RESEARCH ARTICLE

Serum levels of VEGF and bFGF in hypoxic patients with exacerbated COPD

Gordana Pavlisa[1], Goran Pavlisa[2], Vesna Kusec[3], Slobodanka Ostojic Kolonic[4], Asja Stipic Markovic[5], Branimir Jaksic[4]

[1] Special Hospital for Pulmonary Diseases, Zagreb, Croatia
[2] Clinical Institute of Diagnostic and Interventional Radiology, University Hospital Center Zagreb, Croatia
[3] Clinical Institute of Laboratory Diagnosis, University Hospital Center Zagreb, Zagreb, Croatia
[4] Department of Internal Medicine, "Merkur" University Hospital, Zagreb, Croatia
[5] Department of Internal Medicine, University Hospital "Sveti Duh", Zagreb, Croatia

Correspondence: G. Pavlisa, M.D., Ph.D., Intensive care unit, Special Hospital for Pulmonary Diseases, Rockefellerova 3, 10000 Zagreb, Croatia <gpavlisa@net.amis.hr>

Accepted for publication February 25, 2010

ABSTRACT. Hypoxia frequently complicates the course of chronic obstructive pulmonary disease (COPD). Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are the two most potent angiogenic factors and may play a role in adaptation to hypoxia. The aims of the study were to assess the serum levels of VEGF and bFGF and to evaluate their mutual relationship in hypoxic patients with exacerbated COPD. The study group consisted of 50 hypoxic (PaO_2 53 mmHg) patients with exacerbated COPD. Control groups were 30 stable COPD patients with PaO_2 70 mmHg, and 30 healthy blood donors. The serum concentrations of VEGF and bFGF were measured using commercial enzyme-linked immunoassay kits. Patients with exacerbated COPD had significantly higher serum VEGF levels (1,089.16 ± 1,128.03 pg/mL) compared to those with stable COPD (197.68 ± 178.06 pg/mL) ($p < 0.0001$) and healthy blood donor group (257.69 ± 170.4 pg/mL) ($p < 0.0001$). Serum bFGF levels were significantly higher in the exacerbated COPD group (6.15 ± 2.56 pg/mL) compared to control groups ($p = 0.0001$). Basic FGF was undetectable in the stable COPD and blood donor groups. Since VEGF and bFGF correlated significantly with the majority of factors investigated in COPD patients, multivariate analysis was performed. According to the step-wise regression analysis, VEGF was best determined by PaO_2, WBC and IL-6. Basic FGF was best determined by PaO_2 and pH. The highly significant, simple correlation between VEGF and bFGF was lost in multivariate analysis. This suggests that their correlation is not independent, but due to factors that remain in the model after step-wise regression. These are essentially linked to the level of hypoxia. Results of our study suggest that VEGF and bFGF production is stimulated in hypoxic patients with exacerbated COPD. Elevated levels of VEGF and bFGF may activate the process of neoangiogenesis, which may lead to increased perfusion and an improvement in tissue oxygenation in this group of patients.

Keywords: VEGF, bFGF, COPD, hypoxia

Chronic obstructive pulmonary disease (COPD) is frequently complicated by hypoxia. The response to hypoxia occurs through several, well described mechanisms, but none of them alone, or in combination, can fully explain the adaptation to hypoxia. The regulation of gene expression has recently been recognized as an important adaptive response to hypoxia. The cell response to hypoxia is mediated by hypoxia-inducible factor-1 (HIF-1), whose expression rises exponentially in hypoxic conditions. HIF-1 participates in the initiation of transcription of erythropoietin (EPO), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) genes, and in the regulation of metabolic processes by activation of the transcription of several glycolytic enzymes and glucose-carrier-1 [1]. The activation of EPO production and subsequent erythropoesis is the most frequently mentioned model of adaptation to systemic hypoxia. However, only a minor subset of hypoxic COPD patients develop secondary polycythemia and elevated levels of EPO [2]. These data indicate other important mechanisms, such as angiogenesis, that may be involved in the adaptation to hypoxia. VEGF and bFGF are the most potent proangiogenic factors [3]. VEGF is a 45 kDa, dimeric glycoprotein. The biological potency of VEGF depends on its reaction with specific receptors: VEGF R1 and VEGF R2. VEGF R1 and VEGF R2 are expressed on endothelial cells and tumor cells which produce VEGF [4]. Basic fibroblast growth factor is a single-chain, non-glycolysed cation polypeptide with a molecular mass of approximately 18 kDa [5]. VEGF and bFGF mediate a number

