Serum vascular endothelial growth factor and its receptor level in patients with chronic obstructive pulmonary disease

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ABSTRACT. Chronic obstructive pulmonary disease (COPD) is a disorder which encompasses not only morphological changes in parenchyma, central and peripheral airways but also in structural and functional changes of pulmonary vessels. The role of angiogenic factors leading to abnormal pulmonary vessel remodeling remains unclear. We have investigated a cytokine vascular endothelial growth factor (VEGF) known to be involved in angiogenesis, and its soluble receptors (sVEGF R1, sVEGF R2) in the serum of 20 patients with mild COPD and 10 patients with very severe COPD, using sensitive enzyme-linked immunoassays. The control group consisted of 10 healthy volunteers. We found that the concentration of VEGF in the serum of patients with mild COPD was significantly higher (665.31 ± 102.20 pg/mL) in comparison to the control group (318.94 ± 51.40 pg/mL; $p < 0.05$), and there was a strong negative correlation with FEV1 ($r = -0.859$; $p < 0.001$). Additionally, the level of sVEGF R1 in the serum of patients with very severe COPD was also significantly higher (96.60 ± 26.85 pg/mL) than in the control (36.01 ± 3.29 pg/mL; $p < 0.05$) and a positive correlation between the serum level of sVEGF R1 and FEV1 was found ($r = 0.748$; $p < 0.01$). Moreover, we observed an insignificant increase of sVEGF R2 in the serum of patients with mild COPD and those with very severe COPD. These results suggest that VEGF and sVEGF R1 receptor are involved in the development of abnormal pulmonary vascular remodelling in patients with COPD.

Keywords: pulmonary disease, COPD, VEGF, VEGF Receptors, vascular remodelling

Chronic obstructive pulmonary diseasegicold (COPD) is considered to be a chronic inflammatory disorder associated with the remodelling of airway walls [1]. One of the complications of this disease is the structural and functional change in the pulmonary circulation. Pulmonary vascular bed remodelling leads to pulmonary hypertension that is associated with a poorer prognosis [2]. The recent classification of pulmonary hypertension considers primary and secondary types, and among the secondary types, COPD is one of the main causes [2]. Morphological changes in the pulmonary vasculature are generally referred to as a pulmonary vascular remodelling [3]. Chronic hypoxia is a well known cause of pulmonary vascular remodelling and pulmonary hypertension, and it is the major factor implicated in the development of pulmonary hypertension in patients with COPD [4]. Other factors have also been implicated in the pathogenesis of pulmonary hypertension including several cytokines and angiogenic growth factors [3, 4]. Hypoxia-inducible factor-1α (HIF-1α) is one of the pivotal mediators in the response of lung tissue to decreased oxygen availability and it has been strongly implicated in the pathogenesis of pulmonary hypertension [5, 6].

The most potent cytokines involved in vascular remodelling are vascular endothelial growth factor family members (VEGF-A to F). The most common forms of human VEGF-A consists of five different isoforms having 121,145,165,189 and 206 amino acids, derived from alternative splicing of RNA from multiple exons [7]. VEGF 165, the major isoform, is a 45 kDa, heparin-binding glycoprotein. It acts through two closely related receptor tyrosine kinases, VEGF R1 (fms-like tyrosine kinase, Flt-1) and VEGF R2 (kinase domain receptor, KDR in humans or fetal liver kinase, Flk-1) [8]. Similarly to VEGF, VEGF R1 and VEGF R2 are expressed predominantly in

Abbreviations:

COPD	chronic obstructive pulmonary disease
VEGF	vascular endothelial growth factor
sVEGF R1	soluble vascular endothelial growth factor receptor 1
sVEGF R2	soluble vascular endothelial growth factor receptor 2
HIF-1 alpha	hypoxia inducible factor 1 alpha
GOLD	global initiative for chronic obstructive lung disease
FEV1	forced expiratory volume in 1 second
FVC	forced vital capacity
PaO2	partial pressure of arterial oxygen
PCO2	partial pressure of arterial carbon dioxide

vascular smooth muscle and endothelial cells, thus accounting for the specific actions of VEGF in the vasculature. Expression of the VEGF Receptors gene is also regulated by hypoxia. The role of VEGF and its receptors in the pathogenesis of COPD and hypoxia-induced pulmonary hypertension remains unclear. Thus, in this study, we investigated the potential role of VEGF in the pathogenesis of pulmonary vascular components of COPD. The second parameter measured in our study was the concentration of soluble forms of the receptor for VEGF (sVEGF R1 and sVEGF R2).

