Role of Osteoprotegerin and Receptor Activator of Nuclear Factor-κB Ligand in Bone Loss Related to Advanced Chronic Obstructive Pulmonary Disease

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Abstract

Background: Osteoporosis is a common complication of chronic obstructive pulmonary disease (COPD). Recent clinical and biological researches have increasingly delineated the biomolecular pathways of bone metabolism regulation in COPD. We extended this work by examining the specific association and potential contribution of the osteoprotegerin (OPG)/receptor activator of nuclear factor-κB ligand (RANKL) axis to the pathogenesis of osteoporosis in advanced COPD. The aim of this study was to assess the relationships of serum OPG, RANKL, and tumor necrosis factor-alpha (TNF-α) with bone turnover in men with very severe COPD.

Methods: Pulmonary function, T-score at the lumbar spine (LS) and femoral neck (FN), serum OPG, RANKL, soluble receptor of tumor necrosis factor-alpha-I and II (sTNFR-I, sTNFR-II), osteocalcin (OC), and β-CrossLaps (βCL) levels were measured in 45 men with very severe stage COPD and 36 male non-COPD volunteers. COPD patients and healthy controls were compared using an independent *t*-test and Mann–Whitney *U*-test. The Pearson coefficient was used to assess the relationships between variables.

Results: OPG and OC were lower in male COPD patients than in control subjects whereas RANKL, serum βCL, TNF-α, and its receptors were higher. OPG directly correlated with forced expiratory volume in 1 s (FEV1) % predicted ($r = 0.46$, $P < 0.005$), OC ($r = 0.34$, $P < 0.05$), LS ($r = 0.56$, $P < 0.001$), and FN T-score ($r = 0.47$, $P < 0.01$). In contrast, serum RANKL inversely associated with LS and FN T-score ($r = -0.62$, $P < 0.001$ and $r = -0.48$, $P < 0.001$) but directly correlated with βCL ($r = 0.48$, $P < 0.001$). In addition, OPG was inversely correlated with RANKL ($r = -0.39$, $P < 0.01$), TNF-α ($r = -0.56$, $P < 0.001$), and sTNFR-I ($r = -0.40$, $P < 0.01$).

Conclusion: Our results suggest that serum OPG and RANKL levels are inversely associated with bone loss in men with advanced stage COPD.

Key words: Chronic Obstructive Pulmonary Disease; Osteoporosis; Osteoprotegerin; Receptor Activator of Nuclear Factor-κB Ligand; Tumor Necrosis Factor Receptors; Tumor Necrosis Factor-alpha

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), characterized as progressive chronic airflow limitation and associated with an enhanced chronic inflammatory response in the airways and lungs to noxious particles or gases, is not only limited to respiratory symptoms but also leads to extrapulmonary effects, including systemic inflammation, cachexia, muscle dysfunction, and osteoporosis.[1] Molecular and cellular mechanisms of COPD-related osteoporosis are now being increasingly delineated including systemic cytokines, bone-related growth, transcriptional factors, and regulatory proteins and enzymes. Besides these aforementioned factors, there is another group of local factors that has been actively studied in recent years. They are combined in the osteoprotegerin (OPG)/receptor activator of nuclear factor-κB (RANK)/RANK ligand (RANKL) system and regarded as the basic signal mechanisms controlling

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bone resorption in both physiological and pathological conditions.

OPG, a circulating secretory glycoprotein produced by osteoblasts, plays an important role in many physiological processes, particularly in osteoclastogenesis.[2] The RANK, localized at the cell surface of mature osteoclasts and osteoclastic precursors, and its ligand RANKL, a membrane protein, expressed on the surface of osteoblasts and bone stromal cells, are other protagonists, which play key roles in bone resorption processes.[3] Binding of RANKL to RANK stimulates differentiation of osteoclast precursors into mature osteoclasts and also their activation that finally leads to enhanced bone resorption. By blocking interaction between RANK and RANKL onto surface of preosteoclasts, OPG, working as a decoy receptor for RANKL, inhibits the final stage of osteoclast differentiation and bone loss.[2]

In addition, the biological effects of the OPG/RANKL aimed at the regulation of the osteoclastogenesis have been demonstrated *in vitro* and in animal studies.[4,5] With regard to clinical researches, most of them have been devoted to the analysis of the OPG/RANK/RANKL axis contribution to the pathogenesis of osteoporosis in postmenopausal women and in hepatocirrhosis, cardiovascular, and renal pathologies.[6-9] However, the problematics surrounding the regulation of osteoclastogenesis and the involvement of inflammatory mediators, including OPG/RANK/RANKL system in COPD and especially in severe and very severe stages, have been presented without introducing a unique conception. Given that pathogenesis of COPD-related osteoporosis is connected with complex pathophysiological illness patterns, especially with the intensity of the local and systemic inflammatory reactions, the study of tumor necrosis factor (TNF) superfamily's molecules contribution to bone damage in advanced COPD stages is of particular interest. Thus, the purpose of this research was to study the relationship among circulating OPG, RANKL levels, inflammatory markers, and bone turnover in men with very severe COPD to clarify the possible role of the OPG/RANK/RANKL system in bone loss.

