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## **Circulating vascular endothelial growth factor and systemic inflammatory markers in patients with stable and exacerbated chronic obstructive pulmonary disease**

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### **Running head: Circulating VEGF in COPD**

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## Abstract

**Background:** To assess circulating levels of vascular endothelial growth factor (VEGF), a biomarker with prognostic significance in cardiovascular disease, and markers of systemic inflammation in patients with stable and exacerbated chronic obstructive pulmonary disease (COPD).

**Materials and methods:** Lung function parameters, arterial blood gas analysis and circulating levels of VEGF, interleukin-6, tumor necrosis factor-alpha, C-reactive protein, fibrinogen, and peripheral blood neutrophil cell count were assessed in 30 patients on admission to the hospital for acute exacerbation of COPD, in 30 age, sex and body mass index matched patients with stable COPD, and 30 matched controls with normal lung function.

**Results:** Patients with acute exacerbated COPD had higher circulating concentrations of VEGF ( $p < 0.001$ ), interleukin-6 ( $p < 0.05$ ), C-reactive protein ( $p < 0.01$ ), and an increased blood neutrophil cell count ( $p < 0.05$ ) than did patients with stable COPD and healthy controls. VEGF levels in exacerbated COPD correlated with systemic inflammatory markers, such as C-reactive protein ( $r = 0.61$ ,  $p < 0.005$ ), interleukin-6 ( $r = 0.46$ ;  $p < 0.01$ ) and fibrinogen ( $r = 0.39$ ,  $p < 0.05$ ). In patients with stable COPD there was a significant relationship between circulating VEGF levels and FEV1% predicted ( $r = 0.47$ ,  $p < 0.01$ ). Recovery from the exacerbation resulted in a significant reduction in both circulating VEGF levels and markers of systemic inflammation.

**Conclusions:** Circulating levels of VEGF and markers of systemic inflammation are up-regulated in patients with acute exacerbated COPD and decrease after recovery from the exacerbation.

**Word count for abstract: 229**

## Introduction

Vascular endothelial growth factor (VEGF), a soluble heparin binding glycoprotein, is a cytokine with potent angiogenic properties, enhancing vascular permeability and modulating thrombogenicity [1]. VEGF further serves as an endothelial cell survival factor, protecting endothelial cells against apoptosis and delaying endothelial cell senescence [2]. The biological properties of VEGF have led to interest in its role within the lung in both health [3] and disease [4]. In fact recent studies have suggested that the expression of VEGF in lung tissue obtained from patients with chronic obstructive pulmonary disease (COPD) may play a role in the pathogenesis of the disease [5,6]. Kasahara and colleagues [5] have shown that VEGF protein expression is reduced in lung specimens obtained from patients with emphysematous type of COPD. The authors have suggested that the absence of VEGF, which is a trophic factor for endothelial cells, contributes to the development of emphysema. In consistency with these findings, Kanazawa et al. [7] observed a positive relationship between VEGF concentrations in induced sputum samples and FEV1 in patients with stable COPD.

Over the last few years VEGF levels have also been measured in the peripheral circulation as a biomarker of neovascularization and/or vascular remodeling. Several studies reported increased circulating VEGF levels in conditions in which tissue hypoxia and/or inflammation are critical, such as coronary heart disease [8], sepsis [9], cancer [10], and rheumatoid diseases [11]. Furthermore, there is cumulating evidence that circulating VEGF levels have prognostic significance particularly in cardiovascular disease [12], which could provide a mechanism to link the association between inflammation and promotion of cardiovascular disease. Both hypoxia and inflammation are also relevant to COPD. Thus the aims of the present study were threefold: 1. to determine circulating levels of VEGF and systemic inflammation in patients with COPD and appropriate controls 2. to assess the effects of acute deteriorations of respiratory health in COPD on circulating VEGF and systemic inflammatory markers during and after recovery from an acute exacerbation of COPD and 3. to assess the relationship between markers of systemic inflammation, hypoxemia, and lung function parameters with circulating VEGF levels in both stable and exacerbated COPD.

## Methods

The protocol was approved by the Ethics Committee of the Vienna City Council. Informed consent was obtained from each subject.

### Selection of patients

The study population consisted of patients with acute exacerbated COPD admitted to the hospital, patients with stable COPD and control subjects without airflow obstruction.

#### *Patients with acute exacerbated COPD*

During an enrollment period of twelve months a total of 90 patients admitted to the Otto-Wagner-Hospital because of acute exacerbated COPD were screened for eligibility [13]. Acute exacerbation was defined according to recommended international criteria [14]. We excluded patients who had conditions that could potentially affect circulating VEGF levels. Accordingly, 12 patients were excluded due to pneumonia, acute respiratory failure ( $n = 8$ ), heart failure ( $n = 7$ ), acute myocardial infarction ( $n = 2$ ), and interstitial lung disease ( $n = 4$ ). 7 patients were receiving oral anticoagulant therapy, 5 had recent revascularization, 4 had preceding lung surgery, 5 had renal failure, 3 had a history of cancer, and 3 were suffering from an autoimmune disease. Thirty patients with acute exacerbated COPD were included in the final analysis. Suitable patients were recruited into the study within 24 hours of admission. Pre-exacerbation pulmonary function tests were evaluated to corroborate the diagnosis of COPD.