doi: 10.1684/ecn.2010.0193

of processes necessary for angiogenesis [4, 5]. VEGF and bFGF play a significant role during normal angiogenesis, such as wound healing, fetal and neonatal life [4, 6]. Several investigational models have shown that VEGF and bFGF are a part of the adaptive reaction to hypoxia. Their production is enhanced in conditions of local and systemic hypoxia [7]. HIF-1, VEGF and bFGF have been found in the serum and atherosclerotic lesions of patients with coronary artery disease, and play a role in collateral vessel formation [8, 9]. In patients with sleep apnea syndrome and hypoxia, the level of VEGF is elevated [10]. The regulation of these proangiogenic factors in COPD associated with hypoxia is unclear. The aim study were to investigate whether these factors are up-regulated in COPD patients with respiratory failure, and to assess which factors may influence their expression in this complex clinical model.

DONORS AND METHODS

The institutional Ethical Committee approved the study and informed consent was obtained from all of the participants.

Selection of the patients

None of the participants had a history of cancer, autoimmune disease or ischemic heart disease.
The quantitative characteristics of the groups are summarized in *table 1*.

Group 1: Patients with exacerbated COPD

Fifty consecutive patients with exacerbated COPD and hypoxia with partial arterial oxygen pressure (PaO_2) 53 mmHg entered the study. All of the patients had previously diagnosed COPD. The diagnosis was based on the NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria [11]. None of the patients had received continuous oxygen therapy before admission to the hospital. According to the clinical assessment, the onset of the exacerbation occurred at least 24 hours prior to hospitalization in all studied patients.
All patients had the clinical characteristics of respiratory inflammation such as an increase in sputum production, purulent sputum and dyspnea progression. According to the GOLD criteria, 67% of the patients were GOLD IV stage.

Group 2: Patients with stable COPD

Thirty patients with stable COPD with a PaO_2 3 70 mmHg were recruited from a hospital outpatient clinic. Thirteen patients had an arterial oxygen tension over 75 mmHg while breathing air. Stable COPD patients were defined as patients without any exacerbation of COPD during the previous four months, and without any change in respiratory medication during the same period.
The maximum value for the forced expiratory volume in the first second/forced expiratory vital capacity (FEV1/FVC) was 60% of the predicted value. Based on the GOLD criteria, 67% of the patients had moderate, and 33% had severe COPD. All the patients had irreversible airflow limitation.

Group 3: Healthy blood donors

Group 3 consisted of 30 healthy blood donors with arterial oxygen saturation ($SatO_2$) of over 97%, and normal values for the complete blood count. None of them had a history of pulmonary disease.

Laboratory tests

The following tests were performed for all participants: complete blood count, serum VEGF, bFGF and

Table 1
Characteristics of groups and differences between groups

Characteristic	Group 1	Group 2	Group 3	Group 1 to 2	Group 1 to 3	Group 2 to 3
Subjects	n = 50	n = 30	n = 30			
Gender M/F	23/27	19/11	15/15	0.3149	0.7287	0.2974
Age (years)	68.1 ± 9.78	69.43 ± 7.56	57.0 ± 4.44	0.5241	< 0.0001	< 0.0001
PaO_2 (mmHg)	45.42 ± 5.1	75.49 ± 5.22		< 0.0001		
$PaCO_2$ (mmHg)	46.16 ± 8.82	37.7 ± 3.86		< 0.0001		
pH	7.45 ± 0.06	7.43 ± 0.03		0.1682		
$SatO_2$ (%)	80.73 ± 7.49	94.99 ± 1.02		< 0.0001		
FVC (%)	74.9 ± 20.2	92.01 ± 11.35		0.0004		
FEV1 (%)	42.8 ± 20.2	52.47 ± 12.15		0.0029		
RBC ($x10^{12}$/L)	5.34 ± 0.7	4.67 ± 0.35	4.73 ± 0.44	< 0.0001	< 0.0001	0.5605
Hb (g/L)	154.1 ± 22.3	144.2 ± 13.03	143.7 ± 10.5	0.0325	0.0195	0.8782
Hct (ratio)	0.47 ± 0.06	0.37 ± 0.13	0.41 ± 0.03	0.0009	< 0.0001	0.1147
MCV (μm^3)	87.25 ± 6.2	90.28 ± 5.36	86.7 ± 5.6	0.0319	0.6918	0.0151
MCH (pg)	28.81 ± 2.63	30.88 ± 1.81	30.53 ± 2.42	0.0004	0.0048	0.5334
WBC ($x10^9$/L)	10.3 ± 5.2	7.04 ± 1.55	6.8 ± 1.6	0.0015	0.0005	0.5180
Platelets ($x10^9$/L)	287.15 ± 123.05	242.14 ± 64.68	263.2 ± 64.63	0.0718	0.3272	0.2161
IL-6 (pg/mL)	46.72 ± 91.11	1.36 ± 3.76	0.06 ± 0.29	0.0081	0.0065	0.0635

