td>
</tr>
<tr>
<td>Coronary artery disease, No. (%)</td>
<td>12 (21.4)</td>
<td>6 (18.8)</td>
<td>3 (21.4)</td>
<td>3 (30)</td>
<td>0.751</td>
</tr>
<tr>
<td>Type 2 diabetes, No. (%)</td>
<td>7 (12.5)</td>
<td>5 (15.6)</td>
<td>2 (14.3)</td>
<td>0 (0.0)</td>
<td>0.416</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.3 ± 6.8</td>
<td>28.9 ± 5.6</td>
<td>27.1 ± 7.1</td>
<td>19.5 ± 2.9†</td>
<td>0.001</td>
</tr>
<tr>
<td>FFMI, kg/m²</td>
<td>18.4 ± 2.4</td>
<td>19.2 ± 2.0</td>
<td>18.1 ± 2.6</td>
<td>16.1 ± 1.1†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FM (%)</td>
<td>29.6 ± 11.3</td>
<td>33.3 ± 8.7</td>
<td>30.9 ± 10.3</td>
<td>16.2 ± 10.4†</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are given as the mean ± SD. *p < 0.05 versus normal BMD; |p < 0.05 versus osteopenia. BMD — bone mineral density; COPD — chronic obstructive pulmonary disease; MMRC — Modified Medical Research Council scale; BMI — body mass index; FFMI — fat-free mass index; FM — fat mass.

### Table 2  Measurements of hip (total femur), lumbar spine, and total BMD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Entire cohort</th>
<th>Normal BMD</th>
<th>Osteopenia</th>
<th>Osteoporosis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip BMD, g/cm²</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.6 ± 0.1*†</td>
<td>0.001</td>
</tr>
<tr>
<td>Hip, T-score</td>
<td>−1.0 ± 1.5</td>
<td>0.1 ± 0.8</td>
<td>−1.6 ± 0.4</td>
<td>−3.4 ± 0.7†</td>
<td>0.001</td>
</tr>
<tr>
<td>Lumbar spine BMD, g/cm²</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>1.0 ± 0.3</td>
<td>0.7 ± 0.1†</td>
<td>0.001</td>
</tr>
<tr>
<td>Lumbar spine, T-score</td>
<td>−1.3 ± 1.7</td>
<td>−0.4 ± 1.4</td>
<td>−1.8 ± 1.0</td>
<td>−3.5 ± 0.6†</td>
<td>0.001</td>
</tr>
<tr>
<td>Total BMD, g/cm²</td>
<td>1.1 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1*</td>
<td>0.9 ± 0.1†</td>
<td>0.001</td>
</tr>
<tr>
<td>Total T-score</td>
<td>−1.2 ± 1.8</td>
<td>−0.2 ± 1.2</td>
<td>−1.7 ± 0.8*</td>
<td>−3.8 ± 1.2†</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are given as the mean ± SD. *p < 0.05 versus normal BMD; |p < 0.05 versus osteopenia. BMD — bone mineral density.

