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# Longitudinal Measurement of Serum Vascular Endothelial Growth Factor in Patients with Chronic Obstructive Pulmonary Disease

Lucas Boeck Jyotshna Mandal Luigi Costa Michael Roth Michael Tamm
Daiana Stolz

Clinic of Pulmonary Medicine and Pulmonary Cell Research, University Hospital Basel, Basel, Switzerland

**Key Words**
Biomarker · Chronic obstructive pulmonary disease · Emphysema · Serum · Vascular endothelial growth factor

**Abstract**
***Background:*** Impaired vascular endothelial growth factor (VEGF) signaling causes emphysema in animal models. In chronic obstructive pulmonary disease (COPD) patients, alterations in VEGF tissue expression have been observed. We hypothesize that circulating VEGF may be a biomarker to phenotype COPD patients. ***Objective:*** The aim of this study was to investigate VEGF serum levels in stable and exacerbated COPD. ***Methods:*** VEGF serum levels as well as parameters of short- and long-term outcome were assessed and analyzed in two COPD cohorts [PROMISE, n = 117; ProCOLD (PC), n = 191]. ***Results:*** VEGF serum levels at stable COPD were neither related to forced expiratory volume in 1 s nor to the Modified Medical Research Council dyspnea score, 6-min walking distance or BODE index. There was no association between single VEGF levels and COPD exacerbation frequency or mortality at 1 and 2 years of follow-up. In PC an increase in VEGF over time (ΔVEGF) was associated with the exacerbation frequency as well as the 1- and 2-year hospitalization rate (p = 0.046, 0.009 and 0.006, respectively). Furthermore, in PC ΔVEGF was associated with 1- and 2-year survival (p = 0.009 and 0.041, respectively). ***Conclusions:*** Single serum VEGF levels, at stable and exacerbated COPD, were not associated with clinically significant outcomes in COPD. Conversely, the VEGF course seems related to COPD prognosis.


## Introduction

Cigarette smoke is the most important cause of chronic obstructive pulmonary disease (COPD) in the Western world. While most smokers handle this noxious agent without developing significant respiratory disease, the remaining genetically susceptible individuals progress to very different physiological and clinical changes, subsumed under COPD. However, current diagnostic and therapeutic approaches largely ignore this heterogeneity [1, 2]. To improve management, identification of specific COPD phenotypes remains of the greatest importance.

Vascular endothelial growth factor (VEGF) covers a number of proteins, resulting from alternative splicing of a single VEGF gene. The transcription of VEGF is mainly controlled by hypoxia-inducing factor-1α, a hypoxia-responding molecule [3]. VEGF signaling was proposed to be associated with COPD development, COPD sever-


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E-Mail karger@karger.com
www.karger.com/res

Prof. Daiana Stolz, MD, MPH
University Hospital Basel
Petersgraben 4
CH–4031 Basel (Switzerland)
E-Mail daiana.stolz@usb.ch

ity, and distinct clinical and morphological COPD phenotypes such as emphysema. Cigarette smoke reduces the expression of VEGF receptor 2 [4]. Conditional knockout of the VEGF gene or VEGF receptor blockade results in lung cell apoptosis and emphysema [5, 6]. In human lungs, a decreased VEGF expression was associated with emphysema [7]. In contrast, an increased VEGF and VEGF receptor expression was observed in epithelial cells, alveolar macrophages and smooth muscle cells of COPD patients [8]. It was proposed that VEGF is increased in airways of COPD patients, while VEGF expression is decreased in the emphysematous parenchyma of COPD patients. These features may qualify VEGF as a clinically relevant marker for COPD.

The goal of this study was to characterize serum VEGF in stable and exacerbated COPD with regard to disease severity and outcome. To our knowledge, this is the first study investigating VEGF and outcome in COPD.