Values are given as the mean ± SD. Unpaired t- test was used to assess differences between groups with respect to continuous data, and χ^2 test was used regarding nominal data.

interleukin-6 (IL-6). In the COPD patients, the arterial blood gas analyses were performed, while in healthy blood donors, arterial oxygen saturation was measured transcutaneously using fingertip pulse oximetry. In patients with exacerbated COPD, blood samples were taken at the moment of admission to the hospital, before the start of the treatment.

Pulmonary function studies were performed in patients with stable COPD on the day of the blood sample collection. In the exacerbated COPD group, pulmonary function studies were done before the discharge from the hospital.

Blood samples for the arterial blood gas analyses were drawn anaerobically from the radial artery into heparinized syringes and were promptly analyzed. Routine hematological analyses were performed using standard biochemical methods.

The serum samples for the determination of VEGF, bFGF and IL-6 were collected in vacutainer tubes without additive. Within 30 minutes of blood collection, the samples were centrifuged at 1,000 x g for 15 minutes. The serum was carefully transferred to new tubes. Serum samples were frozen within one hour of collection at a temperature of - 70°C. All samples were analyzed after the study period, at the same time. The samples were analyzed using commercially available ELISA kits for VEGF (DVE00), bFGF (HSFB75) and IL-6 (D6050) (R&D Systems, Minneapolis, Minnesota, USA) according to the manufacturer`s instructions. Sensitivity for bFGF was 3 pg/mL, VEGF 9 pg/mL, and for IL-6 0.7 pg/mL.

Statistical analysis

Results were statistically analyzed using parametric and non-parametric tests as appropriate, with "Statistica" and "StatView" (v. 5.0.1. SAS Institute Inc.) software. Results are given as the mean ± SD for continuous data, and as the proportion of the group for nominal data. To assess the difference between groups with respect to continuous data, Student`s t- test was used, and regarding nominal data O_2 test was used. A p value of less than 0.05 was considered statistically significant. The relationship between serum VEGF, bFGF and other continuous data were analyzed by a simple regression test. Since VEGF and bFGF correlated significantly with almost all the factors investigated, multivariate step-wise analysis was performed.

RESULTS

VEGF and bFGF findings in the three study groups

VEGF and b-FGF levels were significantly higher in group 1 compared to groups 2 and 3 ($p < 0.0001$). In the group of patients with exacerbated COPD, the mean VEGF value was 1,089.16 ± 1,128.03 pg/mL. (ranging from 159.53-5,725 pg/mL). The mean bFGFvalue in the exacerbated COPD group was 6.15 ± 2.56 pg/mL (ranging from 2.8-15 pg/mL).

We did not find any statistically significant difference in the VEGF levels between the stable COPD group and blood donor group ($p = 0.1071$). In the stable COPD group, the mean VEGF value was 197.68 ± 178.06 pg/mL (ranging from undetectable levels to 784 pg/mL). In the blood donor group, the mean VEGF value was 257.69 ± 170.4 pg/mL (ranging from 12.48 to 605.73 pg/mL). Basic FGF was undetectable in the stable COPD and blood donor groups *(figures 1 and 2)*.

