DR. ERDAL IN (Orcid ID : 0000-0002-8807-5853)

Article type : Original Article

Endocan as a Potential Biomarker of Disease Severity and Exacerbations in COPD

Erdal İn, M.D.[1] , Mutlu Kuluöztürk, M.D.[1], Teyfik Turgut, M.D.[1] ,
Ayşegül Altıntop Geçkil, M.D.[2] , Nevin İlhan, M.D.[3]

[1] Department of Pulmonary Medicine, Firat University, School of Medicine, Elazig, Turkey
[2] Department of Pulmonary Medicine, Malatya Training and Research Hospital, Malatya, Turkey
[3] Department of Medical Biochemistry, Firat University, School of Medicine, Elazig, Turkey

Corresponding Author:
Assoc. Prof. Dr. Erdal İn
Address: Firat University Faculty of Medicine
Department of Chest Diseases,
23119 Elazig, TURKEY
Tlf: +904242333555/2865
E-mail: inerda@gmail.com

All authors read and approved the final manuscript.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/CRJ.13320

Conflicts of interest: The authors do not have any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. The authors have no conflicts of interest to declare.

Endocan as a Potential Biomarker of Disease Severity and Exacerbations in COPD

Abstract

Introduction: Endocan is a proteoglycan that is regarded as a novel marker of endothelial dysfunction. Endothelial dysfunction in pulmonary vascular bed is known to play an important role for the pathogenesis of COPD.

Objective: This study aimed to determine serum endocan levels in patients with stable COPD and acute exacerbation of COPD (AECOPD) and to test the relationship between serum endocan levels and exacerbations.

Methods: This study enrolled a total of 55 COPD patients, 24 of which had AECOPD and 31 had stable COPD. All patients' basic demographic and clinical data were recorded and blood samples were collected.

Results: Serum endocan levels were significantly higher in the AECOPD group compared to the stable COPD and control groups (for both $p<0.001$) and stable COPD group had higher levels than the control group ($p<0.005$). Additionally, serum endocan levels were negatively correlated with FVC, FEV1, partial oxygen pressure, and oxygen saturation ($r= -0.30$, $p=0.03$; $r= -0.34$, $p=0.01$; $r= -0.34$, $p=0.01$ and $r= -0.36$, $p=0.007$, respectively), and positively correlated with disease duration and systolic pulmonary artery

pressure (r=0.47, p<0.001; r=0.31, p=0.02, respectively). A cut-off value of 434.29 pg/mL for endocan predicted exacerbation with a sensitivity of 79% and a specificity of 84% (AUC: 0.778, 95% Cl 0.648-0.909; p<0.001). Logistic regression analysis revealed that increased endocan levels was independent predictor of COPD exacerbation (OR=9.32, 95%CI, 1.64-52.95; p=0.01).

Conclusion: Endocan may be a novel biomarker for detection of endothelial dysfunction and prediction of exacerbations in patients with COPD.

Key words: Endocan, endothelial cell specific molecule-1, chronic obstructive pulmonary disease, endothelial dysfunction, systemic inflammation.

1. **Introduction**

Chronic Obstructive Pulmonary Disease (COPD) is a preventable and treatable disease characterized by persistent respiratory symptoms and air flow limitation. Having a high mortality, COPD is estimated to be the third major cause of death worldwide in 2020 (1). COPD is characterized by chronic inflammation in airways, lung parenchyma, and pulmonary vascular structures. Furthermore, it is well established that various cytokines and acute phase proteins indicating systemic inflammation increase in COPD, especially in advanced stages and during exacerbations of the disease (2). In addition to inflammation, it has been shown that endothelial dysfunction affecting pulmonary vascular bed has an important role for COPD pathogenesis, and that marker of endothelial dysfunction increase in patients with COPD (3).

Endocan, previously known as endothelial cell-specific molecule-1 (ESM-1), was cloned from the human umbilical vein endothelial cell cDNA library. In humans, it is encoded by a single gene, the *ESM-1* gene, localized on chromosome 5 at the position 5q11.2. Endocan is an endothelial cell-associated proteoglycan with a molecular weight of 50 kDa that is preferentially expressed by renal and pulmonary endothelium. Proangiogenic molecules, such as pro-inflammatory cytokines, which is also known as tumor necrosis factor α (TNF-α), interleukin (IL)-1 and vascular endothelial growth factor (VEGF), play major roles in

endocan's upregulation. Additionally, because endocan reveals as a result of endothelial activation and dysfunction, it is considered to be a novel tissue and blood-based relevant biomarker (4, 5). Endocan also plays significant roles in molecular interactions with various mediators, which are fundamental for the modulation of biological processes such as cell adhesion, proliferation, migration, and neovascularization (6). It was reported in previous studies that endocan had a major role in cancer angiogenesis, prognosis and metastasis, and its serum levels were reported to be elevated in various types of cancers with vascular endothelial involvement (7). In addition to malignancies, several studies defined endocan as a marker of endothelial dysfunction, especially in endothelium-dependent pathological dysfunctions such as cardiovascular diseases (8, 9).