Blood samples for cytokine measurements and arterial blood gas analysis with subjects breathing room air were taken before the initiation of exacerbation therapy. After blood sampling, all patients were treated with nebulized bronchodilator therapy, prednisolone and theophylline. Other treatments such as antibiotics were administered based on clinical need. A second blood sample was collected from the patients in a clinically stable state six weeks after hospital discharge. Spirometric measurements (V6200, Sormedics, Germany) of forced expiratory volume in one second (FEV<sub>1</sub>), forced vital capacity (FVC), FEV<sub>1</sub>/FVC, and peak expiratory flow (PEF) were performed at the follow up visit to confirm the diagnosis of COPD. Predicted values for spirometric parameters were those of an Austrian population [15].

#### *Patients with stable COPD*

Thirty patients with stable COPD were recruited from the hospitals outpatient clinic. These were matched with respect to age, gender, BMI, smoking history (pack-yrs) and FEV<sub>1</sub> to

patients with exacerbated COPD. A stable condition was defined as a stable disease without reported exacerbations and at least three stable visits during the previous 6 months without any changes in respiratory medication and by the absence of an infection prior to inclusion in the study. Based on high-resolution computed tomography and lung function tests performed, most of these patients ( $n = 23$ ) were diagnosed with COPD of emphysema type.

#### *Control subjects*

Thirty healthy subjects matched with respect to age, gender, and BMI to patients with COPD comprised the control group. The controls showed normal pulmonary function.

#### **Blood sampling preparations**

To assess the relative contribution of platelets to VEGF liberation into circulation, we determined VEGF concentrations in serum (VEGFser), thrombin stimulated platelet-rich plasma (VEGFprp), and non-stimulated platelet-poor plasma (VEGFppp). Approximately 30ml venous blood was collected from the subjects' cubital vein and divided into 3-ml plastic blood collection tubes containing either EDTA (Full Blood Count), trisodium citrate (VEGFppp, VEGFprp, IL-6 and TNF- $\alpha$ ) or no anticoagulant (VEGFser). Serum and platelet-poor plasma samples were centrifuged at 10°C for 10 minutes at a relative centrifugal force (g) of 3500. The supernatant was collected, aliquoted, and frozen at -85°C until assay of VEGFppp, IL-6, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ).

VEGFprp was prepared after thrombin-activation. Venous blood was drawn into sodium citrate-containing tubes (0.31% w/v final concentration) and centrifuged at 10°C and 180 g for 15 minutes. The supernatant PRP was separated and 4 U/ml of thrombin was added to 1 ml PRP. After clotting, samples were centrifuged at 10°C for 10 minutes at 3500 g, and the supernatant was removed, aliquoted and stored at -85°C. All blood samples were processed within 30 minutes and frozen within 1 hour after collection. Laboratory staff was blinded to clinical data.

#### **Laboratory measurements**

All measurements were carried out in duplicate as a batch test. The human VEGF immunoassay (R&D Systems, Abingdon, UK) was calibrated with a monoclonal antibody raised against recombinant VEGF165, the major isoform of soluble VEGF. The lower detection limit of the system was 9.0 pg/ml; intra- and inter-assay variations were 6.7% and 8.8%, respectively. IL-6 was measured using high-sensitivity quantitative ELISA kits (R&D

Systems, Abingdon, UK) and results were expressed as pg/ml. TNF- $\alpha$  was measured using high-sensitivity quantitative ELISA kits (R&D Systems, Abingdon, UK). Concentrations below the limit of quantification (LOQ) were set to LOQ/2. Cell counts were performed with an automated hematology analyzer (SE-9500, Sysmex Europe, Norderstedt, Germany). Analysis of the PRP preparations showed them to be 98–100% pure. Plasma fibrinogen was determined according to the Clauss method. C-reactive protein (CRP) levels were assessed using an automated analyzer (Hitachi 917, Boehringer Mannheim, Germany).

### Statistical analysis

Clinical data with normal distribution are presented as mean (SD). Laboratory data are all reported as median (interquartile range) values. Analysis between groups was performed using chi-square test or analysis of variance followed by post-hoc analysis as appropriate. For analysis of follow-up data in patients with exacerbated COPD a Mann-Whitney-U test was performed. Variables which correlated significantly with VEGFser levels in univariate analysis (Spearman's rank correlation) were included in a backward stepwise multiple regression analysis. In these models, we forced in age, sex, BMI, paO<sub>2</sub>, inhaled steroid use, pack-years of smoking, hypertension, and platelet count as covariates to adjust for their potential effects on VEGFser. A p value less than 0.05 was considered statistically significant.