METHODS

Subjects

The study comprised a group of 30 patients with chronic obstructive pulmonary disease (COPD): 14 women and 16 men (age 42-81, mean age 60 ± 7.3).

The diagnosis was based on the NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria [9]. All patients were clinically stable at the time of evaluation. They were divided into 2 groups: the first group of 20 patients with mild COPD (I stage) included 10 men and 10 women (age 42-81, mean age 60 ± 7.8), while the second group of 10 patients with very severe COPD (IV stage) included 4 women and 6 men (age 54-74, mean age 62.9 ± 5.32). The patients with mild COPD presented a predicted FEV1 = 80%, and FEV1/FVC < 70% with or without clinical signs such as chronic cough and sputum production. The patients with very severe COPD were selected according to the presence of FEV1/FVC < 70% a predicted FEV1 < 30%, and clinical signs of right heart failure. All patients with COPD were heavy cigarette users with an average of 40 pack-years (15-80 pack-years) between them. Each patient underwent physical examination.

The control group included 10 healthy smokers with an average of 37 pack-years (17-75 pack-years): 6 women and 4 men (age 48-76, the mean age 61.5 ± 6.16) with a normal pulmonary function. Informed consent was obtained from all subjects participating in the study. The project was approved by the Ethical Committee for Scientific Studies of the Medical University of Lodz, No RNN/145/02/KE.

The general characteristics of the patients and lung function measurements in the groups of patients and in the healthy control group are shown in *table 1*.

Pulmonary function testing

Spirometry was performed using LUNGTEST 1000 (MES, Krakow, Poland) by the same technician at the same time each morning, before breakfast. Pulmonary function values were expressed as a percentage of predicted values [10]. Measured parameters included: FEV1- forced expiratory volume in the first second (% predicted) and FEV1/FVC-forced expiratory volume in the first second to forced vital capacity (%).

Blood sampling and analysis

Blood samples were taken from the antecubital vein in the morning between 7:00 and 8:00 AM after an overnight fast. Blood was processed within one hour of collection, and serum was aliquotted and stored at -70 °C until analysis. The concentrations of VEGF, sVEGF R1 and sVEGF R2 were measured using commercial, enzyme-linked immunoassay kits R&D SYSTEMS (R&D SYSTEMS Inc., 614 McKinley Place NE, Minneapolis, MN 55413, USA) following the manufacturer's instructions. VEGF could be measured in the range 62 to 707 pg/mL, and the sensitivity of assay was less than 9.0 pg/mL. The intra-assay CV was 6.7% and the inter-assay CV was 8.8%. VEGF R1 could be measured in the range 0 to 170.6 pg/mL, and the sensitivity of assay was 5.01 pg/mL. The intra-assay CV was 2.6%, and the inter-assay CV was 7.7% VEGF R2 could be measured in the range 6420 to 24501 pg/mL, and the sensitivity of assay was 4.6 pg/mL. The intra-assay CV was 4.2%, and the inter-assay CV was 7.0%. All measurements were taken in duplicate and averaged.

Statistical analysis

All values are presented as means ± SEM. Statistical analysis was performed among groups by analysis of variance (ANOVA) followed by LSD test (least significance difference). Independent relationships between plasma VEGF, sVEGF R1 and pulmonary function parameters were examined using Pearson's linear correlation analysis. A "p" value of less than 0.05 was considered statistically significant.