The relationship between lung disorders and endocan had been previously studied in the literature. Studies on disorders like pneumonia, acute respiratory distress syndrome, obstructive sleep apnea syndrome (OSAS), and pulmonary embolism have reported important relations between endocan level and disease severity (10-13). Two most recent trials on COPD patients have shown that serum endocan levels increased in both stable COPD and during exacerbations (14,15), and that increase was related to a decline in respiratory function (15).

In the present study, it was aimed to determine serum endocan levels in patients with stable COPD and acute exacerbation of COPD (AECOPD), to evaluate the relationship between serum endocan levels and disease severity, and to analyze role of endocan to predict exacerbation.

2. Materials and Methods

2.1 Design of the Study and the Subjects

This study included a total of 55 COPD patients, of which 24 had AECOPD, and 31 had stable COPD, who presented to the outpatient clinic of Chest Diseases in the Faculty of Medicine of Firat University between September 2017 and December 2017 and who met the inclusion criteria. The control group of the study included 30 healthy individuals, who were matched for age and sex with the study group.

COPD diagnosis was made on the basis of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria; based on spirometry tests (post-bronchodilator $FEV_1/FVC < 0.70$) in individuals who had valid symptoms for the disease (dyspnea, chronic cough or sputum production) and/or was subjected to risk factors (1). AECOPD was defined as an acute worsening of respiratory system symptoms that required additional therapy. In our study, the severity of exacerbation was determined according to GOLD criteria; it was classified as mild (patients who were treated only with short-acting bronchodilators (SABA)), moderate (patients who were treated with antibiotics and/or oral corticosteroids in addition to SABA) and

severe (hospitalized patients). The study participants' demographic and clinical data, and spirometric measurements were recorded. Blood samples of patients diagnosed with COPD were taken after anamnesis, physical examination, and respiratory function tests were completed. In AECOPD cases, collection of blood samples and spirometry tests were conducted prior to the exacerbation treatment.

Patients with acute myocardial infarction, acute coronary syndrome, unstable angina pectoris, heart failure, renal failure, peripheral arterial disease, diabetes, malignancy of any organ, sepsis, respiratory disorder with a potential of causing pulmonary hypertension (pulmonary embolism, chronic thromboembolic pulmonary hypertension, interstitial lung disease, asthma, obesity hypoventilation syndrome, chest wall deformity) were excluded.

2.2 Pulmonary Function Testing

The pulmonary function tests were done using a spirometry device (Medgraphics Ultima CPX 790705-205, St. Paul, MN, USA). The standard spirometric examination was conducted according to European Respiratory Society (ERS) criteria. Forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) are expressed as percentages of predicted values (FEV_1% pred and FVC% pred) according to the prediction equations of the ERS (16).

2.3 Arterial Blood Gas Measurement

Arterial blood gas samples of COPD patients were taken at rest, in a sitting position and breathing room air at the room temperature. Samples were measured by a blood gas analyze device (Rapid lab 348. Biobak., Chiron, Bayer Diagnostic, UK).

2.4 Echocardiography

Echocardiography (ECHO) was performed by sing 3.4 MHz transducer probe with two-dimensional, classical and tissue Doppler. Right ventricle systolic pressure was calculated using the tricuspid regurgitation jet and an estimation of right atrium pressure (RAP) based on inferior vena cava size and collapsibility. Hence, systolic pulmonary artery pressure (sPAP) was calculated by using continuous wave Doppler and applying the Bernoulli equation [sPAP = 4 x (peak TR velocity)2 + RAP] (17).

2.5 Measurement of Serum Endocan Levels

For the study, 8 ml of blood was drawn from the cubital vein. The collected venous blood samples were centrifuged at 2000 RPM, and then, the serum samples were stored at -80 ºC until the day of study. Human Endocan/ESM1 levels were analyzed in the serum samples by using ELISA kit (Catalog no: EK0762,

Boster Biological Technology Co., Ltd., USA) in accordance with kit procedures. The absorbance values were retrieved spectrophotometrically at 450 nm from the ELX800 ELISA reader. For washing the plates, automatic-washer Bio-tek ELX50 (BioTek Instruments, USA) was used. The results were reported in pg/mL. The measurement range was 31.2-2000 pg/mL while the minimum measurable level was <10 pg/mL.

