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DYSREGULATED OSTEOPROTEGERIN/ RECEPTOR-ACTIVATOR OF NUCLEAR FACTOR-κB LIGAND METABOLISM AND BIOCHEMICAL MARKERS OF BONE TOURNOVER IN ADVANCED CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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***Abstract*— Pathogenesis of osteoporosis in advanced stages chronic obstructive pulmonary disease (COPD) is unclear. Therefore, the study of OPG-RANK-RANKL axis contribution to pathogenesis of osteopenic syndrome in COPD is of particular interest.**

Aim: to estimate the relationships between serum OPG, RANKL, TNF-α and bone metabolism markers levels, bone mineral density (BMD) in advanced stages COPD.

Methods: pulmonary function, BMD at the lumbar spine and femoral neck, serum OPG, RANKL, TNF-α and its receptors (sTNFR I, sTNFR II), osteocalcin (bone formation marker), and βCrossLaps (marker of bone resorption) levels were measured in 67 patients with severe and very severe stages COPD and 82 healthy.

Results: 61% COPD patients have osteoporosis and BMD were normal in 12% subjects. Osteopenic syndrome (osteopenia and osteoporosis) was determinated in 44% control individuals. Serum βCrossLaps, TNF-α and its receptors, RANKL levels were significant higher and osteocalcin, OPG concentrations were lower in patients with COPD than in control. The univariate analysis showed that OPG correlated with FEV1 (r=0,63, p<0,001), osteocalcin (r=0,57, p<0,01), both lumbar and femur neck BMD (lumbar: r=0,43, p<0,05 and femur neck: r=0,64, p<0,001), pCO2 (r=-0,39, p<0,05), RANKL (r=-0,67, p<0,001), TNF-a (r=-0,046, p<0,05), and its receptors (sTNFR-I: r=-0,41, p<0,05 and sTNFR-II: r=-0,37, p<0,05). In contrast, we established the inverse relationship between serum RANKL and BMD both at the lumbar spine and at the femur neck (r=-0,48, p<0,01 and r=-0,59, p<0,00 1 respectively); and also direct correlation with βCrossLaps (r=0,42, p<0,05). There were no relationships between serum RANKL levels and pulmonary lung parameters, TNF-α and its receptors.

Conclusion: our results suggest that serum OPG and RANKL levels participate in the pathogenesis of respiratory failure and loss of bone density in patients with advanced stages COPD *(Abstract)*

***Key words*: osteoporosis, chronic obstructive pulmonary disease, TNF-α, OPG, RANKL, osteocalcin, βCrossLaps**

I. INTRODUCTION

Over the past few years, osteoporosis has been recognized as one of the most relevant collateral problems of pulmonology, from medical, social and economic points of view. COPD, one of the most prevalent diseases, representing the fourth leading cause of death worldwide, is characterized as the usually progressive chronic airflow limitation and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases [1]. However, COPD is not only limited to respiratory symptoms, but also leads up to extra-pulmonary system effects and co-morbidities including systemic inflammation, cardiovascular co-morbidity, cachexia, muscle dysfunction, anemia and osteoporosis [2]. Currently, the various risk factors of osteoporosis development in patients with COPD are being discussed, such as the severity of hypoxemia and intensity ventilation disorders, physical inactivity, smoking, poor nutritional status, impaired peripheral muscle function, low fat free mass, hypogonadism, impaired calcium absorption, vitamin D deficiency and glucocorticoid treatment. Molecular mechanisms of osteoporosis development in the COPD have been the center of attention recently. Particularly, the function of cytokines system, growth and transcriptional factors, different regulatory proteins and enzymes have come up for discussion. Besides these aforementioned factors, there is another group of local factors that have been actively studied in recent years. They are combined in the OPG-RANKL-RANK system [3] and regarded as the basic signal mechanisms controlling bone resorption in the physiological and pathological conditions [4].