## Materials and Methods

*Study Population*

Herein, we report the results of two longitudinal COPD trials taking place at the University Hospital Basel, Switzerland. In both studies acute exacerbated COPD (AECOPD) was defined as an acute, sustained worsening of the COPD patient's condition beyond normal day-to-day variation [9]. Moderate AECOPD was defined as an AECOPD requiring systemic corticosteroids and/or antibiotics, whereas severe AECOPD required hospitalization [10]. Mild AECOPDs were not evaluated. Both trials were approved by the institutional review board and registered as the ProCOLD study (PC; Ethics Committee of Basel 232/03, registration No. ISRCTN77261143) and as the PROMISE study (PM; Ethics Committee of Basel 295/07, registration No. ISRCTN99586989). Written informed consent was obtained from all patients.

PC Study

The PC study was a single-center randomized controlled trial. From November 2003 to March 2005, patients hospitalized for acute exacerbations of COPD were recruited. Patients were required to (1) be at an age older than 40 years, (2) meet spirometric COPD criteria and (3) meet the severe AECOPD definition at study inclusion. Patients with immunosuppression, asthma, cystic fibrosis or radiographic infiltrates were excluded. A clinical assessment, lung function and venous puncture were performed at admission, after 14–21 days and after 6 months. Outcome parameters were assessed until 2 years after study inclusion. The primary study objective was to improve antibiotic prescription based on procalcitonin guidance as reported previously [11]. Patients were randomized at the index exacerbation to receive procalcitonin-guided or standard antibiotic therapy. Whereas procalcitonin guidance reduced antibiotic exposure, outcome did not differ in both groups. The evaluation of prognostic markers was a predetermined secondary end point of the study. VEGF serum levels 14–21 days after AECOPD were considered 'stable COPD'. To assess the VEGF course in stable COPD, the difference of VEGF after 14–21 days and 6 months was used. For exacerbation, VEGF levels at admission were employed and compared to VEGF levels after 14–21 days.

PM Study

From November 2008 to October 2011, stable COPD patients were recruited in the Pulmonary Department of the University Hospital Basel. Patients were required to: (1) fulfill GOLD (Global Initiative for Obstructive Lung Disease) grade II–IV criteria [12]; (2) be clinically stable for more than 4 weeks (based on the patients' perception of cough, sputum and dyspnea); (3) be at least 40 years of age, and (4) have a smoking history of at least 10 pack-years. Patients were excluded if: (1) the main respiratory disorder was not COPD; (2) death was expected within 6 months; (3) the patient was immunosuppressed, or (4) a neuromuscular disorder prevented ambulation. The PM was a multicenter observational study performed in 11 European centers. To guarantee fast processing of all blood specimens, only patients from the main study center (University Hospital Basel) were evaluated. A clinical assessment, lung function and venous puncture were performed at study inclusion and every 6 months for 2 years. Outcome parameters were assessed until 5 years after study inclusion. The primary objective was to identify novel markers of outcome in COPD. Therefore, the analysis of VEGF in PM represents a predefined study goal. VEGF serum levels at study inclusion were considered 'stable COPD'. To assess the VEGF course in stable COPD, the difference of VEGF at study inclusion and 1-year follow-up was used. Additional assessments were performed at COPD exacerbation.

*Determination of Serum VEGF Concentration*

After venous puncture, blood samples were immediately centrifuged at 4°C, the serum was stored at –80°C until further processing. The samples were thawed on ice, and VEGF was instantly determined by an enzyme-linked immunosorbent assay (R&D Systems Inc., Minneapolis, Minn., USA). The used assay recognizes VEGF165, VEGF121 and VEGF165b with a detection limit of 31.2 pg/ml as described by the manufacturer. All measurements were carried out in duplicate.