Correlation between parameters analyzed in the COPD patients

Simple correlations of VEGF with other parameters in patients with exacerbated and stable COPD are presented in *table 2*. The data are sorted by the correlation coefficient and significance. VEGF correlated significantly with almost all parameters studied. The strongest

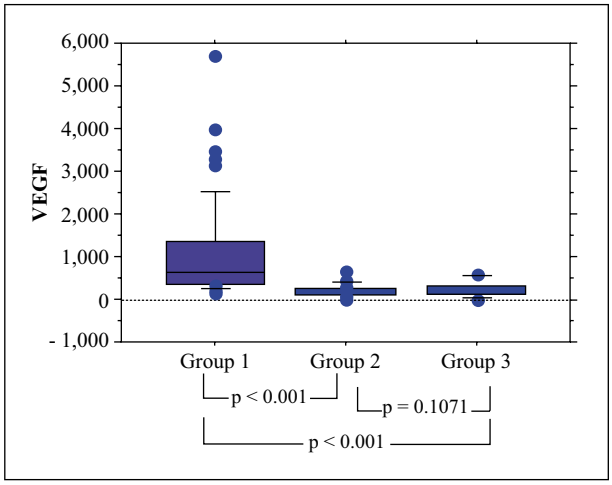

Figure 1
VEGF level in study groups. Statistical differences between groups were assessed with the Mann-Whitney test Box-Whisker standard plots show the following elements: median, 10th, 25th, 75th and 90th percentile.

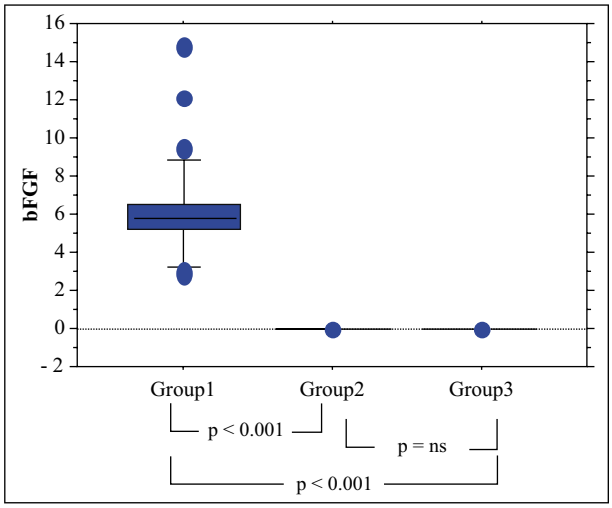

Figure 2
bFGF level in study groups. Statistical differences between groups were assessed with the Mann-Whitney test Box-Whisker standard plots show the following elements: median, 10th, 25th, 75th and 90th percentile.
ns: not significant.

Table 2
Correlation between serum VEGF and selected continuous data

Variables	r	p
PaO_2	- 0.509	< 0.0001
WBC	0.508	< 0.0001
Platelets	0.457	< 0.0001
bFGF	0.455	< 0.0001
RBC	0.280	0.0156
$PaCO_2$	0.236	0.0425
MCH	- 0.230	0.0484
Hct	0.228	0.0507
pH	0.166	0.1565
IL-6	0.140	0.2345
Hb	0.128	0.2763
MCV	- 0.104	0.3788
Age	0.032	0.7892

Correlation between serum VEGF and continuous data were analysed by regression simple test.

correlation of VEGF was with PaO_2 ($r = -0.509$, $p < 0.0001$), white blood count WBC ($r = 0.508$, $p < 0.0001$), platelet count ($r = 0.457$, $p < 0.0001$), and bFGF ($r = 0.455$, $p < 0.0001$).

We found no statistically significant correlation with lung function parameters or GOLD stages of disease when analyzing the overall COPD population or groups 1 and 2 separately.

Also, bFGF significantly correlated with almost all parameters studied *(table 3)*. The strongest correlation of bFGF was with PaO_2 ($r = -0.816$, $p < 0.0001$), VFGF ($r = 0.455$, $p < 0.0001$) and RBC ($r = 0.437$, $p < 0.0001$).

Multivariate analysis

Since several significant correlations were found between parameters studied, multivariate (step-wise regression) analysis was performed to assess their mutual relationship.

Step-wise regression was performed with VEGF and bFGF as dependent variables. Thirteen independent variables were included in the model. These were: PaO_2, arterial carbon dioxide tension ($PaCO_2$), pH, red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), WBC, platelets, IL-6, age, VEGF and/or bFGF.