Osteoprotegerin (OPG), a soluble member of the TNF receptor superfamily expressed by the TNFRSF11B gene, is a circulating secretory glycoprotein, without a transmembrane domain produced by the osteoblasts, and plays a key role in many physiological processes, particularly in osteoclastogenesis [5]. OPG works as a decoy receptor for the

receptor-activator of nuclear factor-κB ligand (RANKL); RANKL, a membrane protein, expressed on the surface of osteoblasts, bone stromal cells, and activated T cells, plays a key role in the process of bone resorption. The receptor-activator of nuclear factor-κB (RANK), localized at the cell surface of mature osteoclasts and osteoclastic precursors, is the third protagonist [6]. Binding of RANKL to RANK stimulates differentiation of osteoclastic precursors into mature osteoclasts, and activation of mature osteoclasts. By blocking interaction between RANK and its ligand RANKL onto surface of preosteoclasts, OPG inhibits the final stage of osteoclastic differentiation and bone resorption [1, 7]. In fact, bone remodeling appears to be mainly controlled by balance of RANKL/OPG.

In vitro and animal studies have demonstrated the capacity of OPG to reduce or prevent bone resorption [8, 9, 10]. Inhibitory action of OPG on the formation of osteoclasts is proves by the results of the findings, which showed that the number of osteoclasts of transgenic mice was considerably reduced by OPG with no effects on the microphages, their precursors [10]. They demonstrate development of an osteoporotic skeletal phenotype, including a high bone-remodeling rate, decreased BMD, and increased incidence of fragility fractures and bone deformities in OPG-knockout mice. Therefore OPG (as RANKL antagonist) and RANKL (as cytokine controlling the osteoclast activity differentiation) are obviously involved in pathogenesis of osteoporosis and other metabolic skeleton diseases [11].

However, most studies are devoted to the analysis of correlation between the OPG-RANK-RANKL axis and osteopenic syndrome in postmenopausal women, hepatocirrhosis, cardiovascular and renal pathologies, and also in patients taking glucocorticoids [7, 12, 13, 14, 15, 16]. However, the study of the role of OPG and RANKL in the formation of osteoporosis in COPD is limited. Pathogenesis of osteopenic syndrome development in COPD is connected not only with the iatrogenic component, but also with pathophysiological illness patterns, demonstrated especially in systemic inflammation. Therefore, the study of OPG-RANK-RANKL axis contribution to pathogenesis of osteopenic syndrome in COPD is of particular interest.

The purpose of our research is to study the correlation between the level of serum OPG, RANKL, inflammatory markers, bone metabolism markers and bone mineral density (BMD) in severe and very severe COPD.

II. PATIENTS AND METHODS

A. Subjects:

In total, 67 clinically stable severe to very severe COPD patients (50 men and 17 women) and age-/gender-matched 62 healthy volunteers were enrolled into the study. All patients had been diagnosed with COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [17]. None of the control group subjects had ever suffered from the pulmonary diseases, neoplasm, and other chronic conditions. All pulmonary lung function tests in this group, were within the normal predictive range.

Inclusion criteria included clinically stable severe and very severe COPD patients for at least 3 months; before the examination the patients received the bone-targeted medications. Exclusion criteria were respiratory disorders other than COPD, lung cancer, known history of the inflammatory disease other than COPD, a COPD exacerbation, cardiovascular diseases (in particular, severe heart failure), endocrine, autoimmune, hepatic or renal disorders, malignancy, blood coagulation disorders or therapy with warfarin.

Height and weight were measured and body mass index (BMI) was calculated.

Smoking exposure was estimated by pack-years.

Numbers of pack-years were calculated using the formula:

Number of pack-years = years of smoking X number of daily smoked cigarettes/20

Information on medical history, smoking status and corticoid use was obtained from patient interviews and medical records. The study protocol was approved by an institutional ethical committee and informed consent was obtained from all the participants.

B. Pulmonary function test

The respiratory function of all the examined patients was appraised using a spiroanalyser Master Screen PFT Jaeger GmbH (Germany) and of bodyplethysmography Master Screen Body Jaeger (Germany) in accordance with European Respiratory Society standards [17]. Pulmonary function tests were performed under stable condition in all patients. The pre- and postbronchodilatator forced expiratory volume in one second (FEV1), vital capacity (VC), forced vital capacity (FVC) and the FEV1/FVC ratio was determined. Single-breath diffusing capacity for carbon monoxide (DLco) was assesses by the use bodyplethysmography. Patients with FEV 1/FVC ratio less than 0.7 were diagnosed as having COPD. According to the inclusion criteria for this study and based on GOLD criteria, the patients with COPD were divided in two groups: I - 36 patients with stage III (FEV 1 30-50 % of predicted), II – 31 with stage IY (less than 30% of predicted).