METHODS

In this cross-sectional study, 45 men with clinically stable very severe COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD)[10] were recruited from outpatient clinics affiliated with a University hospital setting from September 30, 2014, to March 31, 2015. The control group included 36 healthy male non-COPD volunteers who were at their regular medical check-up at the local health center and accepted to participate in this study.

Inclusion criteria included male patients with clinically stable very severe COPD (free of exacerbation for at least 3 months), never accepted the bone-targeted medications. Exclusion criteria were respiratory and inflammatory disorders other than COPD, a COPD exacerbation, cardiovascular diseases (in particular, severe heart failure), endocrine disorders, chronic kidney disease (defined

as an estimated glomerular filtration rate <60 ml/min for >3 months), and significant liver impairment.

The study was approved by the independent interdisciplinary Ethics Committee of Pacific State Medical University and performed in accordance with the principles of the *Declaration of Helsinki*. Each participant completed written informed consent.

Pulmonary function test
The respiratory function of all participants was appraised using a spiroanalyzer MasterScreen™ PFT system (Jaeger GmbH, Hoechberg, Germany) and a bodyplethysmograph MasterScreen body (Jaeger, Germany). Pulmonary function tests were performed under stable clinical conditions in all patients. The pre- and post-bronchodilatator forced expiratory volume in 1 s (FEV_1) % predicted, vital capacity, forced vital capacity (FVC), FEV_1/FVC ratio, and single-breath diffusing capacity for carbon monoxide (DLCO) were determined. COPD patients with a post-bronchodilator FEV_1/FVC ratio of less than 0.7 of the predicted value and FEV_1% <30% of predicted were classified as having very severe stage of COPD GOLD 4 according to the GOLD guidelines.

Bone mineral density measurement
Bone mineral density (BMD) was determined by dual-energy X-ray absorptiometry (DEXA) (GE Lunar Prodigy, Madison, WI, USA) at the lumbar spine (LS) and the femoral neck (FN) and was expressed in standard deviations (SD) of the average reference value for healthy yound adults (T-score). According to the recommendations of the WHO, T-scores between −1.0 and −2.5 SD were considered as osteopenia; T-scores below −2.5 SD were diagnosed as osteoporosis and T-scores more than −1.0 SD conformed to a normal BMD.

Biochemical analyses
Blood samples were obtained from all individuals. Sera samples were separated and stored at −80°C until analysis. Arterial blood samples were obtained by puncture of radial artery to determine arterial oxygen tension (PaO_2) and arterial carbon dioxide tension ($PaCO_2$).

The serum OPG and RANKL levels were both measured using a commercial immune enzyme-linked assay (Biomedica Groupe, Vienna, Austria). The serum tumor necrosis factor-alpha (TNF-α), soluble receptor of tumor necrosis factor-alpha I and II (sTNFR-I and sTNFR-II) were measured in duplicate by a ELISA test system (Quantikine, R&D Systems, Inc., Minneapolis, USA). The serum concentration of the bone metabolism markers β-CrossLaps (βCL) reflecting bone resorption, and osteocalcin (OC), reflecting bone formation, was determined using electrochemiluminiscence immunoassays (ROSHE Diagnostics, Switzerland).

Statistical analyses
Evaluation of probability distribution was performed using the Kolmogorov–Smirnov test. Data were expressed as mean ± standard deviation (SD). Patients with COPD GOLD 4 and healthy controls were compared using independent samples *t*-test and Mann–Whitney *U*-test.

The Pearson coefficient was used for measuring linear correlation between variables. Differences were considered significant at a value of $P < 0.05$. All analyses were performed using Statistical Package of Social Sciences for Windows (version 14.0, SPSS Inc., Chicago, IL, USA).

RESULTS

The characteristics and pulmonary function test results for the male patients with COPD and control group are listed in Table 1. All patients were Caucasian. The mean ages of the male COPD patients and control group were similar whereas bone mass index (BMI) was significantly lower in COPD GOLD 4. There also exist significant differences in pulmonary function among the two groups. There was no significant difference in the proportion of current smokers between COPD patients and non-COPD volunteers, but the smoking index was higher in COPD GOLD 4 [Table 1]. Approximately half of COPD patients were on inhaled corticosteroids and chronic oxygen therapy.