*Statistical Analyses*

Discrete variables are expressed as counts (with percentages) and continuous variables as means ± standard deviation. Comparability between groups was analyzed by the $\chi^2$ test, Fisher's exact test, Student's t test, the Mann-Whitney U test or Kruskal-Wallis test as appropriate. Comparability within groups was analyzed by the paired t test or Wilcoxon's signed-rank test. Pearson's and Spearman's correlations were used to measure the relationship between two continuous variables. The Kolmogorov-Smirnov test was applied to test normal distribution. To assess whether a change in COPD severity, i.e. progressing respiratory disease, is related to a change in VEGF levels, ΔVEGF was associated with outcome parameters. Due to the nonnormal distribution of serum VEGF concentrations, the VEGF course was calculated using log-transformed parameters. ΔVEGF was defined as: logVEGF at baseline – logVEGF after 1 year (PM study) and logVEGF after 2–3 weeks – logVEGF after 6 months (PC study). All tests were 2-tailed, and a p value <0.05 was defined as significant. Data were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, Ill., USA; version 20 for Mac).

## Results

Baseline characteristics of both study cohorts are presented in table 1.

*Absolute VEGF Levels in Stable COPD*

Age and gender were not related to VEGF serum levels in any of the trials (PM: p = 0.8 and 0.08, respectively; PC: p = 0.7 and 0.4, respectively). In the PC cohort, VEGF levels were elevated in patients with cardiac disease (mean ± standard deviation; 490 ± 333 vs. 423 ± 228 pg/ml, p = 0.011) and diabetes (536 ± 288 vs. 439 ± 274 pg/ml, p = 0.033). Conversely, in the PM trial this difference was not evident (p = 0.9 and 0.9, respectively). There was no difference in VEGF levels regarding other comorbidities and medication (table 1). Measures of COPD severity, such as forced expiratory volume in 1 s ($FEV_1$) and GOLD grade, were not related to VEGF levels at stable COPD (PM: p = 0.12 and 0.3, respectively; PC: p = 0.14 and 0.7, respectively). Similarly, GOLD stage, Modified Medical Research Council dyspnea score, 6-min walking distance and BODE index were not associated with VEGF (PM: p = 0.3, 0.7, 0.4 and 0.16, respectively). VEGF levels at different study visits in PC and PM are presented in table 2.

Individual VEGF levels at stable COPD were not associated with the number of subsequent AECOPDs per year and mortality (PM: moderate and severe AECOPDs per year p = 0.7, 2-year mortality p = 0.8, 5-year mortality p = 0.3; PC: moderate and severe AECOPD number p = 0.7, 1-year mortality p = 0.6, 2-year mortality p = 0.6; table 3).

*ΔVEGF Level in Stable COPD*

Several associations between the VEGF course and outcome were identified in stable COPD. In the PM trial, the ΔVEGF was similar regarding the rate of AECOPDs (moderate and severe), severe AECOPDs, 2-year mortality and 5-year mortality (p = 0.6, 0.9, 0.3 and 0.9, respectively; fig. 1). In contrast, in the PC trial, the ΔVEGF was different with respect to exacerbation rate, 1- and 2-year hospitalization rate (p = 0.046, 0.009 and 0.006, respectively). Similarly, the 1- and 2-year survival was decreased in patients with a reduced ΔVEGF (PC: p = 0.009 and 0.041, respectively).

*VEGF at AECOPD*

VEGF serum levels at AECOPD (moderate and severe) were similar to stable COPD VEGF levels in the PM as well as in the PC trial (p = 0.08 and p = 0.6, respectively; fig. 2). There was a positive correlation of VEGF and