Table 4 shows the final step in a step-wise regression analysis of VEGF, and describes variables that remain in the model, while all other variables ended the analysis out of the model. VEGF is independently determined by PaO_2 (F-to-remove = 14.9), WBC (F-to-remove = 23.2) and IL-6 (F-to-remove = 9.3). *Table 5* shows that bFGF is independently determined by PaO_2 (F-to-remove = 151.35) and pH (F-to-remove = 11.26), while other parameters analyzed ended the analysis out of model. A strongly significant, simple correlation between VEGF and bFGF was entirely lost during a step-wise regression analysis. This suggests that the significant, simple correlation between the two angiogenic factors is not independent, but is essentially due to their individual association with hypoxia.

Table 3
Correlation between serum bFGF and selected continuous data

Variables	r	p
PaO_2	- 0.816	< 0.0001
VEGF	0.455	< 0.0001
RBC	0.437	< 0.0001
WBC	0.371	0.0011
Hct	0.371	0.0011
Platelets	0.362	0.0015
$PaCO_2$	0.326	0.0046
pH	0.312	0.0068
MCH	- 0.305	0.0081
IL-6	0.284	0,0141
Hb	0.247	0.0342
Age	- 0.141	0.2294
MCV	- 0.129	0.2734

Correlation between serum bFGF and continuous data were analysed by regression simple test.

DISCUSSION

The present study has shown significantly higher serum VEGF and bFGF levels in exacerbated COPD patients with hypoxia compared to COPD patients with stable disease or healthy blood donors. The strong positive correlation of VEGF and bFGF may indicate that they are simultaneously activated.

Hypoxia is a strong inducer of VEGF release in physiological and numerous pathological situations with consequent neoangiogenesis. Physical strain increases the expression of VEGF in muscles, and this increase correlates with the lactate concentration in venous plasma of the involved muscles [12]. VEGF is up-regulated in ischemic myocardial tissue [8], ischemic brain tissue [13], and ischemic limbs [14]. In patients suffering from sleep apnea syndrome and severe hypoxia, levels of VEGF are elevated [10]. Several investigations have studied serum levels of VEGF in COPD patients. Valipour *et al.* found that patients with exacerbated COPD have higher circulating

Table 4
Stepwise regression analysis of VEGF *versus* 13 independent variables (final step)

	Coefficient	Std. Error	Std. Coeff.	F-to-Remove
Intercept	1,095.496	492.043	1,095.496	4.957
PaO_2	- 24.473	6.345	- 0.375	14.879
WBC	133.021	27.589	0.598	23.247
IL-6	- 4.786	1.568	- 0.362	9.311

Forward entry stepwise regression method was used. F-to-enter was 4.0, F-to-remove was 3.996.

Table 5
Stepwise regression analysis of bFGF *versus* 13 independent variables (final step)

	Coefficient	Std. Error	Std. Coeff.	F-to-Remove
Intercept	- 89.666	30.916	- 89.666	8.412
PaO_2	- 0.176	0.014	- 0.790	151.350
pH	13.895	4.141	0.215	11.260

Forward entry stepwise regression method was used. F-to-enter was 4.0, F-to-remove was 3.996.

VEGF concentrations compared to stable COPD patients and healthy controls [15]. The mean serum VEGF levels in stable COPD patients and healthy controls in their study are comparable to our results. Conversely, the mean serum VEGF levels in patients with exacerbated COPD were higher in our study. Our patients with exacerbated COPD had more severe hypoxia, which may have been the cause of the higher VEGF levels. Alydonyte *et al.* did not find increased expression of serum VEGF in stable COPD patients, and the mean values were comparable to our results [16]. In the study by Kierszniewska-Stepie *et al.*, the serum VEGF concentration was elevated in patients with stable COPD, and even in patients with mild disease [17]. In this study, patients with mild COPD had lower mean PaO_2 compared to our group with stable COPD. The studies mentioned did not address the role of hypoxia as a potential trigger of VEGF synthesis in COPD patients. A statistically significant negative correlation of VEGF with PaO_2 found in our study confirms the hypothesis that hypoxia is strongly associated with VEGF synthesis in this clinical model.

Studies of the concentration dynamics of VEGF are scarce. It has been shown that the concentration of VEGF reaches a maximum 24 hours after an acute myocardial infarction [18]. In our patients, the progression of the disease, as well as the hypoxia lasted longer than 24 hours. Significantly higher parameters of the red blood count in the exacerbated compared to stable COPD group indicate that those patients were possibly hypoxemic prior to the exacerbation with consequently activated erythropoeisis. Also, since this group of patients had more significantly impaired lung function, it is to be expected that the level of hypoxia was lower, even in a stable phase of the disease.