## Results

### Patient characteristics

The characteristics of patients and controls are summarized in Table 1. The groups were well-matched with respect to age, gender, and BMI. By definition lung function parameters were normal in controls and significantly reduced in patients with COPD. There was no significant difference in the proportion of patients with oxygen therapy, hypertension, inhaled bronchodilator or corticosteroid use between patients with exacerbated and those with stable COPD. Mean (SD) duration of hospitalisation for patients with exacerbated COPD was 10.6 (3.7) days.

### VEGF measurements

Coefficients of variation of the duplicate analyses of ELISA were generally less than 5%. The (median [IQR]) serum concentration of VEGF (359 [201–639] pg/ml) was significantly higher ( $p < 0.001$ ) than the corresponding concentration of VEGF<sub>prp</sub> (178 [90–302] pg/ml), despite a higher platelet count in the latter (432 [350–591] platelets  $\times 10^3/\mu\text{L}$  of PRP versus 251 [202–309] platelets  $\times 10^3/\mu\text{L}$  of serum;  $p < 0.01$ ). There was a significant correlation between VEGF<sub>ser</sub> and VEGF<sub>prp</sub> ( $r = 0.681$ ;  $p < 0.001$ ), and between VEGF<sub>ser</sub> and VEGF<sub>ppp</sub> ( $r = 0.474$ ;  $p < 0.01$ ). Correlations did not change significantly when the data from the control subjects was subtracted.

### Circulating VEGF and systemic inflammatory markers in the study groups

The groups were well-matched with respect to hemoglobin concentrations and platelet count (Table 2). Overall, VEGF levels were significantly higher ( $p < 0.01$ ) in patients with acute exacerbated than in patients with stable COPD or controls with normal lung function. These findings were paralleled by an elevation in CRP, peripheral blood neutrophil cell count (PBNC), and circulating IL-6 levels in patients with exacerbated COPD. Fibrinogen levels were significantly higher in patients with COPD compared to controls ( $p < 0.01$  for both), with no significant difference between stable and exacerbated patients. There were no statistically significant differences in TNF- $\alpha$  concentrations between the groups.

### Circulating VEGF and systemic inflammation at clinical recovery

There was a significant reduction in VEGF<sub>ser</sub> levels from 602 [457–883] during acute exacerbation to 490 [378–699] pg/ml ( $p = 0.032$ ) six weeks after hospital discharge (Figure 1). There was a similar decrease in VEGF<sub>prp</sub> and VEGF<sub>ppp</sub> levels (data not shown). The

decrease in circulating VEGF was accompanied by a significant decrease in CRP (6 [1–31] vs. 1 [1–4] mg/L;  $p = 0.013$ ) and fibrinogen concentrations (419 [329–470] vs. 311 [249–401] mg/L;  $p = 0.012$ ). Furthermore, there was a reduction in PBNC and circulating IL-6 levels, however, these changes did not achieve statistical significance.

### **Results of correlation analysis for patients with stable and exacerbated COPD**

For patients with exacerbated COPD, we found positive correlations of VEGF<sub>ser</sub> with CRP, IL-6, PBNC, and fibrinogen (Table 3). Stepwise multiple regression analysis revealed that CRP was the strongest independent predictor ( $p < 0.01$ ) of VEGF<sub>ser</sub>, explaining 38% of the total variability of circulating levels in exacerbated COPD. In patients with stable COPD, VEGF<sub>ser</sub> concentrations correlated positively with FEV1 and PBNC. There was no independent relationship between these variables after adjusting for confounding factors in stable COPD. VEGF<sub>ser</sub> levels were not related to age, gender, BMI, TNF- $\alpha$ , paO<sub>2</sub>, paCO<sub>2</sub>, pH, pack years of smoking, hypertension, LTOT, or inhaled corticosteroid therapy.

## Discussion

This study has investigated circulating levels of VEGF and markers of systemic inflammation in carefully selected patients with stable COPD, patients with acute exacerbated COPD, and age, weight and gender matched controls with normal lung function.

### The origin of circulating VEGF:

Platelets have been proposed as the major source for circulating VEGF [16]. VEGF is released from platelets during blood clotting [17], hemostatic plug formation [18], and in response to inflammatory stimuli [19]. VEGF<sub>ser</sub> concentrations in the present report, however, were significantly higher than VEGF<sub>prp</sub> levels, despite a higher platelet count in the latter. These findings, suggest that in addition to platelets, a further VEGF pool exists in serum that cannot be explained by platelet activation alone. In a previous report from our laboratory, Mittermayer et al. [20] observed a significant increase in circulating VEGF levels in healthy volunteers after experimentally induced systemic inflammation. VEGF levels in that report were related to markers of neutrophil activation rather than clot formation, suggesting a mechanism of VEGF release different from that of the coagulation cascade. There is cumulating evidence that neutrophils contribute to circulating VEGF [21,22]. According to Kusumanto and co-workers [22] approximately 60% of total circulating VEGF resides in neutrophils, whereas only 34% resides in platelets. Although we have not assessed the relative contribution of neutrophils to VEGF liberation into circulation, we observed a strong relationship between VEGF<sub>ser</sub> levels and PBNC, which may support a role for neutrophils in blood-borne VEGF. In disease conditions, the amount of neutrophil-derived VEGF may further increase. Neutrophils may secrete VEGF in response to acute inflammatory stimuli associated with viral [23] and bacterial infections [24], which are frequently associated with exacerbations of COPD.