**Table 1.** Demographic characteristics of the two study cohorts

| | PM cohort (n = 117) | PC cohort (n = 191) |
|---|---|---|
| Age, years | 67±11 | 70±10 |
| Gender female | 34 (29) | 89 (47) |
| Height, cm | 169 ± 8 | |
| Weight, kg | 75±21 | |
| BMI | 26±7 | |
| Comorbidities | | |
| Cardiac disease | 34 (29) | 83 (44)* |
| Diabetes mellitus | 19 (16) | 21 (11)* |
| Renal insufficiency | 18 (15) | 15 (8) |
| Malignancy | 9 (8) | 25 (13) |
| Osteoporosis | 15 (13) | 23 (12) |
| Arterial hypertension | 67 (57) | 44 (23) |
| Liver disease | 12 (10) | |
| Pulmonary hypertension | 19 (16) | |
| Depression | 17 (15) | |
| Adjusted Charlson score | 4.7±2.4 | |
| COPD history | | |
| Current smoker | 40 (34) | 86 (45) |
| Pack-years smoked | 51±25 | 45±28 |
| COPD duration, years | 5.8±2.1 | 10±7 |
| Lung function parameters | | |
| $FEV_1$, % predicted | 50±18 | 45±20[a] |
| FVC, % predicted | 80±18* | 71±21[a] |
| $FEV_1$/FVC, % | 47±15 | 50±15[a] |
| COPD assessment | | |
| GOLD grade 1 | – | 15 (8) |
| grade 2 | 56 (48) | 44 (23) |
| grade 3 | 42 (36) | 66 (35) |
| grade 4 | 18 (15) | 52 (27) |
| GOLD stage A | 13 (11) | |
| stage B | 35 (30) | |
| stage C | 3 (3) | |
| stage D | 63 (54) | |
| MMRC dyspnea score | 1.7±1.1 | |
| 6-min walking distance, m | 372±109 | |
| BODE index | 2.8±2.2 | |
| Bronchitic phenotype | 67 (57) | |
| COPD therapy at study inclusion | | |
| Inhaled anticholinergics | 81 (69) | 97 (51) |
| Inhaled $\beta_2$-agonists | 94 (80) | 163 (85) |
| Inhaled steroids | 100 (85) | 140 (73) |
| Systemic steroids | 11 (9) | 59 (31) |
| Theophylline | 7 (6) | 21 (11) |
| Long-term oxygen therapy | 19 (16) | 27 (14) |

Discrete variables are expressed as counts with percentages in parentheses and continuous variables as means ± standard deviation. All parameters were associated with VEGF serum levels using Pearson's correlation, Spearman's correlation, Student's t test, the Mann-Whitney U test and Kruskal-Wallis test as appropriate. * $p < 0.05$: significant association with VEGF at stable state. BMI = Body mass index; $FEV_1$ = forced expiratory volume in 1 s; FVC = forced vital capacity; MMRC = Modified Medical Research Council.

[a] Presented lung function parameters of the PC study were obtained 14–21 days after study inclusion.

**Table 2.** VEGF levels at stable and exacerbated COPD

| PM cohort | | | PC cohort | | |
|---|---|---|---|---|---|
| baseline (stable) | follow-up | AECOPD | baseline (AECOPD) | follow-up 14–21 days | follow-up 6 months |
| n = 117<br>322±341 | n = 89<br>183±267 | n = 30<br>1,019±1,550 | n = 173<br>438±274 | n = 161<br>437±277 | n = 148<br>436±259 |

**Table 3.** Outcome measures in the two study cohorts

| Outcome | PM cohort (n = 117) | | PC cohort (n = 191) | |
|---|---|---|---|---|
| | measure | p | measure | p |
| Hospitalization of any cause within 1 year | | | 66 (35) | 0.9 |
| Hospitalization of any cause within 2 years | | | 92 (48) | 0.3 |
| AECOPDs (moderate and severe) per year (within 2 years) | 0.9±1.3 | 0.7 | 2.1±1.9 | 0.7 |
| Severe AECOPDs per year (within 2 years) | 0.2±0.4 | 0.7 | | |
| Frequent exacerbator (≥2 AECOPDs/year) | 20 (17) | 0.6 | 97 (51) | 0.6 |
| 1-year all-cause mortality | | | 12 (6) | 0.6 |
| 2-year all-cause mortality | 13 (11) | 0.8 | 37 (19) | 0.6 |
| 5-year all-cause mortality | 38 (33) | 0.3 | | |

Discrete variables are expressed as counts with percentages in parentheses and continuous variables as means ± standard deviation. All parameters were associated with VEGF serum levels using Pearson's correlation, Spearman's correlation, Student's t test, the Mann-Whitney U test and Kruskal-Wallis test as appropriate. p values as assessed by the association with VEGF.