Since the COPD is a complex clinical syndrome, hypoxia may not be the only factor involved with the increased expression of VEGF. Systemic inflammation can influence the VEGF level [19, 20]. Neutrophils are recognized as a significant source of VEGF [21]. Also, inflammatory cytokines, such as IL-6, may induce VEGF expression [22]. COPD is characterized by the chronic inflammation of the tracheobronchial tree, further activated in the case of exacerbation [23]. It is well known that COPD patients exhibit elevated markers of systemic inflammation [24, 25].

In our study, WBC and IL-6 values were, not surprisingly, significantly higher in a group of exacerbated COPD patients compared to stable COPD patients or the blood donor group. A significantly positive correlation, as well as an independent relationship of VEGF with the white blood cell count and IL-6, may suggest that both acute and chronic inflammation contribute to high VEGF level in the model studied. The positive correlation of WBC and VEGF cannot be explained by direct VEGF release from leukocytes under the influence of hypoxia, since white blood cells do not produce significant quantities of VEGF in response to isolated hypoxia [26].

Recent investigations have indicated that VEGF may have a role in the pathogenesis of different manifestations and different stages of stable COPD [27, 28]. In our study, there was no significant correlation between the VEGF levels and lung function parameters. It is possible that in the exacerbated COPD group, systemic hypoxia and inflammation have a much stronger influence on serum VEGF level, than local lung tissue production. Also, in our group of patients with exacerbation, the lung function testing was not performed at the same time as the VEGF measurements.

The most important and consistent finding of our study was that serum bFGF levels are significantly elevated in exacerbated COPD patients, which has been scarcely addressed in the literature. Serum bFGF levels were undetectable in patients with stable disease and in healthy blood donors. Basic FGF synthesis was activated only in patients with significant hypoxia. Therefore, it is probably not a linear phenomenon, but occurs only after a certain level of hypoxia is reached. A strong negative correlation in this model indicates that hypoxia is the most important stimulus of bFGF production. Also, step-wise regression analysis disclosed that the independent correlation is due to hypoxia and acid-base status. Based on these results, we believe that hypoxia is one of the factors leading to its increased expression.

It is well known that the synthesis of bFGF is strongly activated in hypoxic conditions by the regulation of HIF [5]. The increased expression of this factor has been found in states of local and systemic hypoxia. The expression of the bFGF gene is enhanced in skeletal muscles after physical exercise [29]. It has been shown in the model of ischemic myocardium, that monocytes in hypoxia produce bFGF [30]. In patients with solid tumors and concomitant anemia there is a significant correlation between systemic hypoxia and serum b-FGF [31]. In the condition of chronic hypoxia, bFGF is increasingly expressed in the brain tissue [32].

Larsson *et al.* measured bFGF in healthy blood donors [33]. In this study, the levels of bFGF were higher than in our group of healthy blood donors, and levels in women were higher than in men. The mean age of female patients in that study was 43 years, with the expected reproductive cycle in a significant percentage of patients. One of rare situations in which angiogenesis exist in adults is during the female reproductive cycle [6]. The mean age of female patients in our study was 54 years and they were all experiencing the menopause, which may be a reason for the lower levels of bFGF.

Several clinical models have also indicated the association between chronic inflammation and bFGF expression. It has been shown that systemic inflammation in patients on peritoneal dialysis is linked to increased plasma levels of bFGF [34]. Synovial fibroblasts of patients suffering from rheumatoid arthritis produce bFGF [35]. Some studies have suggested that IL-6 could enhance expression of bFGF [36].

However, in contrast to VEGF, in our study, we did not confirm any independent correlation with inflammatory markers.

The highly significant, simple correlation of VEGF and bFGF is completely lost in multivariate analysis, suggesting that their correlation is not independent, but is due to the high correlation with common, causative factor(s) that remain in the model after step-wise regression. Most important of these are essentially linked to the level of hypoxia.