2.6 Statistical Analysis

The statistical analyses were conducted by using IBM SPSS Statistics 21 (Statistical Product and Service Solutions version 21, authorization code: d91314f638c364094170, Armonk, NY, USA) software. The results were presented as mean ± SD. The statistical significance level was determined as $p<0.05$. Student-t test was used for comparing two independent samples. For multiple sample comparisons, One-Way ANOVA test was conducted. Additionally, Tukey test was conducted to determine the importance of any significant difference detected. ANCOVA was used to control for potential confounders such as age, sex, disease duration. Chi-square (X2) test was used to compare the gender distribution between the groups while the Pearson correlation test was used in the evaluation of the parametric values. The *cut-off* value for endocan was determined by using the "Receiver Operating Characteristic" (ROC) analysis method, and sensitivity and specificity values for endocan were determined according to this value. With ROC curve, "Area under Curve" (AUC) value was determined. Binary logistic regression analyses were used for multivariate analysis to assess which variables were predictive of COPD exacerbation, and odds ratios (ORs) were calculated with a 95% confidence interval (CI).

3. RESULTS

3.1 Basic Demographic and Laboratory Data

The study included 55 COPD patients of whom 31 had stable COPD and 24 had AECOPD; a total of 30 healthy controls were also enrolled. There was no significant difference between the three groups with respect to age, sex, or body mass index (BMI) (for age; $p=0.176$, for sex; $p=0.564$, $X^2=1.670$, for BMI; $p=0.334$). It was determined that 15 (62.5%) of the patients out of the 24 patients in AECOPD group had moderate exacerbation while 9 (37.5%) of them had severe exacerbation.

An analysis of basic laboratory parameters demonstrated that the AECOPD group had a significantly higher leukocyte count than both stable COPD group and the control group ($p<0.001$, $p<0.005$, respectively). Hemoglobin levels were significantly higher in the stable COPD group than the controls

($p<0.05$) while hematocrit levels were significantly higher in both COPD groups than the control group (for both groups $p<0.005$) (Table 1).

3.2 Comparison of COPD groups

A comparison of the two COPD groups showed that the AECOPD group had a significantly longer disease duration and significantly higher sedimentation and C-Reaktive protein (CRP) levels (for all parameters $p<0.005$) (Table 1).

An analysis of the basic spirometric data of COPD patients revealed that the AECOPD group had a significantly lower FVC (%), FEV_1 (%), and PEF (%) levels compared to the stable COPD group (for all parameters $p<0.001$).

An analysis of ABG and ECHO data demonstrated that partial oxygen pressure (PaO_2) and oxygen saturation (SaO_2) were significantly lower, and partial carbon dioxide pressure ($PaCO_2$) and sPAP values were significantly higher in the AECOPD group (for all parameters $p<0.001$).

Additionally, mMRC and CAT scores were significantly higher in the AECOPD group (for both parameters $p<0.001$) (Table 2).

3.3 Evaluation of serum endocan levels

The mean serum endocan levels of the controls, stable COPD, and AECOPD were 142.30 ± 109.57 pg/mL, 320.72 ± 188.23 pg/mL, and 584.40 ± 253.77 pg/mL, respectively. According to these results, serum endocan levels were significantly higher in AECOPD group than the other two groups (for all, $p<0.001$). Additionally, serum endocan levels were significantly higher in stable COPD group than the control group ($p<0.005$). Figure 1 shows the serum endocan levels of three groups.

Even when the data was corrected for age, sex and BMI using ANCOVA, serum endocan levels remained significantly higher in AECOPD patients than in stable COPD patients and healthy control subjects (for all, $p<0.001$). Additionally, serum endocan levels remained significantly higher in stable COPD patients than in healthy control subjects ($p<0.01$).

Similarly, even when the data was corrected for disease duration, PaO_2, FEV_1 (%) and sPAP using ANCOVA, serum endocan levels remained significantly higher in AECOPD patients than in stable COPD patients ($p<0.01$).

3.4 Correlation Analysis

There was a negative correlation between serum endocan levels and FVC (%pred) and FEV_1 (%pred) levels (r= - 0.30, p=0.03 and r= - 0.34, p=0.01, respectively), and there was a positive correlation between serum endocan levels and disease duration (r=0.47, p<0.001) (Figure 2).

Additionally, negative correlation was determined between the serum endocan levels and PaO_2 and SaO_2 levels (r= - 0.34, p=0.01 ve r= - 0.36, p=0.007), while positive correlation was observed between the serum endocan levels and sPAP levels (r=0.31, p=0.02) (Figure 3).

3.5 ROC Curve Analysis

When endocan was evaluated with receiver operating characteristic (ROC) curve analysis to estimate exacerbation in patients with COPD, the area under the curve (AUC) value was detected as 0.778 (95% Cl 0.648-0.909; p< 0.001). In addition, the sensitivity and specificity of endocan were found as 79% and 84%, respectively when the optimal cut-off value was specified as 434.29 pg/mL in terms of estimating exacerbation (Figure 4).