Bone mineral density

Our results showed that the T-score in COPD subjects was significantly lower than that in healthy subjects. Based on DEXA T-score, 15 (15/45) male COPD patients had osteopenia and 25 (25/45) had osteoporosis at the LS; 23 (23/45) patients had bone densities in the osteopenic range and 18 (18/45) in the osteoporosis range at the FN; just 4 (4/45) patients had a normal T-score at the LS as well as at the FN. With regard to non-COPD subjects, 4 of them (4/36) had osteoporosis at any area and osteopenia was noted in 9 (9/36) of healthy. We did not find any associations between the age of COPD patients and T-scores. In COPD patients, the LS and FN T-score were positively related to lung function: $FEV_1\%$ predicted ($r = 0.52$, $P = 0.000$ at the LS and $r = 0.48$, $P = 0.001$ at the FN), FEV_1/FVC ($r = 0.50$, $P = 0.000$ at the LS and $r = 0.45$, $P = 0.002$ at the FN), DLCO (LS: $r = 0.37$, $P = 0.012$ and FN: $r = 0.32$, $P = 0.034$), and negatively correlated with $PaCO_2$ (LS: $r = -0.41$, $P = 0.005$ and FN: $r = -0.35$, $P = 0.017$).

Bone metabolism markers

Patients with COPD showed higher serum βCL levels and a lower concentration of OC compared with controls [Table 2]. There was a negative correlation between βCL level and LS T-score ($r = -0.61$, $P < 0.000$) as well as between FN T-score ($r = -0.53$, $P = 0.000$). The level of OC in serum was found to be positively correlated with LS T-score only ($r = 0.34$, $P = 0.023$). The βCL concentration, but not OC level, changes dependant on the degree of airway obstruction ($FEV_1\%$ predicted: $r = -0.52$, $P = 0.000$ and FEV_1/FVC ratio: $r = -0.46$, $P = 0.001$).

Tumor necrosis factor-alpha and its receptors

The serum TNF-α and its receptors levels were higher in comparison with control group [Table 2]. We found an inverse correlation between TNF-α and $FEV_1\%$ predicted ($r = -0.53$, $P < 0.000$), T-score both at the LS

Table 1: Clinical characteristics of male COPD GOLD 4 patients and non-COPD volunteers

| Variables	Non-COPD volunteers (*n* = 36)	Male COPD GOLD 4 patients (*n* = 45)	t/χ^2	*P*
Age (years)	58.9 ± 5.3	60.3 ± 5.4	1.14	0.257
Smoking status				
Former/active, *n*	8/16	18/19	0.03*	0.824
Smoking index (pack/year)	28.6 ± 11.5	43.8 ± 15.2	222.00	0.021
FEV_1 % predicted	101.4 ± 6.5	22.2 ± 6.7	−80.41	0.000
FVC % predicted	98.6 ± 11.2	47.6 ± 7.2	−35.89	0.000
FEV_1/FVC (%)	87.3 ± 6.7	30.4 ± 4.3	−44.51	0.000
DLCO (%)	96.7 ± 12.6	30.3 ± 14.8	−44.68	0.000
PaO_2 (mmHg)	97.4 ± 5.6	61.8 ± 12.4	−35.09	0.000
$PaCO_2$ (mmHg)	38.2 ± 4.6	52.8 ± 9.2	11.29	0.000
BMI (kg/m²)	25.8 ± 3.2	20.1 ± 2.9	−6.19	0.000
Medication, *n* (%)				
Inhaled GCs	NA	23 (51)	NA	NA
Oral GCs	NA	9 (20)	NA	NA
Home oxygen	NA	22 (49)	NA	NA
T-score, LS	0.88 ± 1.48	−2.63 ± 1.59	−10.24	0.000
T-score, FN	1.09 ± 1.37	−2.45 ± 1.39	−11.47	0.000

Data are presented as mean ± SD or number (percentage); *χ^2 values; COPD: Chronic obstructive pulmonary disease; GOLD: Global Initiative for Chronic Obstructive Lung Disease; Pack/year: 1-year smoking 20 cigarettes/day; BMI: Body mass index; FEV_1: Forced expiratory volume in 1 s; FVC: Forced vital capacity; DLCO: Single-breath diffusing capacity for carbon monoxide; SD: Standard deviation; GCs: Glucocorticoids; PaO_2: Arterial partial pressure of oxygen; $PaCO_2$: Arterial partial pressure of carbon dioxide; LS: Lumbar spine; FN: Femur neck; NA: Not available.

Table 2: Serum biochemical parameters in male COPD patients and non-COPD volunteers

Variables	Healthy volunteers (*n* = 36)	Male COPD patients (*n* = 45)	*t*	*P*
β-CrossLaps (ng/ml)	0.35 ± 0.12	0.57 ± 0.21	4.89	0.000
Osteocalcin (pg/ml)	14.4 ± 3.8	11.3 ± 3.9	−3.65	0.001
TNF-α (pg/ml)	9.7 ± 5.8	23.9 ± 4.2	23.56	0.000
sTNFR-I (pg/ml)	142.5 ± 14.9	154.0 ± 16.7	3.29	0.002
sTNFR-II (pg/ml)	188.1 ± 20.1	241.7 ± 26.5	10.32	0.000
OPG (pmol/L)	6.35 ± 0.98	2.47 ± 0.78	−19.22	0.000
RANKL (pg/ml)	343.8 ± 36.2	439.5 ± 55.1	8.73	0.000