### Vascular endothelial growth factor in patients with COPD

VEGF is a potent mediator of angiogenesis, which has multiple effects in lung development and physiology. An increasing number of reports suggest an important role for VEGF in the pathogenesis of COPD [5,6,7]. Kasahara and co-workers [25] previously demonstrated that blockade of the VEGF receptor in rats resulted in emphysema via induction of alveolar septal cell apoptosis. Kanazawa and co-workers [7] extended these observations, as they have shown differential VEGF expression in induced sputum samples from patients with COPD of different phenotypes. While the authors reported significantly lower VEGF sputum concentrations in emphysematous type of COPD, they observed significantly higher VEGF levels in patients with the bronchitis phenotype. Likewise there was either a positive or a

negative relationship between FEV1 and VEGF sputum concentrations in emphysema or chronic bronchitis patients, respectively, suggesting a different role of angiogenesis in each phenotype.

Few recent studies also investigated circulating VEGF levels in patients with COPD [26,27,28,29]. The range of VEGFser concentrations observed in our study population are comparable to those of previously published reports [27,28]. Consistent with our findings, Cheng et al [27] have recently shown no significant differences in circulating VEGF concentrations between patients with stable moderate to severe COPD (FEV1% pred  $47 \pm 16\%$ ; VEGFser  $282 \pm 13\text{pg/ml}$ ) and matched controls (VEGFser  $318 \pm 19\text{pg/ml}$ ) without airflow obstruction. Kierszniewska-Stepien and co-workers [29] similarly reported no significant differences in VEGFser between controls and stable patients with severe COPD, however, the authors observed overall higher VEGFser levels in patients with mild COPD. The latter findings suggest differential VEGF expression at different levels of disease severity. This hypothesis is further supported by the significant positive relationship between VEGFser levels and FEV1 observed in our report. Although it has yet to be determined whether VEGF concentrations in the blood mirror expressions of the cytokine in induced sputum samples, our findings may suggest a similar association between VEGF concentrations and airflow obstruction in patients with emphysematous type of COPD as reported previously [7].

Compared to stable COPD, patients with acute exacerbated COPD in the present report demonstrated higher circulating concentrations of VEGF and increased systemic inflammation. We furthermore observed a significant relationship between markers of systemic inflammation, such as c-reactive protein, interleukin-6 and fibrinogen, with VEGFser levels in acute exacerbated COPD.

Low grade systemic inflammation is common in patients with COPD [30]. The degree of systemic inflammation associated with COPD tends to increase over time [31] and is furthermore upregulated during acute exacerbations [26,32]. Systemic inflammation in COPD has been associated with an increased risk of developing CVD [33]. One mechanism for this may be an increase in procoagulatory factors such as IL-6 and fibrinogen, both which have also been linked to VEGF [19,34,35]. Increased VEGF expression, in turn, may support prothrombotic effects via expression of the plasminogen activator inhibitor, von Willebrand factor, and tissue factor [36,37]. In fact, there is evidence that circulating VEGF levels are elevated in patients with acute coronary syndrome [8,12]. In a report by Heeschen [12], patients with acute myocardial ischemia and serum VEGF levels above 500 pg/ml had the lowest 6-month survival rates. Similarly, Slevin and co-workers [38] demonstrated elevated

concentrations of VEGFser ( $588 \pm 121$ pg/ml) in patients with acute ischemic stroke. Expression of VEGF in that report correlated with both infarct volume and clinical disability. Levels of VEGF detected in acute exacerbated COPD are comparable to those associated with individuals at high risk for cardiovascular morbidity and mortality. The significant relationship in our study between CRP — a marker strongly predictive for the development and prognosis of CVD — and VEGFser further supports this relationship.

