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Original Article

Could detection of VEGF in exhaled breath condensate be more helpful for non-small cell lung cancer?

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Abstract: Background: Given that the morbidity and mortality of lung cancer are the highest among malignant tumours, investigation of tumour markers associated with the development and progression of lung cancer is crucial. This study aims to investigate the clinical significance of vascular endothelial growth factor (VEGF) in exhaled breath condensate (EBC) of patients with non-small cell lung cancer (NSCLC). Methods: We collected EBC samples by using an EBC collector from 132 patients with NSCLC, 62 patients with pulmonary benign lesions and 97 healthy individuals. Enzyme-linked immunosorbent assay was used for detection of VEGF in EBC and serum. Results: The VEGF levels of serum and EBC in the NSCLC group were higher than that in the benign lesions and healthy groups. The VEGF levels in serum and EBC in stages III and IV of the NSCLC group were obviously higher than that in stages I and II. The VEGF levels in serum and EBC of the mortality group were higher than that in the survival group ($P<0.01$). The VEGF level in EBC was positively correlated with that in serum, the correlation coefficient was 0.613 ($P<0.01$). Receiver operating characteristic curve analysis showed that the area under the curve for EBC (0.922) was larger than that for serum (0.802). The diagnostic sensitivity of VEGF level in EBC (87.9%) for lung cancer was higher than that in serum (65.9%). Conclusion: Detection of VEGF in EBC could be more helpful than in serum for the diagnosis, progression monitoring and prognosis of NSCLC.

Keywords: Non-small cell lung cancer, exhaled breath condensate, vascular endothelial growth factor

Introduction

The incidence of lung cancer has significantly increased in recent years with the aging population. The morbidity and mortality of lung cancer have become the highest among malignant tumours [1]. Non-small cell lung cancer (NSCLC) accounts for 80% to 85% of all lung cancer cases. Thus, investigation of tumour markers associated with the development and progression of lung cancer is very important [2]. Exhaled breath condensate (EBC) contains liquid secreted by mucous membranes of the respiratory tract and mixtures of volatile molecules [3], which may provide information on cancers in the airways. Detection of tumour markers in EBC may be a new approach to early diagnosis of lung cancer [4, 5]. Currently, tumour markers in EBC of patients with lung cancer have attracted considerable research attention [6-9].

Vascular endothelial growth factor (VEGF) is related to the angiogenesis, growth and metastasis of many tumours [10]. Given that only a few studies have been conducted on detection of VEGF in EBC [11-14], we investigated the clinical significance of VEGF in EBC of patients with NSCLC by measuring VEGF levels in EBC and serum.

Objects and methods

Research object

We selected 132 patients with NSCLC from the Second Affiliated Hospital of Nantong University from June 2011 to August 2014, which were all pathologically diagnosed as squamous cell carcinoma (59 cases) or adenocarcinoma (73 cases). Staging was based on the TNM classification system of the Union for International Cancer Control 2009 [15]: 14 stage I cases, 32 stage II cases, 45 stage III cases and 41 stage

Table 1. Comparison of the general characteristics in three groups

	NSCLC	Benign lesions	Healthy	*P value*
N	132	62	97	
Age (years)	64.92±8.30	62.74±7.12	63.11±7.91	>0.05
Male/female	87/45	42/20	65/32	>0.05
Smoking (yes/no)	96/36	37/25	46/51	<0.01

Table 2. Comparison of the VEGF levels of serum and EBC in three groups ($\bar{x}$±s, pg/ml)

	N	EBC	Serum
NSCLC	132	45.49±18.52	255.59±115.13
Benign lesions	62	18.01±10.43▲	127.58±62.47▲
Healthy	97	15.48±7.01★,•	140.75±69.94★,•

Note: EBC and serum VEGF levels in NSCLC group as compared with that of the benign lesions groups, ▲*P*<0.01. EBC and serum VEGF levels in the NSCLC group compared with that of the healthy group, ★*P*<0.01. EBC and serum VEGF levels in the benign lesions group compared with that of the healthy group, •*P*>0.05.

IV cases. All patients were followed up; 27 patients died (mortality group) within six months since the collection of specimens, and 105 patients survived (survival group). The exclusion criteria are pulmonary infection, severe hepatic insufficiency, severe renal insufficiency, and inability to cooperate during the experiments. We also selected 62 patients with pulmonary benign lesions as the benign lesions group, which included 21 cases of chronic obstructive pulmonary disease, 29 cases of pneumonia, 6 cases of bronchiectasis, 3 cases of bronchial asthma and 3 cases of lung abscess. In addition, 97 healthy individual were included as the healthy group. No statistical differences in age and gender were found among the three groups. The general characteristics are summarized in **Table 1**. The study was approved by the Ethics Committee of the Second Affiliated Hospital of Nantong University, and all participants signed an informed consent form.