Parameter values are mean ± SD. TNF-α: Tumor necrosis factor-alpha; sTNFR-I and II: Soluble receptor of tumor necrosis factor-alpha-I and II; OPG: Osteoprotegerin; RANKL: Receptor activator of nuclear factor-κB ligand; SD: Standard deviation.

and at the FN [Figure 1a and 1b] and positive relationship with βCL ($r = 0.52$, $P = 0.000$) in men with very severe COPD. Interestingly, the sTNFR-I had a significant negative relationship with $FEV_1\%$ predicted ($r = -0.33$, $P = 0.027$), T-score at the LS only [Figure 1c] and OC levels ($r = -0.42$, $P = 0.004$). A negative correlation was observed between the level of sTNFR-II and T-score at the FN only [Figure 1d], and also positive association with βCL concentration ($r = -0.43$, $P = 0.003$). There was no
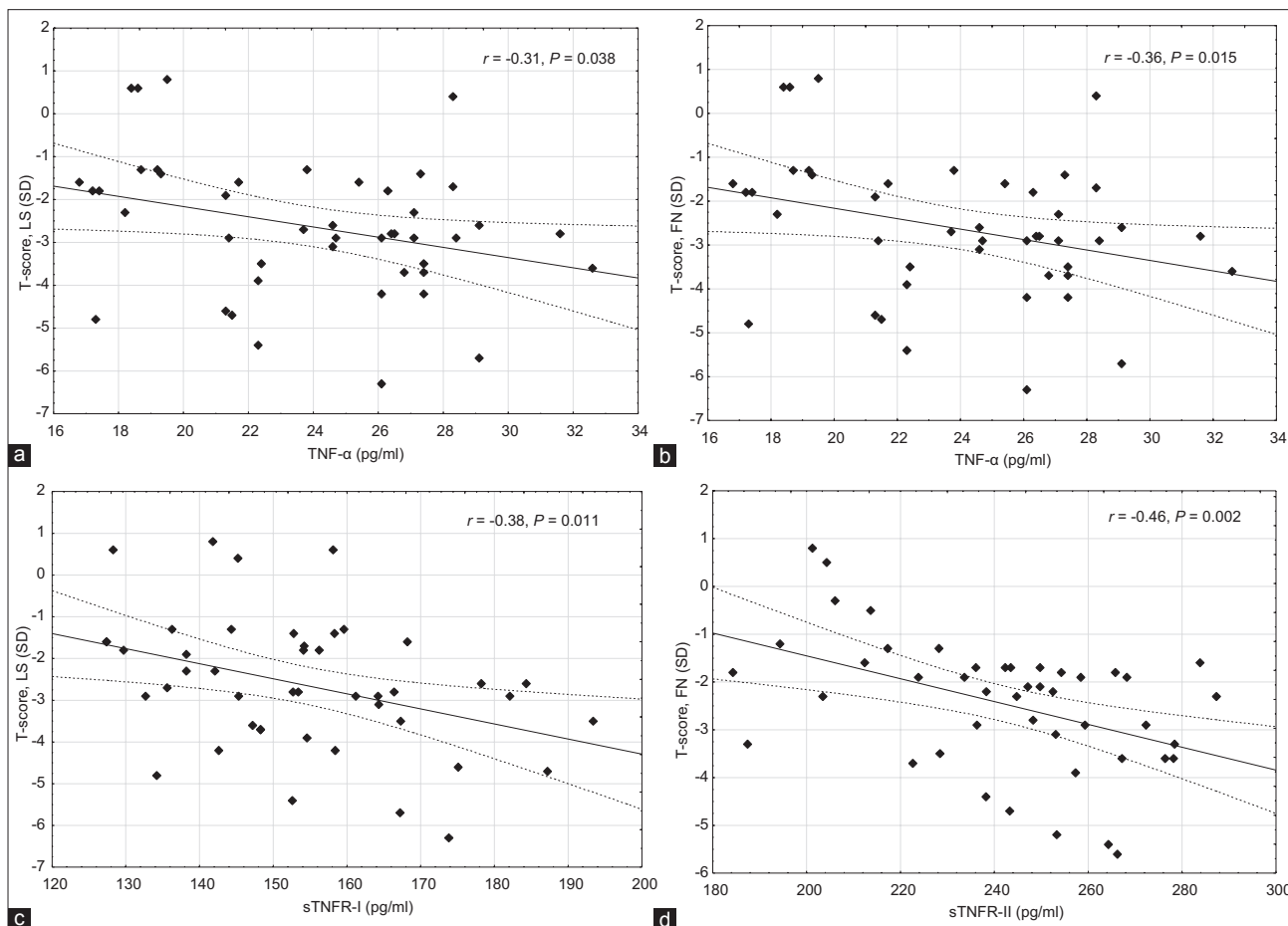
Figure 1: Correlations of TNF-α (a, b) and its receptors sTNFR-I (c), sTNFR-II (d) levels with LS and/or FN T-score from male COPD patients ($n = 45$). TNF-α: Tumor necrosis factor-alpha; sTNFR-I and II: Soluble receptor of tumor necrosis factor-alpha-I and II; LS: Lumbar spine; FN: Femur neck; SD: Standard deviation; COPD: Chronic obstructive pulmonary disease.

correlation between sTNFR-II level and $FEV_1\%$ predicted ($r = 0.07$, $P = 0.003$).