RESULTS

We found that the concentration of VEGF in the serum of patients with mild COPD was significantly higher

Table 1
Characteristics of patients with COPD and healthy controls involved in the study

Subjects data	Controls (n = 10)	Very severe COPD (n = 10)	Mild COPD (n = 20)
Age	61.5 ± 6.1	62.9 ± 5.3	60 ± 7.8
FEV$_1$% predicted	103.78 ± 8.26	25.78 ± 5.2*	83.36 ± 3.5*
FEV$_1$/FVC%	100.56 ± 6.15	59.40 ± 4.22*	66.67 ± 5.09*
Clinical symptoms of heart insufficiency	not present	present	not present
PaO$_2$ mmHg	89.8 ± 2.1	56.2 ± 3.3*	64.7 ± 2.8
PaCO$_2$ mmHg	37.7 ± 0.9	49.02 ± 1.9*†	39.2 ± 1.9

FEV$_1$ = forced expiratory volume in 1 second; FVC = forced vital capacity; PaO$_2$ = partial pressure of arterial oxygen; PCO$_2$ = partial pressure of arterial carbon dioxide. Data represent means ± SEM. * $p < 0.05$ *versus* control, † $p < 0.05$ *versus* mild COPD.

(665.31 ± 102.20 pg/mL) in comparison with the control group (318.94 ± 51.40 pg/mL; $p < 0.05$; *figure 1*). The serum VEGF concentration in patients with mild COPD correlated negatively with FEV1 ($r = -0.859$; $p < 0.001$; *figure 2*). The mean concentration of VEGF in the serum of patients with very severe COPD was also higher than in the control group, but the difference was not significant (466.27 ± 147.89 pg/mL *versus* 318.94 ±51.40 pg/mL; $p > 0.05$). The mean concentration of sVEGF R1 in the serum of patients with very severe COPD (96.60 ± 26.85 pg/mL) was significantly higher than in the control group (36.01 ± 3.29 pg/mL; $p < 0.05$; *figure 3*), and a positive correlation between the serum level of sVEGF R1 and FEV1 was found ($r = 0.748$, $p < 0.01$; *figure 4*). The concentration of sVEGF R1 in the group of patients with mild COPD (60.03 ± 9.23 pg/mL) was also higher than in the control group (36.01 ± 3.92 pg/mL), but the difference was not statistically significant ($p > 0.05$).

The concentration of sVEGF R2 in the serum of patients with mild COPD (14966.78 ± 869.76 pg/mL), and in those with very severe COPD (14737.94 ± 1592.97 pg/mL) was higher than in the control group (13545.61 ± 1073.43 pg/mL). However, there were no statistically significant differences between mild and very severe COPD patients in comparison with the control group ($p > 0.05$; *figure 5*).
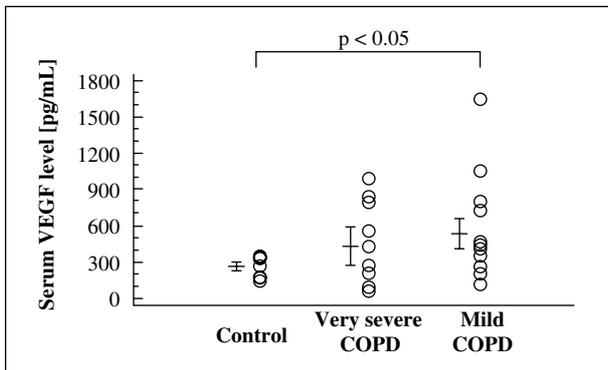

Figure 1
The concentration of VEGF in serum of the control group (C), patients with mild and very severe COPD according to disease severity based on Global Initiative for Chronic Lung Disease guidelines.
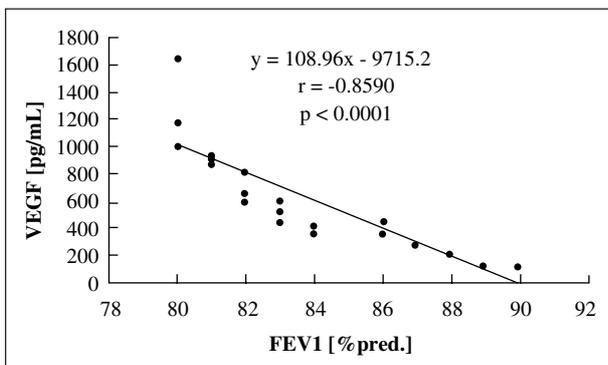