In this study, the patients with exacerbated COPD had significantly higher serum VEGF and bFGF levels compared to the COPD patients with stable disease or the healthy blood donor group. Multivariate analysis disclosed interesting interactions. Step-wise regression analysis showed that hypoxia was the independent factor that contributed to the model, for both VEGF and bFGF. The only two additional factors influencing VEGF were WBC and IL-6, both essentially linked to inflammation. The only additional factor influencing bFGF was alkalosis, the mechanism of which is presently unclear. All other parameters regarding VEGF and bFGF ended the analysis out of the model. A strong, significant correlation between VEGF and bFGF was entirely lost in multivariate step-wise regression analysis. This suggests that their correlation was not independent, but was due to the high correlation with hypoxia, the common factor that remained in the model after step-wise regression. This finding illustrates the predominant role of hypoxia.

The elevated values of VEGF and bFGF in a group of exacerbated COPD patients may indicate that the process of neoangiogenesis has been activated in this group of patients, which may have lead to increased perfusion and an improvement in tissue oxygenation.

Financial support. *This paper was supported by the Ministry of Science and Technology of the Republic of Croatia [Research Grants 0108107 and 108-1081873-1893].*

Disclosure*. None of the authors has any conflict of interest to disclose.*

REFERENCES

1. Gleadle JM, Ratcliffe PJ. Induction of hypoxia-inducible factor-1, erythropoietin, vascular endothelial growth factor, and glucose transporter-1 by hypoxia: evidence against a regulatory role for Src kinase. *Blood* 1997; 89: 503.
2. Pavlisa G, Vrbanic V, Kusec V, Jaksic B. Erythropoietin response after correction of severe hypoxaemia due to acute respiratory failure in chronic obstructive pulmonary disease patients. *Clin Sci (Lond)* 2004; 106: 43.
3. Bussolino F, Albini A, Camussi G, *et al.* Role of soluble mediators in angiogenesis. *Eur J Cancer* 1996; 32A: 2401.
4. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol* 1995; 146: 1029.
5. Klagsbrun M. The fibroblast growth factor family: structural and biological properties. *Prog Growth Factor Res* 1989; 1: 207.
6. Malamitsi-Puchner A, Tziotis J, Tsonou A, Protonotariou E Sarandakou ACreatsas G. Basic fibroblast growth factor: serum levels in the female. *Growth Factors* 2000; 17: 215.
7. Bussolino F, Albini A, Camussi G, *et al.* Hypoxic responses of vascular cells. *Chest* 1998; 114: 25S.
8. Lee SH, Wolf PL, Escudero R, Deutsch R, Jamieson SW, Thistlethwaite PA. Early expression of angiogenesis factors in acute myocardial ischemia and infarction. *N Engl J Med* 2000; 342: 626.
9. Detillieux KA, Cattini PA, Kardami E. Beyond angiogenesis: the cardioprotective potential of fibroblast growth factor-2. *Can J Physiol Pharmacol* 2004; 82: 1044.
10. Imagawa S, Yamaguchi Y, Higuchi M, Neichi T, Hasegawa Y, Mukai HY. Levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea-hypopnea syndrome. *Blood* 2001; 98: 1255.
11. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. GOLD Scientific Committee. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. *Am J Respir Crit Care Med* 2001; 163: 1256.
12. Gustafsson T, Puntschart A, Sundberg CJ, Jansson E. Related expression of vascular endothelial growth factor and hypoxia-inducible factor-1 mRNAs in human skeletal muscle. *Acta Physiol Scand* 1999; 165: 335.
13. Hayashi T, Abe K, Suzuki H, Itoyama Y. Rapid induction of vascular endothelial growth factor gene expression after transient middle cerebral artery occlusion in rats. *Stroke* 1997; 28: 2039.
14. Hochberg I, Hoffman A, Levy AP. Regulation of VEGF in diabetic patients with critical limb ischemia. *Ann Vasc Surg* 2001; 15: 388.
15. Valipour A, Schreder M, Wolzt M, *et al.* Circulating vascular endothelial growth factor and systemic inflammatory markers in patients with stable and exacerbated chronic obstructive pulmonary disease. *Clin Sci (Lond)* 2008; 115: 225.
16. Aldonyte R, Eriksson S, Piitulainen E, Wallmark A Janciauskiene S. Analysis of systemic biomarkers in COPD patients. *COPD* 2004; 1: 155.
17. Kierszniewska-Stepie D, Pietras T. G¢rski P, Stepien H. Serum vascular endothelial growth factor and its receptor level in patients with chronic obstructive pulmonary disease. *Eur Cytokine Netw* 2006; 17: 75.
18. Yin R, Feng J, Yao Z. Dynamic changes of serum vascular endothelial growth factor levels in a rat myocardial infarction model. *Chin Med Sci J* 2000; 15: 154.
19. Alatas F, Alatas O, Metintas M, Ozarslan A, Erginel S, Yildirim H. Vascular endothelial growth factor levels in active pulmonary tuberculosis. *Chest* 2004; 125: 2156.
20. Yano K, Liaw PC, Mullington JM, *et al.* Vascular endothelial growth factor is an important determinant of sepsis morbidity and mortality. *J Exp Med* 2006; 203: 1447.
21. Kusumanto YH, Dam WA, Hospers GA, Meijer C, Mulder NH. Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor. *Angiogenesis* 2003; 6: 283.
22. Cohen T, Nahari D, Cerem LW, Neufeld G, Levi BZ. Interleukin 6 induces the expression of vascular endothelial growth factor. *J Biol Chem* 1996; 271: 736.
23. D'silva L. Cook RJ, Allen CJ, Hargreave FE, Parameswaran K. Changing pattern of sputum cell counts during successive exacerbations of airway disease. *Respir Med* 2007; 101: 2217-20.
24. Wedzicha JA, Seemungal TA, MacCallum PK, *et al.* Acute exacerbations of chronic obstructive pulmonary disease are accompanied by elevations of plasma fibrinogen and serum IL-6 levels. *Thromb Haemost* 2000; 84: 210.
25. Chung KF. Cytokines in chronic obstructive pulmonary disease. *Eur Respir J* 2001; 34: s50.
26. Koehne P, Willam C, Strauss E, Schindler R, Eckardt KU. B_hrer C. Lack of hypoxic stimulation of VEGF secretion from neutrophils and platelets. *Am J Physiol Heart Circ Physiol* 2000; 279: H817.