A recent report [26] similarly assessed several circulating biomarkers including VEGF in patients with COPD during a stable state as well during an exacerbation. In contrast to our findings, however, the authors did not observe a significant difference in median plasma VEGF between baseline (0.01 pg/ml) and exacerbation levels (0.01 pg/ml). Several methodological reasons may account for the discrepancy between our findings: 1. The authors have measured only free plasma levels of VEGF, and may therefore have missed to assess the contribution of platelets and neutrophils to circulating VEGF levels. 2. Hurst and co-workers used an antibody microarray to assess 36 different biomarkers in their patients. Compared to conventional ELISA, the former is basically a qualitative exploratory technique with overall lower sensitivity particularly in the low picogram range, whereas the latter is a more accurate method to specifically quantify protein levels [39,40]. Moreover, the ELISA results in our report were verified by duplicates in all cases. 3. All patients in the present protocol were admitted to hospital because of acute exacerbated COPD, whereas only 3% of the patients studied by Hurst et al. [26] required hospitalization, indicating differences in exacerbation severity and therefore potential differences in the stimulus for VEGF release between the studies.

Finally, we observed that recovery from an exacerbation resulted in a reduction of circulating VEGF levels and systemic inflammatory markers in patients with COPD. Similar results were obtained by McColley et al. [41], who have shown that antibiotic therapy decreased VEGF levels and inflammatory markers in patients with cystic fibrosis. Since all of our patients received systemic steroids and most received antibiotic therapy, we have to acknowledge that we cannot identify the effects of either on VEGF or systemic inflammation. Furthermore the impact of the observed changes in VEGFser levels on patient outcomes has yet to be determined in longitudinal studies.

#### Potential limitations of the study

On a molecular level hypoxia is an important stimulus for VEGF production [1], however, we did not observe a relationship between circulating VEGF levels and arterial oxygenation in

our report. In this context it has to be acknowledged that the hypoxic stimulus for VEGF production was diminished, as practically half of the COPD patients were on LTOT. On the other hand, there is experimental evidence that both platelets and neutrophils, the predominant sources of circulating VEGF, do not release VEGF in response to hypoxia [42]. In addition, it has previously been shown that the transcription of VEGF may be inhibited in patients with chronic hypoxia [43]. Thus, we suggest that hypoxemia associated with COPD seems to not be an independent stimulus for circulating VEGF.

We furthermore have to acknowledge that we did not perform echocardiography to rule out pulmonary hypertension, a potential confounder of circulating VEGF levels in patients with COPD. Tanaseanu and colleagues [44] recently reported elevated VEGF plasma concentrations in COPD patients with pulmonary hypertension compared to those without. Nevertheless, given the close matching of the patient groups and the strong association between changes in systemic inflammation and circulating VEGF levels, it is unlikely that the presence of pulmonary hypertension may have significantly biased our findings.

### Conclusions

Our findings suggest up-regulation of systemic inflammation and circulating VEGF levels in patients with acute exacerbated COPD. Given the compelling evidence implicating vascular factors in the pathogenesis of both chronic lung and cardiovascular disease processes, VEGF may be an important factor linking COPD to cardiovascular pathology.

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## List of abbreviations

BMI: body mass index

CVD: cardiovascular disease

COPD: chronic obstructive pulmonary disease

CRP: C - reactive protein

FEV1: forced expiratory volume in 1 second

FVC: forced vital capacity

IL-6: interleukin 6

IQR: interquartile range

PBNC: peripheral blood neutrophil cells

PEF: peak expiratory flow

TNF- $\alpha$ : tumor necrosis factor alpha

VEGF: vascular endothelial growth factor

VEGFprp: VEGF in platelet rich plasma

VEGFppp: VEGF in platelet poor plasma

## References:

1. Ferrara N. (1999) Molecular and biological properties of vascular endothelial growth factor. *J Mol Med* **77**, 527-543
2. Gerber, H.P., Dixit, V. and Ferrara, N. (1998) Vascular endothelial growth factor induces expression of the antiapoptotic proteins BCL-2 and A1 in vascular endothelial cells. *J Biol Chem* **273**, 13313-13316
3. Kaner, R.J. and Crystal, R.G. (2001) Compartmentalization of vascular endothelial growth factor to the epithelial surface of the human lung. *Mol Med* **7**, 240-246
4. Thickett, D.R., Armstrong, L., Christie, S.J. and Millar, A.B. (2001) Vascular endothelial growth factor may contribute to increased vascular permeability in acute respiratory distress syndrome. *Am J Respir Crit Care Med* **164**: 1601-1605
5. Kasahara, Y., Tuder, R.M., Cool, C.D., Lynch, D.A., Flores, S.C. and Voelkel, N.F. (2001) Endothelial cell death and decreased expression of vascular endothelial growth factor and vascular endothelial growth factor receptor 2 in emphysema. *Am J Respir Crit Care Med* **163**, 737-744
6. Kranenburg, A.R., de Boer, W.I., Alagappan, V.K.T., Sterk, P.J. and Sharma, H.S. (2005) Enhanced bronchial expression of vascular endothelial growth factor and receptors (Flk-1 and Flt-1) in patients with chronic obstructive pulmonary disease. *Thorax* **60**, 106-113
7. Kanazawa, H., Asai, K., Hirata, K. and Yoshikawa, J. (2003) Possible effects of vascular endothelial growth factor in the pathogenesis of chronic obstructive pulmonary disease. *Am J Med* **114**, 354-358
8. Soeki, T., Tamura, Y., Shinohara, H., Tanaka, H., Bando, K. and Fukuda, N. (2000) Serial changes in serum VEGF and HGF in patients with acute myocardial infarction. *Cardiology* **93**, 168-174
9. van der Flier, M., van Leeuwen, H.J., van Kessel, K.P., Kimpen, J.L., Hoepelman, A.I. and Geelen, S.P. (2005) Plasma vascular endothelial growth factor in severe sepsis. *Shock* **23**, 35-38
10. Li, L., Wang, L., Zhang, W., Tang, B., Zhang, J., Song, H., et al. (2004) Correlation of serum VEGF levels with clinical stage, therapy efficacy, tumor metastasis and patient survival in ovarian cancer. *Anticancer Res* **24**, 1973-1979