3.6 Logistic Regression Analysis

The results of binary logistic regression analysis of the potential predictors of COPD exacerbation are shown in Table 3. Multivariate logistic regression analysis revealed that endocan ≥ 434.29 pg/mL (OR: 9.32, 95%CI 1.64-52.95; p=0.01) and sPAP ≥ 35 mmHg (OR: 6.29, 95%CI 1.06-37.49; p=0.04) were independent predictors of COPD exacerbation.

4. Discussion

The results of the present study, which was performed to determine serum endocan levels to demonstrate vascular endothelial dysfunction in COPD patients, indicate that serum endocan levels were significantly higher in both stable COPD and AECOPD patients compared to the control subjects, and this increase was more prominent in the AECOPD patients. Furthermore, serum endocan levels were negatively correlated with FVC, FEV_1, PaO_2 and SaO_2 levels, and were positively correlated with disease duration and sPAP values.

It is believed that endothelial injury occurs by various mechanisms in COPD. These include the direct toxic effect of smoking on endothelial cells, vascular inflammation, increased oxidative stress, autoantibody development against endothelial cells, and reduced activation of the antioxidant pathway in endothelial cells. Furthermore, it is also known that increased release of proinflammatory and vasoconstrictor mediators and reduced release of hemostatic and vasodilatory mediators from vascular endothelium significantly contribute to endothelial dysfunction in COPD (3). Analyzed in various disease states,

endocan is considered a marker of endothelial dysfunction. There are only two recent studies that sought a relationship between COPD and endocan. Pihtili et al. included 47 stable COPD patients and 41 control subjects in their study; they demonstrated a significantly higher serum endocan level in stable COPD patients than the controls. When they grouped patients according to the GOLD staging, however, they observed no significant difference between endocan levels (14). In another study, where 55 stable COPD patients, 31 AECOPD patients, and 27 healthy controls were studied, serum endocan levels of both COPD groups were higher than that of the control group, with the highest level being in the AECOPD group (15). Our study also revealed higher serum endocan levels in both stable and AECOPD patients than the controls. Besides, endocan levels were significantly higher in AECOPD patients than stable COPD patients. These results are in agreement with those reported by the two other studies. In addition, Dai et al. (15) observed a negative correlation between serum endocan levels and FEV_1/FVC, FVC (Lt) and FEV_1/Predictive FEV_1 levels. On the other hand, our study showed a negative correlation between serum endocan levels and FVC (%pred) and FEV_1 (%pred) levels. COPD is a systemic disorder that is also expected to affect vascular endothelium. Although endothelial dysfunction seen in pulmonary vascular bed has also been reported to occur in early stages of COPD, it is also known to be associated with disease severity and FEV_1 levels and to increase in exacerbation periods (18). Our results also support this knowledge. In our study, in the correlation analysis that contains all the COPD patients, it was observed that the duration of the disease was positively correlated with endocan levels. Furthermore, it was determined that AECOPD patients had longer duration of disease compared to stable COPD patients. With the increased duration of disease, it is expected that disease severity and endothelial dysfunction also increase. Thus, it can be stated that the duration of disease, in addition to inflammation, also influences serum endocan levels. Our study revealed higher serum endocan levels in stable COPD patients compared to the controls. Additionally, it was noted that, as spirometric measurements (FVC and FEV_1) were impaired, endocan levels increased and AECOPD patients had higher endocan levels. This increase in endocan levels may be associated with hypoxia-induced increase in pulmonary vascular resistance and oxidative stress in addition to pulmonary vascular inflammation. Hence, our study revealed a positive correlation between serum endocan levels and sPAP and a negative correlation between endocan levels and PaO_2 and SaO_2 levels. Increased sPAP may be related to increased resistance in pulmonary vascular bed secondary to alveolar hypoxemia while reduced PaO_2 and SaO_2 levels may be linked to a more diffuse disease and impaired ventilation/perfusion balance. In addition to other causes, increased resistance of pulmonary vascular bed may intensify endothelial dysfunction and increase endocan release from the endothelium. However, in our study, sPAP tests were conducted with ECHO. While ECHO is the best screening method used in pulmonary hypertension evaluation, the sPAP determined with ECHO in patients with advanced lung diseases may not be able to

reflect the true pressure. In pulmonary hypertension diagnosis, the gold standard method is right heart catheterization (19). Therefore, right heart catheterization and hemodynamic measurements should be conducted to reveal the relationship between endocan and PAP in a better way.