### Table 3  Pulmonary functions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Entire cohort</th>
<th>Normal BMD</th>
<th>Osteopenia</th>
<th>Osteoporosis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1, l</td>
<td>1.6 ± 0.6</td>
<td>1.8 ± 0.6</td>
<td>1.7 ± 0.6</td>
<td>0.9 ± 0.3*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV1, % predicted</td>
<td>53.6 ± 19.2</td>
<td>58.3 ± 19.0</td>
<td>56.1 ± 17.1</td>
<td>35.1 ± 11.3*†</td>
<td>0.002</td>
</tr>
<tr>
<td>FEV1/FVC ratio</td>
<td>48.5 ± 12.2</td>
<td>51.3 ± 11.1</td>
<td>50.4 ± 10.2</td>
<td>36.6 ± 12.2*†</td>
<td>0.002</td>
</tr>
<tr>
<td>RV, l</td>
<td>4.0 ± 1.0</td>
<td>3.8 ± 0.8</td>
<td>4.0 ± 0.92</td>
<td>4.73 ± 1.59</td>
<td>0.211</td>
</tr>
<tr>
<td>TLC, l</td>
<td>7.3 ± 1.4</td>
<td>7.2 ± 1.4</td>
<td>7.5 ± 1.4</td>
<td>7.3 ± 1.7</td>
<td>0.784</td>
</tr>
<tr>
<td>RV/TLC ratio</td>
<td>55.2 ± 9.3</td>
<td>53.2 ± 8.2</td>
<td>54.1 ± 8.4</td>
<td>63.3 ± 3.2*†</td>
<td>0.007</td>
</tr>
<tr>
<td>PaO₂, kPa</td>
<td>9.25 ± 1.58</td>
<td>9.70 ± 1.70</td>
<td>9.08 ± 1.04</td>
<td>8.11 ± 1.23*</td>
<td>0.016</td>
</tr>
<tr>
<td>PaCO₂, kPa</td>
<td>5.10 ± 0.82</td>
<td>5.16 ± 0.78</td>
<td>4.84 ± 0.76</td>
<td>5.29 ± 0.98</td>
<td>0.632</td>
</tr>
<tr>
<td>SaO₂, %</td>
<td>93.0 ± 4.3</td>
<td>94.0 ± 3.8</td>
<td>93.3 ± 2.1</td>
<td>89.8 ± 6.3*</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Values are given as the mean ± SD. *p < 0.05 versus normal BMD; |p < 0.05 versus osteopenia. BMD — bone mineral density; FEV1 — forced expiratory volume in 1 s; FVC — forced vital capacity; RV — residual volume; TLC — total lung capacity; PaO₂ — partial pressure of oxygen; PaCO₂ — partial pressure of carbon dioxide; SaO₂ — oxygen saturation in hemoglobin.
in patients with COPD. Our data demonstrate that patients with osteoporosis of the hip have increased serum OPG levels that predict the total femur T-score independently of age, gender, markers of bone resorption and of the degree of airflow limitation. In previous reports increases in OPG were associated with the severity of COPD [7], and related to hip but not lumbar spine BMD in mild-to-moderate COPD [9]. Nevertheless, to the best of our knowledge our data are the first to demonstrate that increases in circulatory OPG levels reflect also concurrent development of osteoporosis in patients with COPD.

Osteoporosis is a systemic skeletal disease characterized by decreased bone mineral content and microarchitectural deterioration. A recent meta-analysis of 13 studies in patients with COPD indicated the overall mean prevalence of osteoporosis of 35%, ranging from 9 to 69% in different cohorts [3]. In agreement, osteoporosis was diagnosed in 18% of consecutively recruited out-patients with COPD in the present study. Clinical correlates of osteoporosis in COPD include low BMI and body composition measures, reductions in pulmonary function, and therapy with systemic corticosteroids [16–18]. In our cohort, use of systemic corticosteroids represented an exclusion criterion, and therefore such therapy did not interfere with results of the present study. Importantly, patients with osteoporosis in the present cohort had low pulmonary functions as reflected by reductions in FEV₁, in accordance with previous reports [18–20].

Improvements in understanding of pathological processes involved in the process of bone loss in COPD are highly warranted since osteoporosis has serious consequences in such patients. Not only are hip fractures highly prevalent among patients with COPD, they also result in a twofold increased risk of post-operative pulmonary complications [21]. Several mechanisms likely underlie the increased risk of low BMD in patients with COPD, such as systemic inflammation, decreased physical activity owing to dyspnea, and/or other factors leading to proteolysis, reduced bone formation and increased bone resorption [22,23]. OPG, an anti-inflammatory protein derived from osteoblasts, is a soluble member of the tumor necrosis factor receptor superfamily that inhibits osteoclastogenesis by acting as a decoy receptor to competitively inhibit RANKL, and thus preventing the interaction of RANKL with its receptor RANK [24]. In our previous study we observed relationships between adipose tissue leptin and OPG expressions and measures of BMD among patients with COPD [4]. Nevertheless, circulatory OPG levels were not measured and, indeed, the question regarding serum OPG and osteoporosis in patients with COPD across all stages of the disease severity has not been previously approached. The only recent study by Duckers et al. [9] did not include patients with very severe (GOLD IV) COPD, and consequently only two patients in their cohort suffered from hip osteoporosis. Therefore, our observations on increased serum OPG levels among COPD patients with osteoporosis complement and extend these previous findings in several ways. First, although Duckers et al. [9] speculated about a link between OPG and bone resorption markers in COPD, we have demonstrated that OPG levels predicted total femur T-score independently of bone resorption as reflected by the lack of an association between OPG and β-crosslaps in either univariate or multivariate analyses. Second, our observations point at a possible role of OPG as a biomarker of osteoporosis in COPD. This could be clinically meaningful, since in the recent guidelines for the management of