11. Taylor PC. (2005) Serum vascular markers and vascular imaging in assessment of rheumatoid arthritis disease activity and response to therapy. *Rheumatology* **44**, 721-728
12. Heeschen, C., Dimmeler, S., Hamm, C.W., Boersma, E., Zeiher, A.M. and Simoons, M.L. (2003) Prognostic significance of angiogenic growth factor serum levels in patients with acute coronary syndromes. *Circulation* **107**, 524-530
13. Celli, B.R., MacNee, W., ATS/ERS Task Force (2004) Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J* **23**, 932-946
14. Rodriguez-Roisin R. (2000) Toward a consensus definition for COPD exacerbations. *Chest* **117**, 398-401
15. Forche G. (1986) First comprehensive spirometric studies of new reference values for unrestricted use. *Wien Med Wochenschr Suppl* 1-36
16. Gunsilius, E., Petzer, A., Stockhammer, G., Nussbaumer, W., Schumacher, P., Clausen, J., et al. (2000) Thrombocytes are the major source for soluble vascular endothelial growth factor in peripheral blood. *Oncology* **58**, 169-174
17. Webb, N.J., Bottomley, M.J., Watson, C.J. and Brenchley, P.E. (1998) Vascular endothelial growth factor (VEGF) is released from platelets during blood clotting: implications for measurement of circulating VEGF levels in clinical disease. *Clin Sci* **94**, 395-404
18. Weltermann, A., Wolzt, M., Petersmann, K., Czerni, C., Graselli, U., Lechner, K., et al. (1999). Large amounts of vascular endothelial growth factor at the site of hemostatic plug formation in vivo. *Arterioscler Thromb Vasc Biol* **19**, 1757-1760
19. Caine, G.J., Lip, G.Y., Stonelake, P.S., Ryan, P. and Blann, A.D. (2004) Platelet activation, coagulation and angiogenesis in breast and prostate carcinoma. *Thromb Haemost* **92**, 185-190
20. Mittermayer, F., Pleiner, J., Schaller, G., Weltermann, A., Kapiotis, S., Jilma, B., et al. (2003) Marked increase in vascular endothelial growth factor concentrations during *Escherichia coli* endotoxin-induced acute inflammation in humans. *Eur J Clin Invest* **33**, 758-761
21. Gaudry, M., Bregerie, O., Andrieu, V., El Benna, J., Pocard, M.A. and Hakim, J. (1997) Intracellular pool of vascular endothelial growth factor in human neutrophils. *Blood* **90**, 4153-4161

22. Kusumanto, Y.H., Dam, W.A., Hospers, G.A., Meijer, C. and Mulder, N.H. (2003) Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor. *Angiogenesis* **6**, 283-287
23. Ghildyal, R., Dagher, H., Donniger, H., de Silva, D., Li, X., Freezer, N.J., et al. (2005) Rhinovirus infects primary human airway fibroblasts and induces a neutrophil chemokine and a permeability factor. *J Med Virol* **75**, 608-615
24. Van der Flier, M., Coenjaerts, F., Kimpen, J.L.L., Hoepelman, A.M. and Geelen, S.P.M. (2000) Streptococcus pneumoniae induces secretion of vascular endothelial growth factor by human neutrophils. *Infect Immun* **68**, 4792-4794
25. Kasahara, Y., Tuder, R.M., Taraseviciene-Stewart, L., Le Crass, T.D., Abman, S., Hirth, P.K., et al. (2000) Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J Clin Invest* **106**, 1311-1319
26. Hurst, J.R., Donaldson, G.C., Perera, W.R., Wilkinson, T.M.A., Bilello, J.A., Hagan, G.W., et al. (2006) Use of plasma biomarkers at exacerbation of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **174**, 867-874
27. Cheng, S.L., Wang, H.C., Yu, C.J. and Yang, P.C. (2008) Increased expression of placenta growth factor in chronic obstructive pulmonary disease. *Thorax* Published online 2008 January 17 as doi: 10.1136/thx.2007.087155
28. Aldonyte, R., Eriksson, S., Piitulainen, E., Wallmark, A. and Janciauskiene, S. (2004) Analysis of systemic biomarkers in COPD patients. *COPD* **1**, 155-164
29. Kierszniewska-Stepien, D., Pietras, T., Gorski, P. and Stepien, H. (2006) Serum vascular endothelial growth factor and its receptor level in patients with chronic obstructive pulmonary disease. *Eur Cytokine New* **17**, 75-79
30. Gan, W.Q., Man, S.F.P., Senthilselvan, A. and Sin, D.D. (2004) Association between chronic obstructive pulmonary disease and systemic inflammation: a systemic review and a meta-analysis. *Thorax* **59**, 574-580
31. Donaldson, G.C., Seemungal, T.A., Patel, I.S., Bhowmik, A., Wilkinson, T.M., Hurst, J.R. (2005) Airway and systemic inflammation and decline in lung function in patients with COPD. *Chest* **128**, 1995-2004
32. Wedzicha, J.A., Seemungal, T.A., MacCallum, P.K., Paul, E.A., Donaldson, G.C., Bhowmik, A. et al. (2000) Acute exacerbations of chronic obstructive pulmonary disease are accompanied by elevations of plasma fibrinogen and serum IL-6 levels. *Thromb Haemost* **84**, 210-5