Specimen collection

We collected EBC of all of the participants by using an EBC collector (HAAK EK20 EcoScreen), which was produced by Germany Eric Jaeger Company. The collector was pre-cooled for 20 min, and the participants were required to clean their mouth before the test, wear nose clip and breathe quietly by biting mouthparts. After 20 min of quiet breath, the expiratory air turned into snow-like substance by condensation. The collection tubes were then gathered, which contained 1-3 ml of EBC after the melting of specimen, and then transferred to centrifuge tubes and stored in a refrigerator at -70°C. At the same time, fasting venous blood samples from all of the participants were collected and centrifuged for serum extraction. The samples were then stored in a refrigerator at -20°C. All samples were analyzed within a week.

Detection method

VEGF levels in serum and EBC were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits, in accordance with the manufacturer's protocols. The ELISA kits were purchased from Bender Med Systems GmbH (Austria).

Statistical analysis

SPSS13.0 statistical software was used for statistical analysis. All data were presented for the normal distribution test and expressed as mean ± standard deviation ($\bar{x}$±s). Moreover, t test was used to compare two samples and chi-squared test was used to compare count data. Correlation analysis was employed to describe the relevance of the VEGF in EBC and in serum. Statistical significance was defined as P<0.05. Specificity and sensitivity for diagnosis of lung cancer was analyzed using receiver operating characteristic (ROC) curves. The upper left point where the area under the curve (AUC) was at maximum was selected for the best critical value of diagnosis. The corresponding density values for the cut-off value were considered the reference critical values of VEGF for diagnosis of lung cancer.

Results

Comparison of EBC and serum VEGF levels among the NSCLC, benign lesions and healthy groups

The VEGF levels of serum and EBC in the NSCLC group (255.59±115.13 pg/ml, 45.49±18.52 pg/ml) were higher than that in the benign

Table 3. Relationship between EBC level of VEGF and patient clinical characteristics

Characteristics		N	EBC (pg/ml)	*p* value
Pathologic type	Adenocarcinoma	73	42.85±17.18	>0.05
	Squamous carcinoma	59	48.75±19.71	
Pathologic stage	I+II	46	30.54±13.36	<0.01
	III+IV	86	53.48±15.77	
Death or survival	Survival group	105	40.17±15.86	<0.01
	Death group	27	66.16±12.79	

Table 4. Relationship between serum level of VEGF and patient clinical characteristics

Characteristics		N	Serum (pg/ml)	*p* value
Pathologic type	Adenocarcinoma	73	250.51±108.15	>0.05
	Squamous carcinoma	59	261.87±123.88	
Pathologic stage	I+II	46	169.02±87.73	<0.01
	III+IV	86	301.89±100.66	
Death or survival	Survival group	105	229.26±109.41	<0.01
	Death group	27	357.95±72.25	

lesions (127.58±62.47 pg/ml, 18.01±10.43 pg/ml),all *P*<0.01. The VEGF levels of serum and EBC in the NSCLC group (255.59±115.13 pg/ml, 45.49±18.52 pg/ml) were higher than that in the healthy group (140.75±69.94 pg/ml, 15.48±7.01 pg/ml), all *P*<0.01. EBC and serum VEGF levels in the benign lesions group (127.58±62.47 pg/ml, 18.01±10.43 pg/ml) compared with that of the healthy group (140.75±69.94 pg/ml, 15.48±7.01 pg/ml), all *P*>0.05 (**Table 2**).

EBC and serum VEGF levels in NSCLC patients

The EBC VEGF level in adenocarcinoma of the NSCLC group (42.85±17.18 pg/ml) compared with Squamous carcinoma (48.75±19.71 pg/ml), *P*>0.05. The EBC VEGF level in stages III and IV of the NSCLC group (53.48±15.77 pg/ml) was obviously higher than that in stages I and II (30.54±13.36 pg/ml), *P*<0.01. The VEGF level in EBC of the mortality group (66.16±12.79 pg/ml) were higher than that in the survival group (40.17±15.86 pg/ml), *P*<0.01 (**Table 3**).