In COPD, exacerbations affect health status and course of disease negatively while leading to hospitalization (1). COPD is characterized by a systemic inflammation accompanied by the pulmonary vascular remodeling in addition to abnormal inflammatory responses in the lungs, and these inflammatory processes are known to be aggravated by exacerbations (20, 21). In COPD patients, it was observed that several biomarkers were increased at the onset of exacerbation and these biomarkers were decreased in the treatment process (22). In the current study that investigated endothelial nitric oxide (eNO) pathway, it was reported that in stable COPD; eNO synthase function was disrupted and this disruption was temporarily aggravated during exacerbations, and partially improved by systemic steroid treatment (23). In another study, the effectiveness of potential biomarkers was investigated to diagnose exacerbations early in COPD patients. In this study, which investigated several biomarkers in L-arginine pathway, it was discovered that both asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) distinguished AECOPD patients with high sensitivity and specificity (AUC: 0.81, $p = 0.001$; AUC: 0.91, $p < 0.001$, respectively). In the study conducted by Ruzsics et al., it was stated that ADMA and SDMA could be helpful agents in the early diagnosis of AECOPD (24). Similarly, our study also studied the *cut-off* level of endocan to predict acute exacerbations. A *cut-off* level of 434.29 pg/mL had a sensitivity of 79% and a specificity of 84% for predicting acute exacerbations (AUC: 0.778, $p< 0.001$). Also, it is shown that increased endocan levels was found an independent predictor of COPD exacerbation according to logistic regression analysis. According to our study results, endocan may be a rapidly conclusive and useful marker both for detection of endothelial dysfunction and prediction of acute exacerbations in COPD. However, there is an insufficient body of literature knowledge to support our results. Hence, studies with larger sample size and follow-up data should be conducted to determine the importance of endocan in COPD and support our findings.

In our study, it was determined that the serum endocan levels in control, stable COPD and AECOPD groups were 142.30 ± 109.57 pg/mL, 320.72 ± 188.23 pg/mL, and 584.40 ± 253.77 pg/mL, respectively. Compared to our study, Dai et al. (15) found higher endocan levels both, in controls (434.8±18.98 pg/mL) and stable COPD (509.7±18.25 pg/mL), while lower levels in AECOPD (524.7±27.18 pg/mL). In terms of endocan levels, the differences determined between the two studies could be related to the analysis methods of serum samples (ELISA &Magnetic Luminex Screening Assay**)** and the differences in the populations investigated. Moreover, in terms of endocan levels, it was observed that there were significant levels of differences between the control groups of the two studies. However, when control groups of studies that

investigated different patient populations in the literature were investigated, it was observed that rather different values were determined between 111.8 pg/mL and 546.74 pg/mL (25-27). Thus, it could be stated that there is no standardization for endocan levels of healthy control cases. For such a standardization, a rather wide series of studies are required. Furthermore, when both AECOPD cases of both studies were investigated, it was observed that the patients in our studies were distinctively more severe patients compared to those in the study of Dai et al. (15). In COPD patients, as the severity of disease increases, the endothelial dysfunction is also expected to increase. This may explain the reason why the endocan levels were lower in control and stable COPD groups while being higher in the AECOPD group. Similarly, compared to our study, the serum endocan levels were determined to be high (860.1±259.8 pg/mL in the COPD group and 647.3±316.9 pg/mL in the control group) in the study conducted by Pihtili et al. (14). In this study, in which only stable COPD patients and control cases were included, the methods adopted and the populations investigated were similar to those in our study. However, the most important difference between this study and our study was that diabetic patients were included in both the control and COPD groups. Another difference was that patients who were smoking were included in the control group of that study. It is known that both diabetics and smoking leads to endothelial dysfunction (3, 27). In the literature, several studies reported that endocan levels were determined to be higher in diabetic patients compared to control groups (27, 28). In our study, we formed the control group from patients who did not smoke and did not have any disease. Furthermore, diabetes mellitus which can lead to endothelial dysfunctions, was accepted as exclusion criteria in our study. So, these findings can explain the reason of low endocan levels determined in both the control group and stable COPD patients in our study.

Study limitations

Our study had relatively small numbers of COPD patients and controls. The target number of subjects could not be reached especially in the AECOPD group. Furthermore, re-study of endocan levels at a stable period after exacerbation among patients with AECOPD would more clearly establish its relationship with exacerbations.

In conclusion, this study demonstrated that serum endocan levels were increased in COPD patients and this increase was correlated with a decline in respiratory function and COPD exacerbations. Endocan can be a biomarker for detection of endothelial dysfunction and prediction of exacerbation among patients with COPD.

Conflict of Interests

The authors do not have any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. The authors have no conflicts of interest to declare.

Author Contributions

Conception and design of the study: İn, Kuluöztürk, İlhan, Turgut
Acquisition of data: İn, Kuluöztürk, İlhan
Analysis and interpretation of data: Kuluöztürk, İn, İlhan, Altintop
Drafting the article: İn, Altintop, Turgut
Revising it critically for important intellectual content: İn
Final approval of the version to be submitted: İn

Ethics

The study was conducted in accordance with the Helsinki Declaration, and it was approved by the Ethical Committee of the Medicine Faculty of Firat University (issue:116672/13.11.15). The subjects who participated in the study provided their written consent to be included in the study.

Data availability statement:

Data openly available in a public repository that issues datasets with DOIs.