Serum osteoprotegerin and receptor activator of nuclear factor-κB ligand concentrations

As shown in Table 2, the serum OPG levels were significantly lower and the RANKL levels were significantly higher in men with very severe COPD compared to those in the control group [Table 2]. With regard to the group with advanced COPD, OPG significantly correlated with $FEV_1\%$ predicted ($r = 0.46$, $P = 0.001$) and bone formation marker ($r = 0.34$, $P = 0.023$). As shown in Figure 2, we observed a positive relationship between the level of OPG in serum and both LS and FN T-scores [Figure 2a and 2b]. Furthermore, the level of OPG was found to be negatively correlated with $PaCO_2$ ($r = -0.30$, $P = 0.043$), RANKL [Figure 3a], TNF-a [Figure 3b], and its receptor sTNFR-I [Figure 3c]. In contrast, we established an inverse relationship between the RANKL concentration and T-score both at the LS and at the FN [Figure 2] and also direct correlation with βCL ($r = 0.48$, $P = 0.001$), and sTNFR-II [Figure 3d]. There were no relationships between serum RANKL levels and pulmonary lung parameters, TNF-α and its receptor sTNFR-I.

DISCUSSION

Osteoporosis is frequently present in patients with COPD, which ultimately results in a higher risk of bone fracture.[11-15] The multicentric TOwards a Revolution in COPD Health study, which included 658 COPD patients, reported osteoporosis in 23% and osteopenia in 43% patients at the hip or the LS on DEXA scan.[12] In another relevant study, vertebral fractures were detected in 40% of 2981 COPD patients.[16] Moreover, impaired lung function has been shown to be an independent predictor of osteoporosis and increased prevalence of osteoporotic fractures in COPD patients in several studies.[12,17-19] It has previously been demonstrated that patients with advanced stages of COPD have lower BMD and more frequently have osteoporosis.[11] In our study, the prevalence of osteoporosis in subjects with advanced stages of COPD was higher than those in control subjects. Our finding is in line with several previous studies.[11,13,14,19] Furthermore, our study has again confirmed that male patients with osteoporosis had worse lung function, as evidenced by lower $FEV_1\%$ and DLCO values, and raised $PaCO_2$ levels.[19]