The serum VEGF level in adenocarcinoma of the NSCLC group (250.51±108.15 pg/ml) compared with Squamous carcinoma (261.87 ±123.88 pg/ml), *P*>0.05. The serum VEGF level in stages III and IV of the NSCLC group (301.89±100.66 pg/ml) was obviously higher than that in stages I and II (169.02±87.73 pg/ml), *P*<0.01. The VEGF level in serum of the mortality group (357.95±72.25 pg/ml) were higher than that in the survival group (229.26 ±109.41 pg/ml), *P*<0.01 (**Table 4**).

Correlation between EBC and serum VEGF levels

The VEGF level in EBC was positively correlated with that in serum, the correlation coefficient was 0.613 (*P*<0.01) (**Figure 1**).

Figure 1 shows that the VEGF levels in serum and EBC are linearly and positively correlated; the correlation coefficient was 0.613 (*P*<0.01).

Analysis of the sensitivity and specificity of VEGF level for diagnosis of lung cancer

Receiver operating characteristic curve analysis showed that the area under the curve for EBC (0.922) was larger than that for serum (0.802) (**Figure 2**). The diagnostic sensitivity of VEGF level in EBC (87.9%) for lung cancer was higher than that in serum (65.9%) (**Table 5**).

Discussion

VEGF is produced mainly by the pulmonary vascular endothelial cells, bronchial epithelial cells, alveolar epithelial cells and lung inflammatory cells. VEGF is a kind of growth factor that has specific effects on vascular endothelial cells. VEGF can regulate the formation of blood vessels and lymphatic vessels, as well as increase the permeability, promote growth and metastasis of tumours, by combining with receptors located in the endothelial cell surface of vascular and lymphatic cells. VEGF also promotes tumour growth directly by combining with receptors on tumour cell surface in an autocrine way. Studies showed high VEGF expression in cancerous tissues and serum of lung cancer patients; increased VEGF expression in lung cancer is related to increased degree of malignancy of lung cancer, high risk of lymph node metastasis and poor prognosis [16].

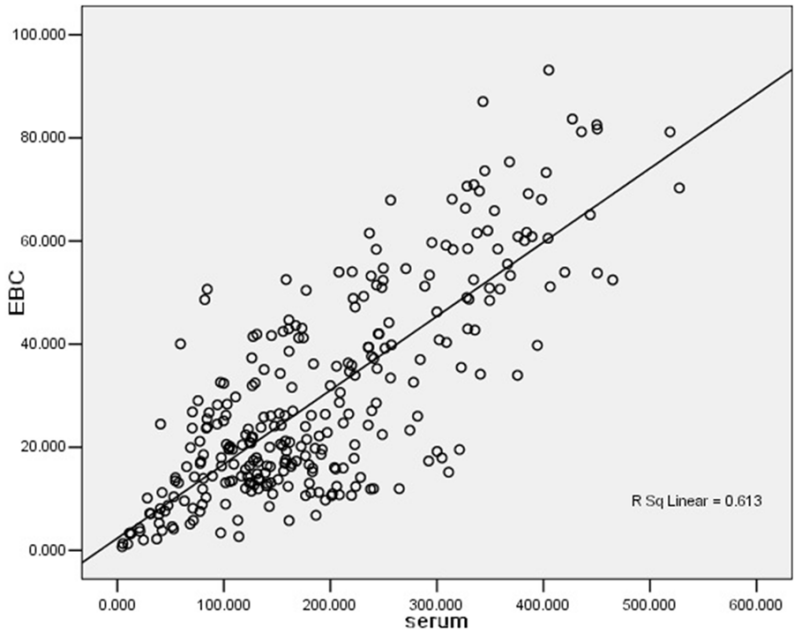

Figure 1. Correlation scatter diagram of EBC and serum level of VEGF (pg/ml).