COPD duration as well as VEGF and leukocyte count (PC: p = 0.046 and 0.005, respectively) at AECOPD. Furthermore, patients who presented with an increased amount of sputum had elevated VEGF levels (PC: p = 0.045). Neither VEGF levels at AECOPD nor the VEGF course after AECOPD were related to measures of COPD severity or outcome. VEGF levels at AECOPD did not differ regarding exacerbation frequency, hospitalization and survival.

## Discussion

Herein we report that single VEGF serum levels in stable and exacerbated COPD are not associated with markers of disease severity or outcome. However, increasing VEGF levels in patients with stable COPD might be related to poor prognosis.

An association between VEGF and airway obstruction was reported previously. Serum VEGF and $FEV_1$ correlated positively in 30 patients with stable COPD [13]. However, there was no difference in VEGF between stable COPD patients and healthy subjects [13, 14]. Elevated VEGF serum levels in COPD patients were reported in 2 other trials [15, 16]. Another study demonstrated elevated VEGF serum levels in patients with mild COPD only, though negatively correlating with $FEV_1$ [17]. We did not observe any association between single VEGF levels and established markers of COPD severity. In both trials VEGF levels did not correlate with $FEV_1$. Furthermore, there was no association between VEGF and diffusion capacity, walking distance, GOLD grade (1, 2, 3, 4), GOLD stage (A, B, C, D), BODE index and symptom score. Data of VEGF levels at COPD exacerbations are inconsistent. Whereas elevated serum VEGF levels were reported [13, 18], other studies did not observe this difference [19]. We compared