References

1. Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management and prevention of chronic obstruvtive pulmonary disease, updated 2017. http://www.goldcopd.org.

2. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. Eur Respir J. 2009;33:1165-85.

3. Polverino F, Celli BR, Owen CA. COPD as an endothelial disorder: endothelial injury linking lesions in the lungs and other organs? (2017 Grover Conference Series). Pulm Circ. 2018;8(1):2045894018758528.

4. Lassalle P, Molet S, Janin A, et al. ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines. J Biol Chem.1996;271(34):20458-64.

5. Kechagia M, Papassotiriou I, Gourgoulianis KI. Endocan and the respiratory system: a review. Int J Chron Obstruct Pulmon Dis. 2016;11:3179-87

6. Kali A, Shetty KSR. Endocan: a novel circulating proteoglycan. Indian J Pharmacol. 2014;46(6):579-83.

7. Yang J, Yang Q, Yu S, et al. Endocan: A new marker for cancer and a target for cancer therapy. Biomed Rep. 2015;3(3):279-83.
8. Balta S, Mikhailidis DP, Demirkol S, et al. Endocan a novel inflammatory indicator in newly diagnosed patients with hypertension: a pilot study. Angiology. 2014;65(9):773-7.
9. Kose M, Emet S, Akpinar TS, et al. Serum Endocan Level and the Severity of Coronary Artery Disease: A Pilot Study. Angiology. 2015:66(8):727-31.
10. Kao SJ, Chuang CY, Tang CH, et al. Plasma endothelial cell-specific molecule-1 (ESM-1) in management of community-acquired pneumonia. Clin Chem Lab Med. 2014;52(3):445-51.
11. Tang L, Zhao Y, Wang D, et al. Endocan levels in peripheral blood predict outcomes of acute respiratory distress syndrome. Mediators Inflamm. 2014;2014:625180.
12. Altintas N, Mutlu LC, Akkoyun DC, et al. Effect of CPAP on new endothelial dysfunction marker, endocan, in people with obstructive sleep apnea. Angiology. 2016;67(4):364-74.
13. Kuluöztürk M, İn E, İlhan N. Endocan as a marker of disease severity in pulmonary thromboembolism. Clin Respir J. 2019;13(12):773-80.
14. Pihtili A, Bingol Z, Kiyan E. Serum endocan levels in patients with stable COPD. Int J Chron Obstruct Pulmon Dis. 2018;13:3367-72
15. Dai L, He J, Chen J, et al. The association of elevated circulating endocan levels with lung function decline in COPD patients. Int J Chron Obstruct Pulmon Dis. 2018;13:3699-706.
16. Quanjer PH, Tammeling GJ, Cotes JE, et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl**.** 1993;16:5-40.
17. Rudski LG, Lai WW, Afilalo J. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography. J Am Soc Echocardiogr. 2010;23(7):685-713.
18. Green CE and Turner AM. The role of the endothelium in asthma and chronic obstructive pulmonary disease (COPD). Respiratory Research. 2017;18(1):20

19. Galiè N, Humbert M, Vachiery JL, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS). Eur Respir J. 2015;46(4):903-75.
20. Van Eeden S, Leipsic J, Paul Man SF, et al. The relationship between lung inflammation and cardiovascular disease. Am J Respir Crit Care Med. 2012;186:11-6.
21. Wedzicha JA, Donaldson GC. Exacerbations of chronic obstructive pulmonary disease. Respir Care. 2003;48:1204-13.
22. Koutsokera A, Stolz D, Loukides S, et al. Systemic biomarkers in exacerbations of COPD: the evolving clinical challenge. Chest. 2012;141(2):396-405.
23. Csoma B, Bikov A, Nagy L, et al. Dysregulation of the endothelial nitric oxide pathway is associated with airway inflammation in COPD. Respir Res. 2019;20(1):156.
24. Ruzsics I, Nagy L, Keki S, et al. L-Arginine Pathway in COPD Patients with Acute Exacerbation: A New Potential Biomarker. COPD 2016;13(2):139-45.
25. Lee YH, Kim JS, Kim SY, et al. Plasma endocan level and prognosis of immunoglobulin A nephropathy. Kidney Res Clin Pract. 2016;35(3):152-9.
26. Efe SC, Demirci K, Ozturk S, et al. Serum endocan levels in patients with cardiac syndrome X. Herz. 2018;43(4):359-63.
27. Lv Y, Zhang Y, Shi W, et al. The Association Between Endocan Levels and Subclinical Atherosclerosis in Patients With Type 2 Diabetes Mellitus. Am J Med Sci. 2017;353(5):433-8.
28. Ekiz-Bilir B, Bilir B, Aydın M, et al. Evaluation of endocan and endoglin levels in chronic kidney disease due to diabetes mellitus. Arch Med Sci. 2019;15(1):86-91.