### Table 4 Serum markers of bone turnover and inflammatory and endocrine parameters.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Osteoporosis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, mg/l</td>
<td>5.0(5.0, 8.0)</td>
<td>5.0(5.0, 6.1)</td>
<td>5.2(3.3, 7.6)</td>
<td>6.5(5.0, 17.1)</td>
<td>0.210</td>
</tr>
<tr>
<td>Neutrophil count, ×10⁹/l</td>
<td>4.4 ± 1.4</td>
<td>4.2 ± 1.1</td>
<td>4.4 ± 1.5</td>
<td>5.1 ± 2.1</td>
<td>0.277</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>3.6(2.9, 6.9)</td>
<td>3.6(2.8, 9.1)</td>
<td>3.5(2.7, 4.9)</td>
<td>4.6(3.1, 6.9)</td>
<td>0.653</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>13.4(10.7, 16.5)</td>
<td>14.3(12.1, 22.1)</td>
<td>12.3(11.0, 14.4)</td>
<td>9.3(8.3, 13.8)</td>
<td>0.134</td>
</tr>
<tr>
<td>Osteocalcin, pg/ml</td>
<td>18.2(13.6, 24.0)</td>
<td>16.7(13.9, 22.9)</td>
<td>16.6(11.8, 20.7)</td>
<td>27.3(19.1, 34.4)</td>
<td>0.017</td>
</tr>
<tr>
<td>β-crosslaps, μg/l</td>
<td>0.26(0.14, 0.47)</td>
<td>0.24(0.14, 0.36)</td>
<td>0.23(0.12, 0.48)</td>
<td>0.67(0.38, 0.84)</td>
<td>0.025</td>
</tr>
<tr>
<td>P1NP, μg/l</td>
<td>31.7(24.5, 46.7)</td>
<td>29.4(24.5, 41.5)</td>
<td>31.0(18.5, 35.2)</td>
<td>50.2(26.9, 77.6)</td>
<td>0.125</td>
</tr>
<tr>
<td>Parathormone, pmol/l</td>
<td>30.1(5.2, 46.4)</td>
<td>26.7(4.9, 42.4)</td>
<td>9.5(5.2, 51.0)</td>
<td>40.7(23.1, 47.6)</td>
<td>0.637</td>
</tr>
<tr>
<td>TSH, IU/l</td>
<td>1.78(1.15, 2.31)</td>
<td>1.78(1.23, 2.20)</td>
<td>2.07(1.08, 2.83)</td>
<td>1.31(0.51, 1.96)</td>
<td>0.246</td>
</tr>
<tr>
<td>fT4, pmol/l</td>
<td>15.6(14.1, 17.2)</td>
<td>15.4(14.0, 17.2)</td>
<td>16.0(14.8, 16.8)</td>
<td>16.8(14.2, 18.2)</td>
<td>0.885</td>
</tr>
</tbody>
</table>

Values are given as median (interquartile range). *p < 0.05 versus normal BMD.

BMD = bone mineral density; CRP = high sensitivity C-reactive protein; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-α; P1NP = type 1 amino-terminal propeptide of procollagen; TSH = thyroid-stimulating hormone; fT4 = free thyroxine.

![Figure 1](image_url) Plasma osteoprotegerin (OPG) levels in patients with chronic obstructive pulmonary disease and different femur bone mineral density (BMD) categories. *p < 0.05.
COPD, patients with this condition represent a target group for treatment of osteoporosis [1]. Importantly, our observation of increased circulatory OPG in patients with osteoporosis in agreement with other chronic diseases with different disease-specific mechanisms of bone loss such as chronic heart failure [10] or end-stage kidney failure [11]. Since OPG is a protein with protective effects against bone loss it has been speculated that its increases in such clinical conditions may represent a mechanism that counter-acts disease-specific mechanisms of increased bone loss such as systemic inflammation [6] or increased tissue breakdown and proteolysis [7].