Figure 2
Correlation between serum VEGF concentration and FEV1 in patients with mild COPD. The correlation coefficient (r) was obtained using linear regression (Pearson's) analysis.
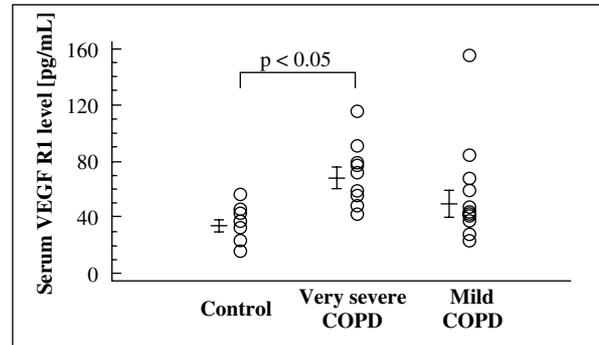

Figure 3
The concentration of sVEGF R1 in serum of the control group (C), patients with mild and very severe COPD according to disease severity based on Global Initiative for Chronic Lung Disease guidelines.
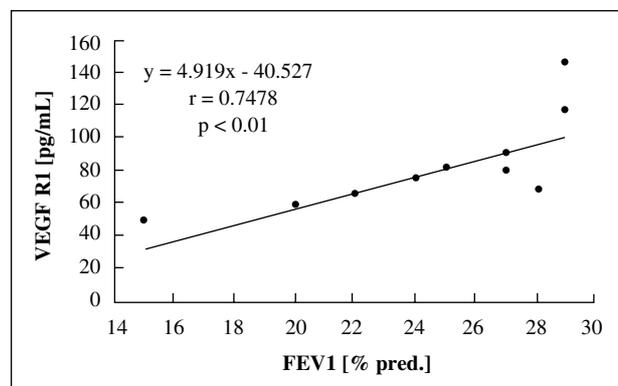

Figure 4
Correlation between serum sVEGF R1 concentration and FEV1 in patients with very severe COPD. The correlation coefficient (r) was obtained using linear regression (Pearson's) analysis.
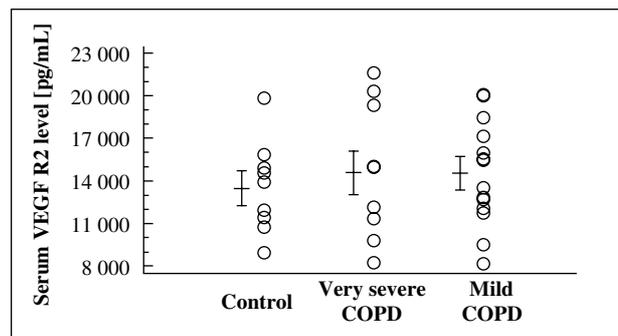

Figure 5
The concentration of sVEGF R2 in serum of the control group (C), patients with mild and very severe COPD according to disease severity based on Global Initiative for Chronic Lung Disease guidelines.

DISCUSSION

Chronic obstructive pulmonary disease is associated with structural and functional changes in the pulmonary parenchyma, central and peripheral respiratory track and pulmonary circulation. Endothelial and smooth muscle cells proliferation of the vascular wall leading to pulmonary vessel remodelling and pulmonary hypertension is characteristic of COPD [1, 2, 11]. Recently, an interesting study was conducted that indicates the crucial contribution of angio-

genic growth factors in the pathogenesis of COPD [12]. In fact, enhanced expression of VEGF in vascular and airway smooth muscle cells and alveolar epithelium of patients with COPD (examined by immunohistochemistry) has been found [13]. It appears that the vascular endothelial growth factor (VEGF), discovered by Ferrara and Henzel in 1989 [14], has a crucial effect on endothelial cells proliferation, apoptosis and vascular wall remodelling [15].

It has been recognized that the fixed narrowing of small airways, emphysema and luminal obstruction may all contribute to the airflow limitation in COPD. The airflow limitation is usually associated with chronic airway inflammation characterized by an influx of inflammatory cells in the small airways and alveolar wall [16, 17]. Airway inflammatory cells and alveolar epithelial cells may be a major source of inflammatory mediators, cytokines and growth factors including VEGF. Recently, several investigations were conducted studying the pathophysiological role of VEGF in lung diseases. VEGF expression is up-regulated by hypoxia [18] and overexpression of VEGF was also proposed to contribute to the pathogenesis of hypoxic pulmonary hypertension [19], which is the principal mechanism underlying COPD.