Nevertheless, other studies have suggested that the increased prevalence of osteoporosis in COPD patients is only partly
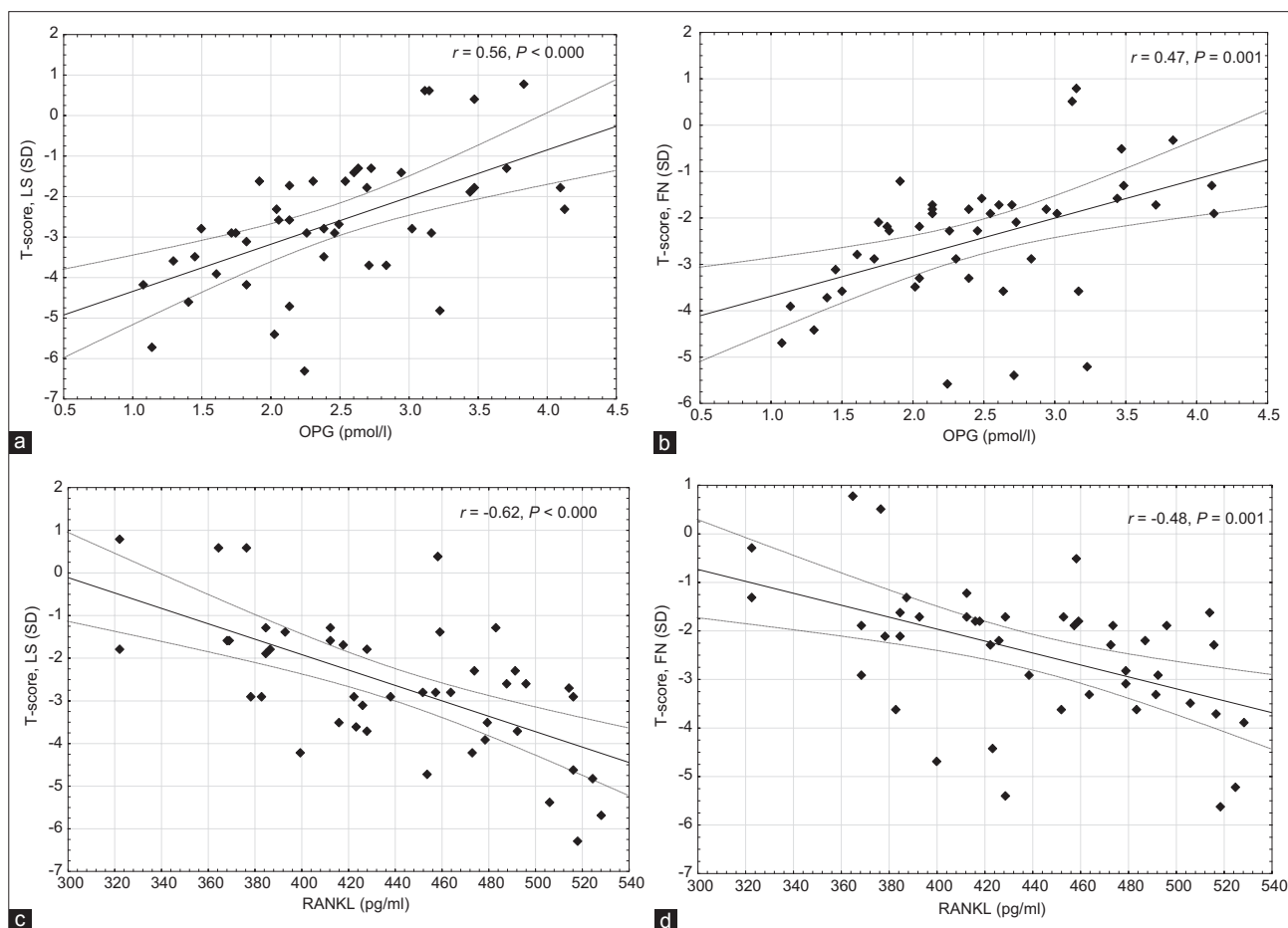
Figure 2: Correlation of OPG and RANKL with LS (a and c) and/or FN T-score (b and d) from male COPD patients ($n = 45$). OPG: Osteoprotegerin; RANKL: Receptor activator of nuclear factor-κB ligand; LS: Lumbar spine; FN: Femur neck; SD: Standard deviation; COPD: Chronic obstructive pulmonary disease.

dependent on the degree of airflow limitation and that there are additional factors involved in bone health.[20,21]

Indeed, chronic inflammation leads to the production of cytokines including the activation of the TNF-α system, growth factors, acute phase proteins, and mobilization of circulating cells that stimulate bone turnover and osteoclast-related resorption. With regard to systemic inflammation, increased concentrations of circulating proinflammatory mediator TNF-α and its direct correlation with the functional lung parameters have been reported in COPD patients.[22,23] In addition, the elevated TNF-α concentration is associated with osteoclastic-mediated resorption via activation of osteoclast surface receptors (thereby inducing activity of mature osteoclasts and the differentiation of their precursors),[24,25] and is recognized as a strong predictor of osteoporosis. TNF-α also heightens vascular molecules's adhesion to the osteoblasts and as a result leads to the intensified accumulation of osteoclast precursors in the area of bone formation.[26] In pretransplant patients with COPD, Førli et al.[27] reported a direct relationship between TNFR-II and the blood resorption marker. However, Vondracek et al.[19] did not find any difference in serum concentrations of TNF-α or its receptors TNFR-I and TNFR-II among patients with or without osteoporosis, which did not correlate with

BMD or bone turnover markers in men with severe COPD. Our present study demonstrated that serum TNF-α and its receptors levels were higher in male COPD patients than in control subjects. Moreover, there is a negative correlation between TNF-α and its receptors concentrations with airway obstruction and BMD T-score, and any relationships with bone turnover that conforms to the literary data.[24-27]