Osteoblasts and stromal cells in the skeletal tissue secrete OPG which in its soluble state blocks the RANK/RANKL interaction [5,6], and thereby acts as a physiological regulator of (increased) bone turnover. In general, skeletal tissue represents the main source of circulatory OPG levels [24]. Indeed, our former findings of reduced fat-tissue OPG expressions in patients with COPD and osteoporosis [4] combined with the results of the present study suggest that adipose-tissue does not substantially contribute to circulatory OPG levels in COPD.

No relationships were observed between serum RANKL levels and hip or lumbar BMD in COPD patients in the present study, in contrast to previous report [8]. Nevertheless, severe concerns might have been raised in the interpretation of the serum RANKL levels in that study since the proportion of membrane-bound RANKL may vary considerably and a certain percentage of patients may even have undetectable RANKL levels in the systemic circulation [25]. Indeed, this was the case in one patient in our cohort.

Interestingly, circulatory OPG was related to hip but not lumbar osteoporosis in our study, in agreement with previous reports in terminal stages of disorders other than COPD such as chronic heart failure [10] or renal failure [11]. Differences in bone composition and in the pattern of BMD loss between the hip and lumbar spine [26] may underlie this discrepancy. In addition, among patients with COPD, physical deconditioning and reduction in weight-bearing demands exert greater effects on the hip than on the lumbar spine, [3,9] thus contributing to the progression of hip osteoporosis rather than to lumbar BMD loss. There are some limitations in the present study such as its cross-sectional nature that does not allow for revealing causal relationships between OPG and osteoporosis. Longitudinal studies to evaluate the time course of OPG levels changes in association with measurements of BMD are warranted. Furthermore, limited number of patients and a small number of women represent another limitation. However, circulatory OPG levels in the present study were comparable to those seen previously in COPD [9] and chronic heart failure patients [10], and the observed increases in circulatory OPG (~30%) in osteoporotic COPD patients parallel those reported in patients with osteoporosis in other advanced chronic diseases [10,11]. We therefore believe that our results are robust to gain some understanding on the role of OPG in COPD-related osteoporosis. Also, it has to be underlined that concomitant measurements, within one group of patients with COPD, of body composition, BMD, and of circulatory OPG and RANKL levels are unique and represent strength of the study.

In conclusion, our results suggest that osteoporosis is associated with increased circulatory levels of OPG in patients with COPD, and therefore OPG might serve as a biomarker of this COPD-related comorbidity. Further studies are needed to analyze the interactions between OPG and complex processes of bone resorption in more details.

**Conflict of interest**

No conflict of interest to disclose.

**Acknowledgments**

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**References**


<table>
<thead>
<tr>
<th>Variable</th>
<th>β coefficient</th>
<th>SE</th>
<th>Standardized β coefficient</th>
<th>95% Confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>−1.984</td>
<td>2.011</td>
<td>−</td>
<td>−5.997 to 1.983</td>
<td>0.329</td>
</tr>
<tr>
<td>Age</td>
<td>−0.005</td>
<td>0.027</td>
<td>−0.023</td>
<td>−0.057 to 0.048</td>
<td>0.849</td>
</tr>
<tr>
<td>Gender</td>
<td>0.782</td>
<td>0.656</td>
<td>0.137</td>
<td>−0.527 to 2.080</td>
<td>0.239</td>
</tr>
<tr>
<td>FEV₁</td>
<td>0.023</td>
<td>0.009</td>
<td>0.300</td>
<td>0.005 to 0.041</td>
<td>0.015</td>
</tr>
<tr>
<td>Log β-crosslaps</td>
<td>−1.541</td>
<td>0.554</td>
<td>−0.341</td>
<td>−2.627 to −0.508</td>
<td>0.008</td>
</tr>
<tr>
<td>OPG</td>
<td>−0.214</td>
<td>0.081</td>
<td>−0.315</td>
<td>−0.377 to −0.054</td>
<td>0.011</td>
</tr>
</tbody>
</table>

SE — standard error of β coefficient; FEV₁ — forced expiratory volume in 1 s; OPG — osteoprotegerin.
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