C. BMD measurement

Bone mineral density was determined by dual-energy X-ray absorptiometry (“Prodigy” of the firm “Lunar”, USA) at the lumbar spine (L2-L4) and no dominant femoral neck (FN) and was expressed as a T-score (standard deviations (SD) from a young, sex-specific reference mean BMD). According to recommendations of the World Health Organization (WHO), T-score between -1 to -2.5 SD was considered as osteopenia; T-score below -2.5 SD was diagnosed osteoporosis and T-score below -2.5 SD allied with one or several atraumatic fractures denoted severe osteoporosis; and T-score more than -1.0 SD was conformed to the normal BMD.

D. Assessment of fractures

The examined patients were carried out through the retrospective fracture frequency analysis. Quantity and localization of the fractures were registered. The presence of

vertebral fractures was evaluated using thoracic and lumbar spine X-rays. Lumbar spine images were unavailable in 47 patients. The history of the non-traumatic, non-vertebral fractures (hip, forearm or rib fractures) has been revealed from the medical record and patient interview.

E. Biochemical analyses

In all patients, peripheral venous blood simples from the antecubital vein were collected and were stored at -80°. The serum OPG and total RANKL levels were mesured using a commercial immune-enzyme liked assay (Biomedica Groupe, Vienna, Austria). TNF-α in the blood serum and its receptors sTNFR-I and sTNFR-II were measured using commercially test system (sandwich ELISA, R&D System, USA). The concentration of the bone metabolism markers in the blood serum β CrossLaps (β CL) reflecting bone resorption, and osteocalcin (OC) reflecting bone formation, were determined using by electrochemiluminiscence immunoassays (ROSHE Diagnostics, Switzerland). At the time of venous blood simple collection, arterial blood sample was obtained by puncture of radial artery to determine arterial oxygen tension (PaO2) and arterial carbon dioxide tension (PaCO2).

F. Statistical analyses

Data were expressed as mean ± standard deviation (SD). The Kolmogorov-Smirnov test of normality was applied. The Student's t-test was used for comparisons of continuous variables between those with COPD and control group. The Kolmogorov-Smirnov test of normality was applied. The Pearson product correlation coefficient was used to examine correlations between variables and BMD. A p-value less than 0.05 was considered to be statistically significant. All analyses were performed using the Statistical Pack-age for the Social Science (SPSS) software version 14.0 (SPSS Inc., USA).

III. RESULTS

A. Demographic and clinical characteristics of the patients:

The baseline demographic data and clinical characteristics of all patients are listed in Table 1. The mean ages of the COPD patients and control group were similar whereas BMI was significantly low in stage IY. There were significant differences between spirometric pulmonary function between COPD patients and non-COPD volunteers (p<0,001). As expected, all the parameters of respiratory function decreased in advanced stages were receiving home oxygen therapy and had been exposed to corticosteroids. The proportion of current smokers was the lowest in COPD stage IY, but there was no significant difference in pack-years smoked.

B. Bone mineral density and fractures:

Our results showed that the T-score in COPD subjects was significantly lower than that in healthy. The osteopenic syndrome (according to the WHO criteria, T-score -1 SD) has been found in 57 (85 %) COPD patients: 41 (61%) had osteoporosis at the any area, 52 (78%) patients had osteopenia at the any site, and just 8 (12%) patients had the normal BMD at the lumbar spine as well as at the femoral neck. Six of the 62 non COPD subjects had osteoporosis at the any area and 21 (34%) had osteopenia. It should be noted that the smokers with COPD had the lower T-score both at the L2-L4 and at FN as compared with the nonsmokers (p<0,05) (data not shown). We didn't find any associations between the age and the sex of the COPD patients and T-score. In subpopulation, the BMD was lower in COPD stage IY than in COPD stage III, without statistical significance (Table 1). The overall prevalence of vertebral fracture was 48% (32/67) and the prevalence of non-traumatic, non-vertebral fractures were observed in 36% (24/67) COPD patients (Table 1). In univariate analyses, the BMD L2-L4 and BMD FN was positively related to lung function: FEV1 (r=0,63, p<0,001 at L2-L4 and r=0,57, p<0,001 at FN), FEV1/FVC (r=0,59, p<0,01 at L2-L4 and r=0,51, p<0,01 at FN) and negatively correlated with pCO2 (r=-0,43, p<0,05 at L2-L4 and r=-0,39, p<0,05 в FN) and DLCO (r=-0,47, p<0,05 at L2-L4 and r=-0,38, p<0,05 at FN).