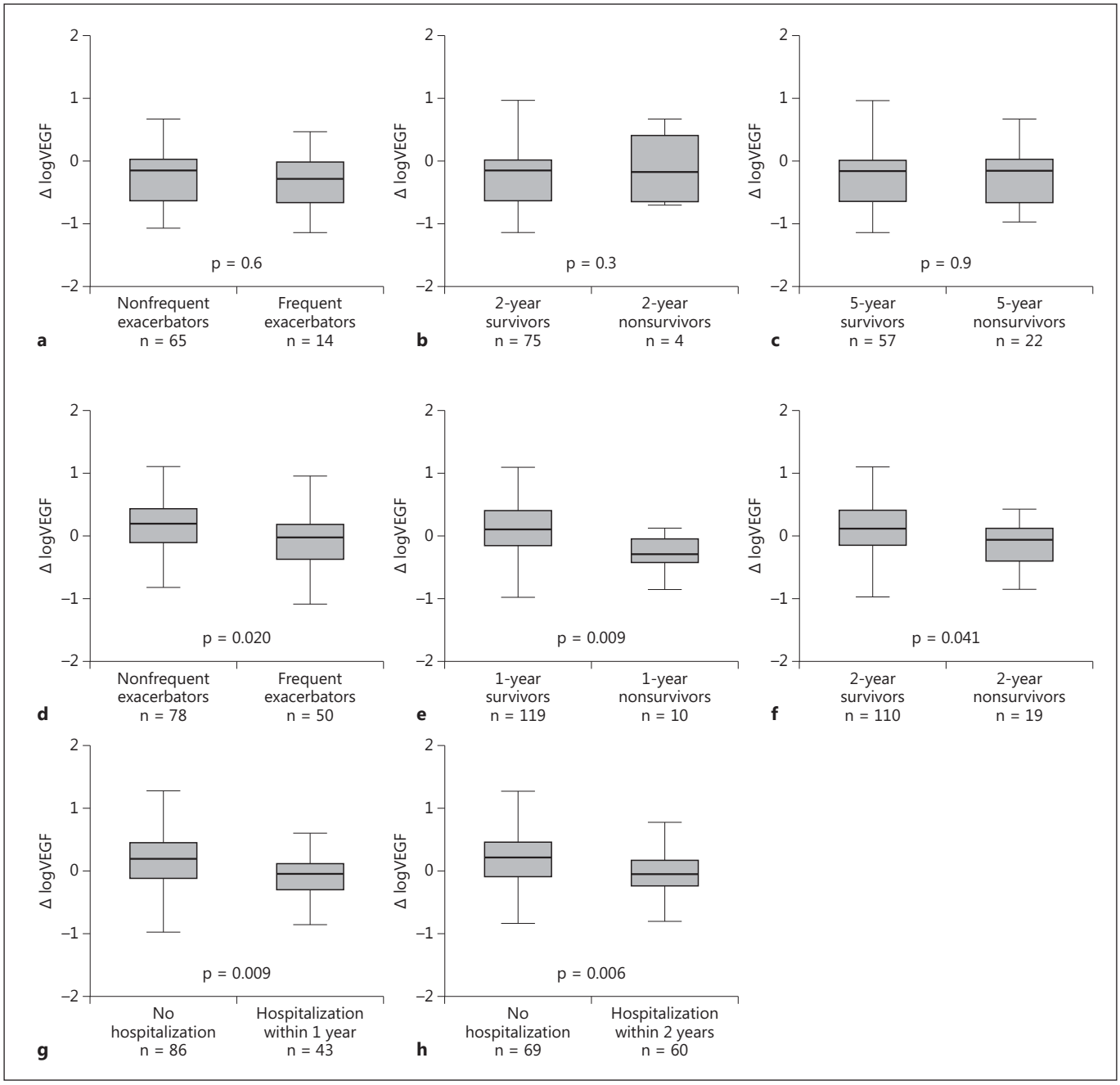


**Fig. 1.** Course of VEGF serum levels during stable COPD in association with outcome. ΔVEGF levels of the PM (**a–c**) and PC trials (**d–h**) are presented with regard to important COPD outcome measures. Comparability between groups was analyzed by Student's t test or the Mann-Whitney U test.

VEGF serum levels in stable and exacerbated COPD within patients. However, in both trials VEGF levels at COPD exacerbation did not differ from stable COPD.

Similarly, VEGF levels in respiratory secretions are conflicting. Most studies indicate that VEGF in sputum or epithelial lining fluid of central and peripheral airways is lower in COPD and decreases with increasing severity [14, 20, 21]. Controversially, elevated sputum VEGF levels were observed in COPD patients, negatively correlating with $FEV_1$ [19]. Kanazawa et al. [22] reported elevat-

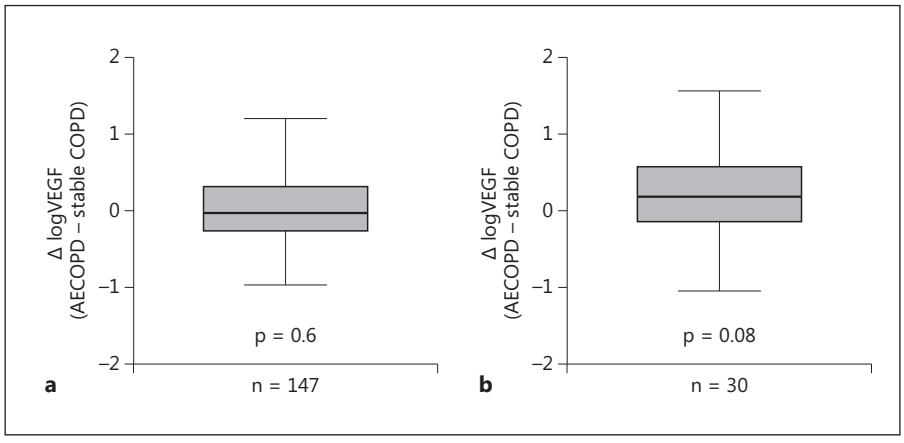


**Fig. 2.** Difference of VEGF serum levels between stable and exacerbated COPD. Comparability within groups was analyzed by the paired t test or Wilcoxon's signed-rank test. **a** PC. **b** PM.

ed VEGF sputum levels in chronic bronchitis compared to normal controls, negatively correlating with $FEV_1$, whereas VEGF sputum levels were reduced in emphysema positively correlating with $FEV_1$. Herein, we did not identify a difference in VEGF levels regarding chronic bronchitis or diffusion capacity, a marker of emphysema. This is in line with results of a previous trial observing similar VEGF levels in patients with and without emphysema, as defined by high-resolution computed tomography [23].