Figure Legands:

Figure 1. The comparison of serum endocan levels in all study groups. capacity (FVC), and **(c)** forced expiratory volume in the first second (FEV_1) in patients with COPD.

Figure 2. Correlation between serum endocan levels and **(a)** diseases duration, **(b)** forced vital capacity (FVC), and **(c)** forced expiratory volume in the first second (FEV_1) in patients with COPD.

Figure 3. Correlation between serum endocan levels and **(a)** partial arterial oxygen pressure (PaO_2), **(b)** oxygen saturation, and **(c)** systolic pulmonary artery pressure (PAP) in patients with COPD.

Figure 4. Receiver operating characteristic (ROC) curve analysis of the utility of endocan to predict acute exacerbation of COPD.

Table 1. Comparison of the demographical and laboratory data of patients with COPD and controls.

Table 2. Comparison of various parameters of patients with stable and acute exacerbation of COPD.

Table 3. Results of binary logistic regression analysis of the potential predictors of COPD exacerbation.

Table 1. Comparison of the demographical and laboratory data of patients with COPD and controls.

	Controls (n=30)	Stable COPD (n=31)	AECOPD (n=24)
Age, years	59.9 ± 4.9	62.1 ± 9.5	64.1 ± 9.9
Sex, male/female	21/9	26/5	18/6
BMI, kg/m^2	26.3 ± 2.3	25.3 ± 5.5	24.6 ± 4.8
Disease duration, years	-	4.3 ± 2.3	6.2 ± 2.2**
Smoking, pack-years	-	43.1 ± 21.5	45.0 ± 26.3
Wbc, x10^9 /L	6.5 ± 1.9	8.5 ± 2.1 [c]	11.3 ± 4.4 [a,] **
Hb, g/dl	13.6 ± 0.9	14.6 ± 1.6 [c]	14.5 ± 1.9
Hct, %	40.8 ± 5.6	46.1 ± 5.2 [b]	46.3 ± 5.7 [b]
Plt, x10^9/L	240.8 ± 53.8	243.2 ± 59.5	269.3 ± 71.9
CRP, mg/dL	-	7.4 ± 5.3	53.8 ± 63.3**
ESR, mm/h	-	11.4 ± 9.8	23.2 ± 15.6**
Endocan, pg/mL	142.3 ± 109.6	320.8 ± 188.2 [b]	584.4 ± 253.8 [a,] *

BMI:Body mass index, WBC: White blood cell, Hb: Hemoglobin, Hct: Hematocrit, Plt: Platelet, CRP: C-Reaktive protein, ESR: Erythrocyte sedimentation rate.

When comparing controls with Stable COPD or control with AECOPD;

[a] p<0.001, [b] p<0.005, [c] p<0.05.

When comparing Stable COPD with AECOPD;

** p<0.001, ** p<0.005.*

Table 2. Comparison of various parameters of patients with stable and acute exacerbation of COPD.

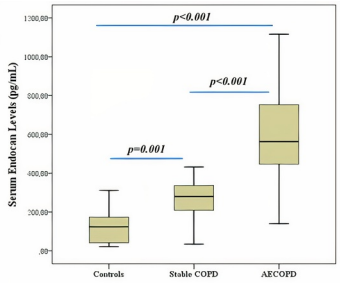
	Stable COPD (n=31)	**AECOPD (n=24)**	***p***
PFT			
FVC, %	67.1 ± 12.5	51.7 ± 16.8	**<0.001**
FEV1, %	53.5 ± 12.7	37.5 ± 13.8	**<0.001**
FEV1/FVC	60.1 ± 7.9	54.3 ± 11.2	**0.03**
PEF, %	49.7 ± 14.5	32.9 ± 10.7	**<0.001**
ABG			
Ph	7.4 ± 0.02	7.4 ± 0.05	0.20
PaO_2, mmHg	68.8 ± 10.6	54.6 ± 12.4	**<0.001**
SaO_2, %	90.5 ± 7.9	80.4 ± 10.9	**<0.001**
$PaCO_2$, mmHg	40.9 ± 4.1	49.7 ± 9.2	**<0.001**
ECHO			
EF, %	54.9 ± 6.1	53.6 ± 4.7	0.38
sPAP, mmHg	35.8 ± 11.9	48.5 ± 15.4	**0.001**
RVD, mm	24.4 ± 3.3	25.8 ± 3.9	0.15
Scales			
mMRC	2.2 ± 1.1	3.4 ± 0.7	**<0.001**
CAT	18.2 ± 8.4	25.5 ± 4.1	**<0.001**

PFT: pulmonary function tests, FVC: forced vital capacity, FEV1 : forced expiratory volume in 1 second, PEF: peak expiratory flow, ABG: Arterial blood gases, Ph: Potential Hydrogen, $PaCO_2$: Partial carbon dioxide pressure, PaO_2: Partial oxygen pressure, SaO2: Oxygen saturation, saturation ECHO:Echocardiography, EF: Ejection Fraction, sPAP: Systolic pulmonary artery pressure, RVD : Right ventricle diameter, CAT: COPD Assessment Test, mMRC: Modified British Medical Research Council.