C. Bone metabolism markers:

This research established that βCL concentration in serum blood was significant higher in COPD patients in compared with control group (Table 2). The mean value of βCL was almost 2.5 times over the analogous parameter in non-COPD volunteers. The circulation of the bone formation biochemical marker OC was clearly lower in COPD patients than in the control (Table 2). The univariate analysis showed the negative correlation between βCL level and T-score at the L2-L4 (r=-0,64, p<0,001) as well as between T-score at the FN (r=-0.56, p<0.01). There was a positive association between serum OC level and T-score at the L2-L4 only (r=0,39, p<0,05). The βCL concentration, but not OC level, change depended on the degree of airway obstruction and its level correlated negatively with FEV1 (r=-0,53, p<0,01) and FEV1/FVC ratio (r=-0,46, p<0,01). The circulation biomarkers of bone formation and bone resorption were not related to BMD at the FN or at the lumbar spine in healthy volunteers. In addition, there was no association between biochemical bone turnover markers and smoking pack-year history.

D. TNF-a and its receptors:

In patients with severe and very severe COPD the serum TNF-α concentration and its receptors levels were evidently higher in comparison with control group (Table 2). We found inverse correlation between TNF-α and FEV1, OPG (r=-0,53, p<0,01), T-score both at the L2-L4 (r=0,49, p<0,01) and at the FN (r=0,46, p<0,05); and positive relationship with the bone resorption marker βCL (r=0,48, p<0,05). Interesting, the correlation analyses revealed that sTNFR-I and sTNFR-II had a significant direct correlation with T-score at the L2-L4 only (r=0,41 and r=0,44, p<0,05).

E. Serum OPG and RANKL concentrations:

The OPG levels in the blood serum were detected significantly lower in patients with severe and very severe COPD to those in control group (p<0.01) (Table 2). The RANKL levels were significantly higher in COPD patients as compared to healthy patients (p<0.05). It's interesting that COPD smokers had lower concentration of the serum OPG than nonsmokers (data not shown, p<0,05). This fact confirms once again the possible influence of the smoking on the bone

metabolism. In univariate analyses, OPG significantly positively correlated with FEV1 (r=0,63, p<0,001), parameter of bone formation (r=0,57, p<0,01), both lumbar and femur neck T-score (L2-L4: r=0,43, p<0,05 and FN: r=0,64, p<0.001, respectively) and was negative association with pCO2 (r=-0,39, p<0,05), RANKL (r=-0,67, p<0,001), TNF-a (r=-0,046, p<0,05), and its receptors (sTNFR-I: r=-0,41, p<0,05 and sTNFR-II: r=-0,37, p<0,05). In contrast, we established the inverse relationship between the RANKL concentration in the blood serum and T-score both at the L2-L4 and at the FN (r=-0,48, p<0,01 and r=-0,59, p<0,00 1 respectively); and also direct correlation with marker of bone resorption (r=0,42, p<0,05). There were no relationships between serum RANKL levels and pulmonary lung parameters, TNF-α and its receptors.