The OPG/RANK/RANKL system has been shown to have pleiotropic effects on bone metabolism,[3,5,7,11,28] vascular and immune systems[7,16,26] and has led to a new molecular perspective on osteoclast biology and bone homeostasis. Any modification in the RANKL/ OPG can induce either excessive bone resorption or, in contrast, excessive bone formation. Dysregulation of the RANKL/RANK/OPG system can be associated with certain pathological conditions, such as postmenopausal osteoporosis, bone turnover-associated osteolysis, and certain bone metastatic tumors, immune disease, rheumatoid arthritis, or cardiovascular pathology.[4,6,7,9,28,29] Interestingly, the data relating OPG, RANK, and RANKL with bone metabolism in different conditions have shown conflicting results. Mezquita-Raya et al.[12] established that reduced serum OPG levels correlated positively with BMD at the LS and were associated with postmenopausal fractures.
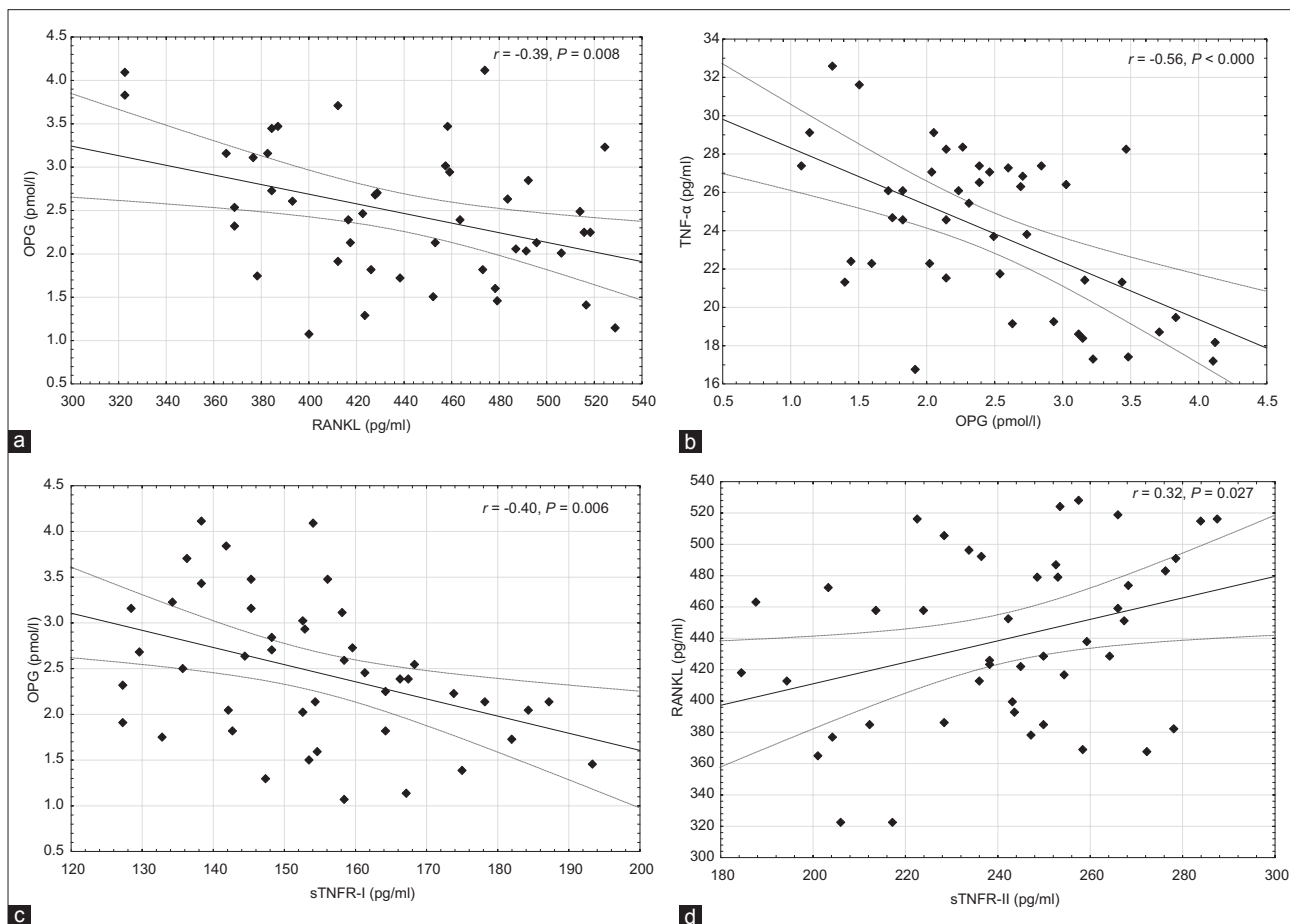
Figure 3: Relationships between OPG and RANKL levels (a), between OPG and TNF-α (b), between OPG and sTNFR-I (c), and correlation of RANKL and sTNFR-II (d) concentrations in men with COPD (*n* = 45). OPG: Osteoprotegerin; RANKL: Receptor activator of nuclear factor-κB ligand; TNF-α: Tumor necrosis factor-alpha; sTNFR-I and II: Soluble receptor of tumor necrosis factor-alpha-I and II; COPD: Chronic obstructive pulmonary disease.