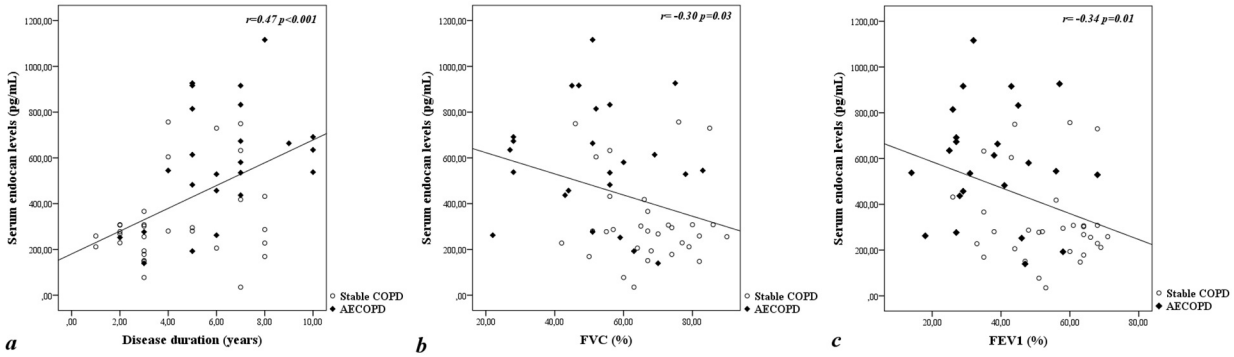
Table 3. Results of binary logistic regression analysis of the potential predictors of COPD exacerbation.

Independent variables	OR value	95% CI	*p*-value
Age (≥ 70 yrs)	0.62	(0.13-2.94)	0.55
Sex (male)	0.42	(0.05-3.96)	0.45
BMI (< 18.5 kg/m^2)	0.69	(0.13-3.67)	0.67
Disease duration (≥ 5 yrs)	0.89	(0.12-6.61)	0,91
PaO_2 (< 60 mmHg)	4.77	(0.74-30.64)	0.09
FVC (<60% pred.)	1.02	(0.09-12.05)	0.99
FEV_1 (< 50% pred.)	3.21	(0.27-38.83)	0.36
sPAP (≥ 35 mmHg)	6.29	(1.06-37.49)	**0.04**
Endocan (≥ 434.29 pg/mL)	9.32	(1.64-52.95)	**0.01**

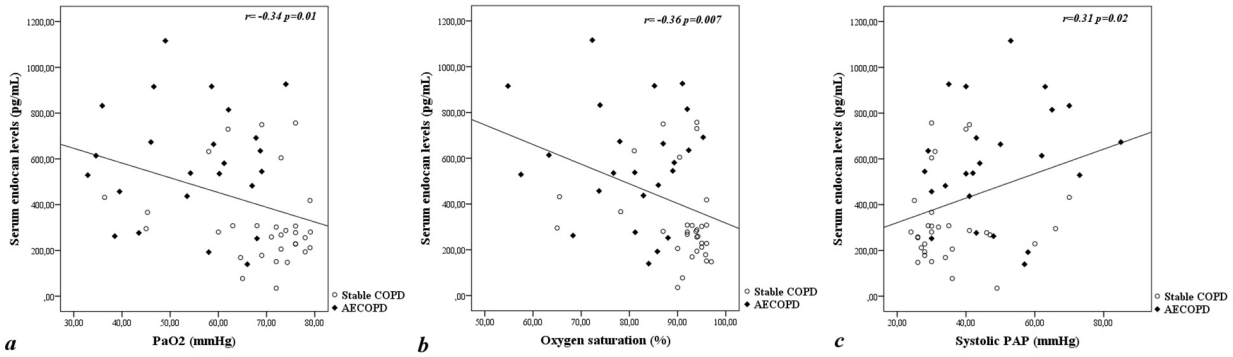
OR: odds ratio, CI: confidence interval, BMI:Body mass index, PaO_2: Partial oxygen pressure, FVC: forced vital capacity, FEV1 : forced expiratory volume in 1 second, , sPAP: Systolic pulmonary artery pressure.



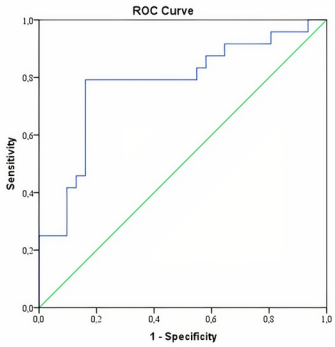
crj_13320_f1.jpg



crj_13320_f2.jpg



crj_13320_f3.jpg



crj_13320_f4.jpg