IV. DISCUSSION

Osteoporosis is one of the extrapulmonary effects of COPD. Indeed, the prevalence of osteoporosis, osteopenia and vertebral fractures in patients with COPD has been reported an overall of 35.1% (range 9–69%), of 38.4% (range 27–67%) [18] and 24-63 % [19], respectively. Most studies demonstrated inferior hip and spine BMD among those with COPD compared to controls without COPD [20, 21, 22, 24]. In a cross-sectional study, Silva et al. evaluated the BMD in clinically stable COPD patients by DEXA scan and reported osteoporosis and osteopenia each in 42% of patients. Bhattacharyya et al. studied BMD by ultrasound bone densitometer in a small number of advanced COPD patients and reported osteopenia/osteoporosis in 27 (73%) patients [20]. The multicentric TORCH (TOwards a Revolution in COPD Health) study, which included 658 COPD patients, had reported osteoporosis in 23% and osteopenia in 43% patients at the hip or the lumbar spine on DEXA scan. So, previous studies reported that BMD in COPD patients was lower than in healthy subjects and the increase of COPD severity was associated with the reduction of mean values of BMD [21]. It have been demonstrated that patients with advanced stages COPD have lower BMD and T-score and more frequently have osteoporosis [22]. In our study the reduction of T-score has been found in about 85% of our patients with COPD advanced stages. The prevalence of osteoporosis in severe to very severe COPD was higher than those in control healthy (61% versus about 10%). The degree of lung function reduction and severity of COPD is found to correlation with bone loss. Our finding is in line with several other studies [22, 23]. The significant increase in prevalence of osteoporosis in COPD patients over a period of 3 years and the increase of the vertebral fractures over time demonstrated in the study of Gaat-Verboom et al. [24]. However, some studies are showing the negative results in terms of dependency on pulmonary function parameters [25, 26]. These results suggest that the increased prevalence of osteoporosis in COPD patients is only partly dependent on the degree of airflow limitation and there are more factors involved in the bone health.

It's known, one of the consequences of osteoporosis is atraumatic fractures. Many studies to date have shown an increased risk of fractures in patients with COPD. Hip fractures are the most serious and devastating among osteoporosis-induced fractures because of their high disability, morbidity and mortality. Moreover hip fracture is a strong risk factor for future vertebral and non-vertebral fractures. De Luise et al. in the Danish cohort study reported a 60–70% higher risk of death following hip fracture in patients with COPD than those without COPD [27]. Also Regan et al. showed poor outcome in COPD patients who had hip fractures. COPD was diagnosed in 47% of the 12,646 patients with hip fracture. The mortality rate increased in severe COPD [28]. Much attention has been directed toward the negative outcomes of hip fractures; however vertebral fractures are often results in kyphosis, chronic pain and impaired respiratory function. Majumdar et al. demonstrated the prevalence of vertebral fractures in COPD patients with acute exacerbation [29]. Nuti et al. detected vertebral fractures by lateral chest radiograph in 40% of 2,981 COPD patients [30]. Watanabe at al. indicated increased prevalence of vertebral fractures in COPD patients and an association of lower FEV1 with multiples vertebral fractures [31]. The prevalence of vertebral fracture (48%) and the prevalence of non-traumatic, non-vertebral fractures (36%) in COPD patients observed in our present study.

Interestingly, eleven of our sixty seven COPD patients without osteoporosis had the atraumatic fractures and abnormal levels of bone metabolism markers in the serum. It can be assumed, in COPD the increase in prevalence of bone fragility is most likely due to deterioration of the microarchitecture, without a significant impact on bone mineral density. Indeed, chronic inflammation leads to the production of cytokines, different growth factors, an increased acute phase proteins and other circulating cells identified in various studies, an activation of the TNF-α system, stimulating bone turnover and osteoclast related resorption, which is associated with an bone fragility [1, 19]. Patients with GOLD stages III and IV showed a lower bone formation rate than those with GOLD stages I and II. These microarchitectural changes are responsible for reduced bone strength and increased risk of fractures. With regards to systemic inflammation, increased concentrations of circulating inflammatory mediators, such as TNF-α, IL-1, Il-6 have been reported in COPD patients [2].

According to the research dates an elevated level of TNF-α and its direct correlation with the functional lung parameters observed in COPD [32, 33]. It is known that TNF-α is related to the molecular myopathy markers and at the same time it is involved in the process of the bone resorption [32]. In addition, the elevated TNF-α concentration is recognized as a strong predictor of the osteoporosis, whose heightened activation in the COPD intensifies osteoclastic-mediated resorption via activation of osteoclast surface receptors, inducing activity of mature osteoclasts and the differentiation of their precursors [34]. Besides TNF-α heightens vascular molecules expression of adhesion on the osteoblasts and as a result leads to the intensified accumulation of the osteoclast precursors in the area

of the bone formation [35]. In pre-transplant patients with COPD Forli et al. reported a direct relationship between TNF-α receptor II and the resorption marker βCTX [36]. Our present study demonstrates that serum TNF-α and its receptors levels were higher in COPD patients than control subjects. In COPD group, TNF-α and its receptors concentrations correlated negatively with airway obstruction and BMD, and positively with bone resorption marker that conforms to the literary data [35, 36, 37].