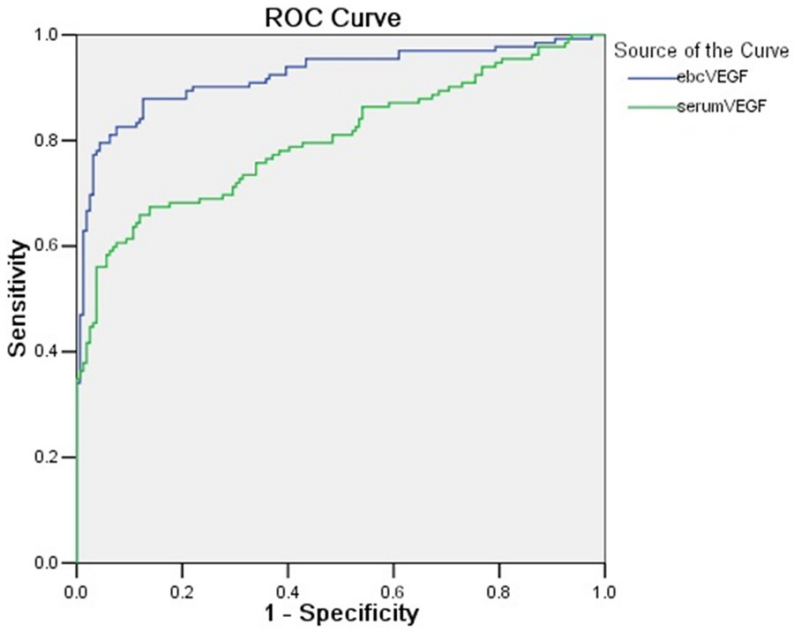

Figure 2. The ROC curves of VEGF in the diagnosis of lung cancer.

EBC analysis is a new technique for detection of respiratory biochemical components without interference from the physiological or pathological processes of the respiratory tract [17]. The process of collecting EBC is completely non-invasive [18] and does not damage the bronchial mucosa. Moreover, EBC detection results are reliable and can be repeated because EBC can be collected directly from the airways [19]. Results of the current study showed a good correlation between EBC and serum VEGF levels, with a correlation coefficient of 0.613. Thus, VEGF levels can be detected in EBC for diagnosis of NSCLC.

In this study, ELISA was used for detection of VEGF. Results showed that the VEGF levels of serum and EBC in the NSCLC group were higher than that in the healthy group. This result is consistent with that in studies by Gessner and Urbaniak [12, 13]. Thus, VEGF in EBC can be used as a tumour marker in diagnosis of lung cancer. We also found that the serum and EBC VEGF levels in the NSCLC group were higher than that in the benign lesions group. By comparison, no significant difference in serum and EBC VEGF levels between the benign lesions group and the healthy group. These results indicated that VEGF detection in EBC could be employed in the differential diagnosis of benign and malignant lung diseases.

The EBC VEGF levels in stage III and stage IV of the NSCLC group were considerably higher than that in stage I and stage II. This finding indicated that the progress of the disease could be evaluated according to the VEGF level in EBC of NSCLC patients. We believe that high VEGF level in EBC of NSCLC patients indicates later stage of the tumour, wide range of infiltration and distant metastasis [14].

The patients with NSCLC were divided into survival group and mortality group according to the

Table 5. The sensitivity and specificity of the VEGF levels in serum and EBC for the diagnosis of lung cancer

	Area under ROC curve	Critical value	Sensitivity	Specificity
EBC-VEGF	0.922	24.60 pg/ml	87.9%	87.4%
Serum-VEGF	0.802	212.07 pg/ml	65.9%	88.1%

prognosis. Results showed that the VEGF levels in serum and EBC of the mortality group were higher than that in the survival group. Thus, VEGF level in EBC can be used to evaluate the prognosis of patients with NSCLC. Meanwhile, we found no significant difference in EBC VEGF level between the adenocarcinoma and squamous carcinoma groups. This finding suggests that VEGF level in EBC is not closely related to the pathological type of lung cancer.

Moreover, ROC curve analysis shows that the AUC for EBC (0.922) was larger than the AUC for serum (0.802). The diagnostic sensitivity of EBC VEGF level in lung cancer (87.9%) was higher than that of serum (65.9%). This result shows that the detection of VEGF levels in EBC may be more significant than in serum for diagnosis of NSCLC. This superiority may be related to high VEGF expression in the lung because alveolar type II epithelial cells are the main source of VEGF. Normally, the level of VEGF in lung tissue epithelial inner liquid, which is the physiological "savings pool" of VEGF, is 500 times that in plasma [20]. Under normal conditions, lung epithelial inner fluid with high concentration of VEGF cannot penetrate into blood to protect alveolar epithelial cells. VEGF is a metabolite of cell secretion, a kind of typical exocrine protein, which may be transported directly to EBC from the surface of tumour cells.

In conclusion, we confirmed that VEGF detection in EBC of patients with NSCLC is feasible. VEGF level in EBC, which was positively correlated with VEGF level in serum, showed an important value in the diagnosis, progression monitoring and prognosis of NSCLC. Given that elevated levels of VEGF can be detected in various tumours, detection of VEGF level in serum should be confirmed by other examinations. By contrast, VEGF level in EBC has specificity for diagnosis of lung cancer because of it mainly comes from the lungs. VEGF detection in EBC offers unique advantages in auxiliary diagnosis of lung cancer. Further investigation on the application of the current approach is recommended.

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Disclosure of conflict of interest

None.

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