27. Kasahara Y, Tuder RM, Taraseviciene-Stewart L, *et al.* Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J Clin Invest* 2000; 106: 1311.

28. Kanazawa H, Asai K, Nomura S. Vascular endothelial growth factor as a non-invasive marker of pulmonary vascular remodeling in patients with bronchitis-type of COPD. *Respir Res* 2007; 8: 22.

29. Olfert IM, Breen EC, Mathieu-Costello O, Wagner PD. Skeletal muscle capillarity and angiogenic mRNA levels after exercise training in normoxia and chronic hypoxia. *J Appl Physiol* 2001; 91: 1176.

30. Panutsopulos D, Zafiropoulos A, Krambovitis E, Kochiadakis GE, Igoumenidis NE, Spandidos DA. Peripheral monocytes from diabetic patients with coronary artery disease display increased bFGF and VEGF mRNA expression. *J Transl Med* 2003; 1: 6.

31. Porta C, Imarisio I, De Amici M, *et al.* Pro-neoangiogenic cytokines (VEGF and bFGF) and anemia in solid tumor patients. *Oncol Rep* 2005; 13: 689.

32. Ganat Y, Soni S, Chacon M, Schwartz ML, Vaccarino FM. Chronic hypoxia up-regulates fibroblast growth factor ligands in the perinatal brain and induces fibroblast growth factor-responsive radial glial cells in the sub-ependymal zone. *Neuroscience* 2002; 112: 977.

33. Larsson A. Sköldenberg E, Ericson H. Serum and plasma levels of FGF-2 and VEGF in healthy blood donors. *Angiogenesis* 2002; 5: 107.

34. Stompór T, Zdzienicka A, Motyka M, Dembińska-Kieć A, Davies SJ, Sulowicz W. Selected growth factors in peritoneal dialysis: their relationship to markers of inflammation, dialysis adequacy, residual renal function, and peritoneal membrane transport. *Perit Dial Int* 2002; 22: 670.

35. Bucala R, Ritchlin C, Winchester R, Cerami A. Constitutive production of inflammatory and mitogenic cytokines by rheumatoid synovial fibroblasts. *J Exp Med* 1991; 173: 569.

36. Jee SH, Chu CY, Chiu HC, Huang YL, Tsai WL, Liao YH. Interleukin-6 induced basic fibroblast growth factor-dependent angiogenesis in basal cell carcinoma cell line *via* JAK/STAT3 and PI3-kinase/Akt pathways. *J Invest Dermatol* 2004; 123: 1169.