The OPG/RANK/RANKL system has been shown to have pleiotropic effects on bone metabolism [3, 5, 7, 11], vascular and the immune systems [7, 16, 35], 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, bone tumor and certain bone metastatic tumors, immune disease, rheumatoid arthritis or cardiovasculaire pathology [4, 6, 7, 9, 14, 15, 16]. However, the OPG, RANK, and RANKL axis is most studied in cardiovascular pathology, and in postmenopausal women with osteoporosis. Interestingly, the data of the different researches concerning the relations of the OPG, RANK, and RANKL with bone metabolism showed however the conflicting results. So, Mezquita-Raya et al. established serum OPG level reduction, which correlated positively with BMD just at the lumbar spine and was associated with the availability of the fractures got by women in postmenopausal period [12]. The other researches showed the similar data in which the patients with the normal BMD had the higher OPG level in comparison with the analogous rate in the osteopenic syndrome [34, 37]. Warren et al., on the contrary, found that there was no correlation between the OPG level and the BMD in postmenopausal women [38]. The same results are got by Khosla et al. and Penisi et al. [13, 39]. However, only in a few studies have the authors examined the serum OPG/RANK/RANKL concentrations in COPD patients. Bai et al. examined the level of inflammatory cytokines and OPG/RANK/RANKL protein levels in 80 stable male COPD patients. The authors observed that serum TNF-α, RANKL and the ratio of RANKL/OPG levels were significantly higher in the COPD/emphysema with low BMD group than in other two groups (control and COPD with normal BMD). Opposite, the OPG level did not significantly differ among these three groups [40]. In the study of Duckers et al., the serum level of OPG was greater in COPD patients who combined with osteopenia/osteoporosis than those with normal BMD and inversely related to hip BMD, but the level of RANKL and the ratio of RANKL/OPG were not determined [41]. Pobeha et al. showed the elevation serum OPG level in COPD patients with osteoporosis compared with normal hip BMD. No differences were observed in RANKL levels [42]. In contrast, Eagan et al. noted significantly lower OPG concentration in COPD patients compared to the control [43]. But the both studies of Eagan and Bai revealed that the balance of the OPG/RANK/RANKL system in COPD patients is destroyed and manifests a dominant trend for RANKL [40, 43]. The date from our observation reveal the decrease of serum OPG concentration decrease and the increase of RANKL level in patients with advanced stages COPD. Therewith we could note the positive correlation between the OPG level and BMD in the different skeleton areas; serum RANKL concentration correlated negatively with both T-score at the lumbar spine and at the femur neck.

The investigations concerning the relationship between OPG and the bone metabolism markers showed the contradictory results. Thus Rogers et al. established the negative correlation between OPG and the bone formation markers [44]. In contract, according to data of Pobeha et al., there were no relation between plasma OPG and RANKL levels and bone turnover markers in COPD patients [42]. In our present research we established the dysregulation of OPG/RANKL system in relation to bone metabolism markers in patients with advanced stages COPD, which could indicate increasing resorptive and decreasing formation processes in the COPD.

The expression of mRNA of the OPG gene takes place in the different tissues especially in the lungs, heart, kidneys, liver, placenta, skin, bowels and bones. Regulation of the OPG synthesis is realized by a number of the growth factors, sex and bone-specific hormones, cytokines, most of whom take part in the bone tissue remodeling [35]. It's interesting to note that proinflammatory mediators, particularly TNF-α, influence on the regulation of the regulatory protein receptor system of the cytokine network of the TNF-α family, particularly OPG receptor, RANK, RANKL, osteoclast differentiation factor and TNF-dependent inducible cytokine. Moreover, it's established the possibility of TNF-α to heighten the RANKL expression that could lead up to the imbalance in the OPG/RANKL system, therefore intensifying the bone tissue resorption in COPD [40]. The increased TNF-α level is supposed to correlate with the OPG activity that is showed in our research.