In contrast to the cross-sectional design of previous studies, we performed a longitudinal study, allowing the assessment of short- and long-term outcome as well as the change of serum levels over time. VEGF serum levels at stable or exacerbated COPD were not associated with the COPD exacerbation frequency and mortality. However, in one study the VEGF course during stable COPD was associated with clinically relevant outcomes in COPD. Interestingly, an increase in VEGF levels was related to poor outcome. In line with these results we identified elevated VEGF levels with increasing COPD duration. Three arguments may explain this finding. As mentioned above, increased VEGF levels may reflect the bronchitic phenotype of COPD, associated with more and severer exacerbations [24]. The observation that increased sputum production at AECOPD is associated with elevated VEGF levels supports this notion. Second, serum VEGF is not necessarily related to VEGF levels of sputum. Other compartments of the COPD lung, such as alveoli and pulmonary vasculature may contribute to elevated VEGF serum levels, but not to VEGF in airway secretions. Third, although most studies revealed decreasing VEGF levels in sputum and airways with increasing disease severity, contrary reports challenge these findings [19]. Several trials reported elevated serum VEGF in COPD, some of them negatively correlating with $FEV_1$, i.e. positively correlating with COPD severity [13, 15, 16]. Moreover, since the main stimulant of VEGF is hypoxia, via hypoxia-inducible factor [3], an increase in VEGF serum levels in severer disease seems plausible. This may also explain the worse outcome in patients with increasing VEGF levels.

To our knowledge this is the largest evaluation of VEGF in COPD so far. Nevertheless, several limitations merit consideration. First, VEGF has a very short half-life prone to measurement errors. To overcome this bias, blood samples were centrifuged cool and frozen immediately after venous puncture. To ensure optimal quality only blood samples of a single center were used. Second, we do not know whether VEGF serum levels adequately reflect VEGF levels in alveoli and airways. In any case, the determination of VEGF in bronchoalveolar lavage fluid or sputum tends to be more tedious and would hardly be useful in the clinical routine. There exist several differences regarding the two study cohorts. Whereas the PC study included only patients with exacerbated COPD, in the PM trial patients were included exclusively at stable COPD. Thus, PC patients may have been more prone to recurring exacerbations and inflammation [25, 26]. Moreover, in the PC we considered stable patients 14–21 days after exacerbation; however, a proportion may not have reached clinical stability, possibly affecting the results. It is noteworthy that VEGF levels in the PC were very similar in stable and exacerbated COPD, whereas in the PM there was a trend to higher VEGF levels during exacerbation. Importantly, in the PC an increase in VEGF levels was associated with poor outcome. Finally, we cannot rule out that the VEGF course, and the associated clinical outcome, does not directly reflect COPD activity

but rather the severity of associated comorbidities. In any case, VEGF change over time was significantly associated with all-cause mortality. Further studies would have to assess the influence of specific comorbidities such as cardiovascular disease and malignancy in the levels of this biomarker. Strategies combining several disease markers will be necessary to address the complexity of COPD [27, 28]. Novel tools such as the omics approaches are promising for stratifying clinically relevant COPD phenotypes [29].

We conclude that a single VEGF serum level does not reflect COPD severity and outcome, neither at the stable state nor at exacerbation. Thus, single VEGF serum levels are unlikely to be a clinically relevant marker in COPD. However, increasing VEGF levels might identify patients with a poorer prognosis. This finding warrants future investigations.


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## Financial Disclosure and Conflicts of Interest

The authors have no financial or nonfinancial conflicts of interest related to this paper. The sponsors of this investigator-initiated project were not involved in study design, conduction, statistical analysis and approval of the manuscript.

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