Although these findings were supported by other studies,[25,30] this was not uniformly the case.[13,31,32] Importantly, one potential explanation for this discordance is that the serum OPG/RANK/RANKL concentrations have not been concomitantly measured. Bai *et al.*[33] observed that serum TNF-α, RANKL, and the ratio of RANKL/OPG levels were significantly higher in COPD/emphysema patients with low BMD group compared to control groups whereas OPG levels did not differ significantly between these groups. In the study of Duckers *et al.*,[34] the serum level of OPG was greater in COPD patients who combined with osteopenia/osteoporosis than those with normal BMD and was inversely related to hip BMD but not lumbar BMD in those with mild-to-moderate COPD. The level of RANKL and the ratio of RANKL/OPG were not determined. Pobeha *et al.*[35] showed an elevated serum OPG level in COPD patients with osteoporosis compared with normal hip BMD. No differences were observed in RANKL levels. In contrast, Eagan *et al.*[36] noted significantly lower OPG concentrations in COPD patients compared to controls groups. However, both studies of Bai *et al.*[33] and Eagan *et al.*[36] revealed that the balance of the OPG/RANK/RANKL system in COPD patients is destroyed and manifests a dominant trend toward increased RANKL. Crucially, the results from most of these

studies were conflicting and inconclusive despite evaluating patients with COPD over a wide range of severity of airways obstruction and determining circulating inflammatory markers in relation to osteoporotic status. This discrepancy of behavior of inflammatory markers may be explained by the fact that there are differences in the severity of the airflow obstruction, oxidative stress, and perfusion ratio in COPD patients.[37] The data from our observations revealed that decreased serum OPG concentration and increased RANKL levels were both associated with advanced COPD stage. In addition, OPG levels positively correlated whereas RANKL levels negatively correlated with BMD T-score at the LS and at the femur neck.

The investigations concerning the relationship between OPG and bone metabolism markers have shown contradictory results. Indeed, while Rogers *et al.*[38] established a negative correlation between OPG and the bone formation markers, the data from Pobeha *et al.*[35] showed no relation between plasma OPG and RANKL levels and bone turnover markers in COPD. In the current study, we have established that the dysregulation of OPG/RANKL system, associated with degree of airflow limitation and hypercapnia, may play an important role in the disturbance of bone turnover in men with advanced COPD stage, resulting in increased

resorptive and decreased formation processes in very severe COPD. The expression of mRNA of the OPG gene takes place in different tissues, especially in the lungs, heart, kidneys, liver, skin, bowels, and bones. Regulation of OPG synthesis is realized by a number of growth factors, sex and bone-specific hormones, and cytokines, most of which take part in bone remodeling.[26] It is interesting to note that proinflammatory mediators, particularly TNF-α, influence the regulation of the regulatory protein receptor system of the cytokine network of the TNF-superfamily, particularly OPG receptor, RANK, RANKL, osteoclast differentiation factor, and TNF-dependent inducible cytokine. In addition, it is possible that TNF-α may increase RANKL expression, thereby dysregulating OPG/RANK/RANKL system and aggravating bone tissue resorption in COPD.[33] Our data confirmed that increased TNF-α levels correlated negatively with the OPG activity in the very severe COPD patients studied.

It is well known that chronic use of systemic glucocorticoid can both influence the serum level of inflammatory cytokines (included TNF-superfamily members) and accelerate bone loss.[39] In our study, only 20% of the COPD group used systemic glucocorticoid therapy. Notably, there were no clear differences in either two evaluated skeletal sites between our patients using or not using systemic steroids (data not shown). However, given the relatively small proportion of patients receiving systemic corticoids, our finding of a nonsignificant contribution to accelerated bone loss in men with very severe COPD must be interpreted with care. As for the inhaled glucocorticosteroids only for triamcinolone acetonide a link with the frequency of fractures was proved, others are within discussion.[40] In our study, patients did not receive triamcinolone.

Our study includes some limitations. First, the investigation was conducted with a relatively small number of subjects and, therefore, the results of this study should be interpreted with caution. Second, we included only men with very severe stage COPD so that our results might not be valid to patients with others stages of COPD. Third, we did not analyze the various risk factors, including pharmacological agents, which have contributed to the development of osteoporosis and osteopenia in these patients. The most crucial factors in this inflammatory process with effects on bone are smoking and use of the corticosteroids.[41] In our study, the roles of systemic/inhaled corticosteroids and smoking were not detailed. However, although the use of oral glucocorticoids are a highly significant risk factor for osteoporosis, previous studies have shown that their predictive value is relatively poor in COPD patients.[42,43] It was also observed that the risk of developing osteoporosis in COPD patients using inhaled corticosteroids was almost the same as those not using inhaled corticosteroids.[44] Finally, in this study, we did not perform a multivariate linear regression analysis to determine the independent factors associated with bone loss due to the relatively small sample size. On the other hand, the strength of the present study was that we excluded women and important confounders related to many common comorbidities and chronic inflammation both of which can significantly impact on bone metabolism.

In conclusion, the current study confirms that an imbalance in the OPG and RANKL axis is associated with the development of osteoporosis in advanced COPD stage, either through an increase in RANKL or a decrease in OPG. Thus, the OPG, RANKL, and RANK systems are critical in the physiological mechanism bone cell activity, remodeling, and mineralization in many diseases, including COPD. Further research of the pathophysiological aspects of osteoporosis development in COPD is required to further delineate the specific causal contribution of each of these factors to COPD-related bone loss with the ultimate aim of identifying new and more effective therapeutic targets for lung disease-associated osteoporosis.

Conflicts of interest
There are no conflicts of interest.

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