33. Sin, D.D. and Man. S.F. (2003) Why are patients with chronic obstructive pulmonary disease at increased risk of cardiovascular disease? The potential role of systemic inflammation in chronic obstructive pulmonary disease. *Circulation* **107**, 1514-1519
34. Cohen, T., Nahari, D., Cerem, L.W., Neufeld, G. and Levi, B.Z. (1996) Interleukin 6 induces the expression of vascular endothelial growth factor. *J Biol Chem* **271**, 736-741
35. Valipour, A., Litschauer, B., Mittermayer, F., Rauscher, H., Burghuber, O.C. and Wolzt, M. (2004) Circulating plasma levels of vascular endothelial growth factor in patients with sleep disordered breathing. *Respir Med* **98**, 1180-1186
36. Nemerson Y. (1988) Tissue factor and hemostasis. *Blood* **71**,1-8
37. Brock, T.A., Dvorak, H.F. and Senger, D.R. (1991) Tumor-secreted vascular permeability factor increases cytosolic Ca<sup>2+</sup> and von Willebrand factor release in human endothelial cells. *Am J Pathol* **138**,213-221
38. Slevin, M., Krupinski, J., Slowik, A., Kumar, P., Szczudlik, A. and Gaffney, J. (2000) Serial measurement of vascular endothelial growth factor and transforming growth factor-beta 1 in serum of patients with acute ischemic stroke. *Stroke* **31**, 1863-1870
39. Angenendt, P. (2005) Progress in protein and antibody microarray technology. *Drug Discov Today* **10**, 503-11
40. Kusnezow, W. and Hoheisel, J.D. (2002) Antibody microarrays: promises and problems. *Biotechniques* **Suppl**, 14-23
41. McColley, S.A., Stellmach, V., Boas, S.R., Jain, M. and Crawford, S.E. (2000) Serum vascular endothelial growth factor is elevated in cystic fibrosis and decreases with treatment of acute pulmonary exacerbation. *Am J Respir Crit Care Med* **161**,1877-1880
42. Levy AP. (1999) A cellular paradigm for the failure to increase vascular endothelial growth factor in chronically hypoxic states. *Coron Artery Dis* **10**, 427-430
43. Koehne, P., Willam, C., Strauss, E., Schindler, R., Eckardt, K.U. and Buhner, C. (2000) Lack of hypoxic stimulation of VEGF secretion from neutrophils and platelets. *Am J Physiol Heart Circ Physiol* **279**, H 817-824
44. Tanseanu, C., Tudor, S., Tamsulea, I., Marta, D., Manea, G., Moldoveanu, E. (2007) Vascular endothelial growth factor, lipoprotein-associated phospholipase A2, sP-selectin and antiphospholipid antibodies, biological markers with prognostic value in pulmonary hypertension associated with chronic obstructive pulmonary disease and systemic lupus erithematosus. *Eur J Med Res* **12**, 145-151

## Figure Legend

### Figure 1 - VEGFser concentrations in patients with acute exacerbated COPD at admission and clinical recovery

The line across each boxplot and the scatter diagram represents the median. The lines that extend from the top and the bottom of each box represent the lowest and highest observations still inside the region defined by the lower and upper limit of confidence. The circle outside the box represents an outlier. Statistical difference was assessed by Mann Whitney-U test.