In the present study, we demonstrated for the first time that the serum VEGF concentration was elevated in patients with the mild COPD stage. In addition, the serum VEGF level correlated negatively with a decline in FEV1. Thus, elevated VEGF concentration, perhaps under the influence of hypoxia, may be associated with the development of microvasculature abnormalites in the highly vascularized lung tissues in patients with mild COPD. The VEGF concentration in patients with very severe COPD was also higher, but it did not reach a level of statistical significance. The reason why the level of VEGF in severe COPD is not significantly higher than in healthy control subjects remains unclear. However, we hypothesize that the destruction of alveolar walls due to emphysema (aside from hypoxia) may contribute to impaired VEGF production by lung tissue in patients with severe COPD. Furthermore, recent observations have indicated that emphysema may lead to loss of the pulmonary vascular bed and the increase of alveolar septal cell apoptosis [20].

Our results may indicate the contribution of VEGF to the development of vascular remodelling at the early stage of COPD. These results are in agreement with the immunohistochemical data showing intense VEGF expression and increased VEGF mRNA content (analysed by reverse transcription polymerase chain reaction) within the pulmonary arteries, which were examined in lung tissue segments collected from patients with mild COPD [12]. An attempt has been made to evaluate the VEGF concentration in the saliva of patients with COPD and severe COPD. The VEGF concentration increase appears to activate the anti-apoptotic proteins in pulmonary vessel endothelium simultaneously preventing them from programmed death [21].

With reference to recent research, it has been concluded that pulmonary vessel endothelium dysfunction, observed in patients at initial stages of the disease, results from the decreased release of nitrogen oxide in the endothelial cells of the carotid artery. For this reason the weakening of endothelium leads to accelerated pulmonary vessel remodelling [12]. Endothelium seems to play a pivotal role in tone regulation, cell growth control and apoptosis of arterial wall cells. Hence, the weakening of endothelium function may initiate the vessel remodelling process at an early stage of the disease. It has been postulated recently that COPD-associated pulmonary vascular changes may reveal itself at the initial stages of the disease even in the absence of hypoxemia, and it may occur among smokers without clinical manifestations [12]. Therefore, it can be speculated that smoke is involved in the increase of the VEGF concentration in serum [22]. Moreover, it is generally acknowledged that smoke constituents increase the mRNA VEGF expression and proteins in carotid artery endothelium [23, 24].

In view of the recent studies, there seems to be increased VEGF expression in muscular pulmonary vessels among patients with mild COPD and among smokers with regular pulmonary function in comparison with non-smokers. This expression is strictly related to the vascular wall thickness. However, among the patients with serious pulmonary emphysema, the immunohistochemical VEGF expression in pulmonary vessels and the protein content in pulmonary parenchyma was low despite advanced vascular remodelling [25].

Recent evidence is presented showing that not only vascular VEGF expression and its production in the lung tissue, but also blockade, or intrinsic malfunction of endothelial VEGF Receptors, all have implications in the pathogenesis of COPD [26]. In our examinations, we evaluated the serum concentrations of soluble forms of VEGF Receptors (sVEGF R1 and sVEGF R2). VEGF R2 is considered to be the primary mediator of endothelial cell division in extra-uterine life, which is not observed in cases of VEGF and VEGFR1 binding. The measurement of concentrations of the soluble forms of VEGF Receptors in the serum of patients at different stages of COPD, has not been conducted yet. In the present study, the significant increase in the concentration of sVEGF R1 receptors in the serum of patients with very severe COPD was observed. Additionally, the serum VEGF R1 levels showed a significantly positive correlation with a sensitive parameter of airflow obstruction such as FEV1. This may confirm the results of observations obtained by other authors, which indicate the potential role of the VEGF R1 receptor in the mechanism of vascular remodelling and pulmonary hypertension induction leading to very severe COPD [27, 28]. On the other hand, we did not observed any significant changes in the sVEGF R2 receptor concentration in either mild or very severe COPD patients. The lack of statistically significant changes in the serum concentrations of sVEGF R2 may indicate the minor role of this receptor in the pulmonary vessels in the course of this syndrome.

In conclusion, our results suggest that the VEGF and VEGF R1 system dysfunction may be involved in the autocrine and/or paracrine mechanisms of vascular remodelling in patients with COPD. The determination of angiogenic cytokines in the blood serum may also be used as a measure of the severity of the disease.

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