V. CONCLUSION

In conclusion, the present study confirms that imbalance between OPG and RANKL can be responsible for the development of osteoporosis in advanced stages COPD, either through an increase in RANKL and/or a decrease in OPG. Thus OPG, RANKL and RANK make the system, which is the physiological mechanism of the osteoclastogenesis regulation in many diseases, particularly in the COPD, having a delicate and differentiated influence on the bone cell activity, its remodeling and mineralization. The further research of the pathophysiological aspects of the osteoporosis development in the COPD will lead, in its turn, to the deeper comprehension of mechanisms underlying COPD-related bone loss that is necessary for the search of the new and more effective programs of osteoporosis therapy in lung diseases.

TABLE I. **CLINICAL CHARACTERISTICS OF PATIENTS WITH COPD AND HEALTHY SUBJECTS**

Parameters	*Control*	*Total COPD*	*COPD stage III*	*COPD stage IY*
Number (n)	62	67	36	31
Age, years	61,2±7,2	64,1±7,1	63,8±7,3	64,4±6,7
Male/Female	46/16	50/17	26/10	24/7
Smoking status (current/ex/ never smokers)	16/12/34	35/26/5	23/10/3	12/16/2
Smoking Index (pack-years)	44,2±29,6	58,9±31,6	63,4±32,1	54,3±33,1
FEV1, %	98,3±6,8	31,7±6,5*	39,2±6,4*	22,2±6,7*
FVC, %	97,6±11,2	69,2±7,5*	64,2±5,2*#	47,6±7,2*#
ОФВ1/FVC, %	85,6±6,8	37,8±5,6	45,3±6,7*	30 ± 4,3*#
DLCO, %	104,7±14,2	54,3±16,1*	46,3±13,2*	37,3±15,6*
BMI, kg/m²	25,8±3,2	20,7±2,1	22,3±2,4	19,1±1,7*
- inhaled GCs - oral CGs - home oxygen	- - -	32 (48%) 17 (25%) 29 (23%)	15 (41%) 8 (22%) 9 (25%)	17 (54%) 9 (29%) 13 (42%)
Т-критерий, L2-L4, SD	1,3±0,4	-3,8±0,7*	-3,3±0,6*	-4,2±0,9*
Т-критерий, FN, SD	1,7±0,7	-3,0±0,5*	-2,7±0,6*	-3,3±0,5*
Vertebral fractures	2 (3%)	32 (48%)	14 (39%)	18 (58%)
Non vertebral fractures	4 (6%)	24 (36%)	11 (31%)	13 (42%)

Note: Data are presented as mean±standard deviation; * - Pairwise comparisons with control group as the reference (p<0,001); # - difference statistically significant between III and IY stages COPD (p<0,05).
Abbreviations : COPD, chronic obstructive pulmonary disease; BMI, body mass index; FEV1, forced expiratory volume in one second; FVC, forced volume capacity; DLCO: single-breath diffusing capacity for carbon monoxide; SD, standard deviation ; GCs, glucocorticoids

TABLE II. **SERUM BIOCHEMICAL PARAMETERS IN COPD PATIENTS AND HEALTHY VOLUNTEERS**

Parameter	***Control (n=62)***	***Total COPD (n=67)***	***P value***
βCrossLaps, µg/l	0,27±0,12	0,86±0,16	p<0,01
Osteocalcin, pg/ml	17,2±2,7	6,7±3,1	p<0,05
TNF-α, pg/ml	7,8±3,2	22,6±3,6	p<0,01
sTNFR-I, pg/ml	131,6±11,7	166,2±7,3	p<0,05
sTNFR-II, pg/ml	252,4±21,1	183,2±13,6	p<0,01
OPG, pmol/l	2,23±1,16	6,82±1,14	p<0,01
RANKL, pg/ml	357,8±42,3	487,6±36,5	P<0,05

Abbreviations: TNF-α, tumor necrosis factor-α; sTNFR-I and II, soluble receptor TNF-α I and II ; OPG, osteoprotegerin ; RANKL, receptor-activator of nuclear factor-κB ligand

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