## Tables

**Table 1 - Characteristics of study population**

	<b>Exacerbated COPD</b> (n = 30)	<b>Stable COPD</b> (n = 30)	<b>Control Subjects</b> (n = 30)
Age, years	62 (9)	60 (7)	59 (8)
BMI, kg/m <sup>2</sup>	25 (6)	25 (5)	26 (4)
Male	77%	70%	70%
Pack years	57 (39)	58 (27)	27 <sup>†</sup> (18)
Hypertension	26 %	30%	20%
LTOT	50%	43%	n.a.
% of patients with inhaled corticosteroids	27%	30%	n.a.
% of patients with inhaled bronchodilators	100%	100%	n.a.
FEV1, L	0.92 (0.4)	0.97 (0.4)	3.50 <sup>†</sup> (0.4)
FEV1, % pred	34 (14)	34 (9)	84 <sup>†</sup> (6)
FEV1/FVC, %	42 (13)	39 (11)	82 <sup>†</sup> (8)
PEF (L/s)	2,90 (1,2)	3,12 (0,6)	9.8 <sup>†</sup> (1,5)
paO <sub>2</sub> , mmHg	52 (10)	61 <sup>‡</sup> (8)	87 <sup>†</sup> (5)
paCO <sub>2</sub> , mmHg	47 (10)	42 <sup>‡</sup> (6)	40 <sup>†</sup> (6)

Nonparenthesized values are means; parenthesized values are standard deviations.

† indicates  $p < 0.01$  versus both other groups.

‡ indicates  $p < 0.05$  versus patients with exacerbated COPD.

BMI = body mass index; FEV1 = forced expiratory volume in 1 second; FVC = forced vital capacity; PEF = peak expiratory flow; LTOT = long term oxygen therapy; paO<sub>2</sub>, paCO<sub>2</sub> = arterial oxygen and carbon dioxide tensions;

**Table 2 - Median (interquartile ranges) circulating cytokine concentrations and other laboratory markers in the study population**

	<b>Exacerbated COPD (n = 30)</b>	<b>Stable COPD (n = 30)</b>	<b>Control Subjects (n = 30)</b>
VEGFser, pg/ml	602 <sup>†</sup> (457–883)	229 (151–310)	238 (134–363)
VEGFprp, pg/ml	275 <sup>†</sup> (169–431)	118 (42–188)	155 (61–210)
VEGFppp, pg/ml	22 <sup>‡</sup> (13–28)	16 (13–19)	11 (5–21)
IL-6, pg/ml	3.5 <sup>‡</sup> (0.8–6.2)	2.2 <sup>††</sup> (1.7–2.9)	1.2 (0.7–1.4)
TNF- $\alpha$ , pg/ml	1.0 (0.7–1.3)	1.3 (0.9–2.3)	1.4 (1.0–1.7)
CRP, mg/l	6.0 <sup>†</sup> (1–31)	4.0 (2–6)	1.5 (0–5)
Fibrinogen, mg/dl	419 <sup>††</sup> (329–470)	424 <sup>††</sup> (358–459)	360 (326–393)
PBNC $\times 10^3$ cells/ $\mu$ L	9.5 <sup>‡</sup> (6–12)	7.0 <sup>††</sup> (5–9)	4.0 (4–6)
Hemoglobin, g/dl	14.0 (13–15)	15.0 (13–16)	14.3 (13–15)
Platelet count $\times 10^3$ cells/ $\mu$ L	279 (194–356)	273 (218–316)	268 (196–300)

<sup>†</sup> indicates  $p < 0.01$  and <sup>‡</sup> indicates  $p < 0.05$  versus stable COPD and healthy controls

<sup>††</sup> indicates  $p < 0.05$  versus healthy controls

CRP = C-reactive protein; IL-6 = interleukin 6; PBNC = peripheral blood neutrophil cell count; TNF- $\alpha$  = tumor necrosis factor alpha; VEGFser = VEGF serum concentrations.

**Table 3 - Correlation coefficients of VEGFser with inflammatory markers, lung function parameters and oxygenation**

	Exacerbated COPD	Stable COPD
	VEGFser (pg/ml)	
CRP, mg/L	0.61*	0.15
PBNC $\times 10^3$ cells/ $\mu$ L	0.46 <sup>†</sup>	0.46 <sup>†</sup>
IL-6, pg/ml	0.46 <sup>†</sup>	0.07
Fibrinogen, mg/dl	0.39 <sup>‡</sup>	0.02
TNF- $\alpha$ , pg/ml	0.21	0.02
FEV1, L/s	-0.23	0.36 <sup>‡</sup>
FEV1, % pred	-0.28	0.47 <sup>†</sup>
PaO2, mmHg	0.25	0.09

\* p < 0.005, <sup>†</sup> p < 0.01, <sup>‡</sup> p < 0.05

CRP = C-reactive protein; FEV1 = forced expiratory volume in 1 second; IL-6 = interleukin 6; PBNC = peripheral blood neutrophil cell count; TNF- $\alpha$  = tumor necrosis factor alpha; VEGFser = VEGF serum concentrations.

