

## Original Article

# Clinical significance of TGF- $\beta$ 1 in exhaled breath condensate and serum from patients with non-small cell lung cancer

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**Abstract:** Objective: To investigate the clinical significance of the detection of transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) in exhaled breath condensate (EBC) and serum from patients with non-small cell lung cancer (NSCLC). Methods: EBC samples from 152 NSCLC patients and 117 healthy volunteers (normal control group) were collected with a breath condenser. Blood samples of the two groups were also collected. Each sample was analysed by enzyme-linked immunosorbent assay (ELISA) method. Data were analysed by statistical methods. Results: The TGF- $\beta$ 1 levels in EBC and in serum from NSCLC group ( $20.77 \pm 7.62$  ng/ml,  $50.70 \pm 23.96$  ng/ml) were higher than from the healthy group ( $9.65 \pm 3.38$  ng/ml,  $20.90 \pm 6.47$  ng/ml),  $P < 0.05$ . The TGF- $\beta$ 1 levels in EBC and in serum in phase III and IV stages of NSCLC group were higher than those in phase I and II stages. The TGF- $\beta$ 1 levels in EBC and in the serum of the death group were higher than those of the survival group. The EBC TGF- $\beta$ 1 levels were positively correlated with the serum TGF- $\beta$ 1 levels with a correlation coefficient of 0.571 ( $P < 0.05$ ). The sensitivity and specificity of EBC-TGF- $\beta$ 1 test were 80.3% and 94%, respectively. The sensitivity and specificity of serum-TGF- $\beta$ 1 test were 79.6% and 95.7%, respectively. The sensitivity of the combined two-way detection was 82.2%, and its specificity was 99.1%. Conclusion: Monitoring changes in TGF- $\beta$ 1 levels in EBC or in serum is helpful in the early diagnosis, evaluation, determination of treatment course and prognosis of NSCLC. The combined detection of two pathways could compensate for each other's deficiencies and improve sensitivity.

**Keywords:** Exhaled breath condensate, non-small cell lung cancer, transforming growth factor- $\beta$ 1, combined detection

## Introduction

Lung cancer is one of the most common malignant tumours in humans. The curative effect and prognosis of lung cancer depends on its early diagnosis [1]. Non-small cell lung cancer (NSCLC) accounts for 80% to 85% of lung cancer cases [2]. If NSCLC is diagnosed in I A phase, the 5-year survival rate increases to 80% instead of the current 15% [3-5]. Therefore, early warning markers of lung cancer with strong specificity and high sensitivity should be identified for use in early diagnosis. In recent years, the detection of tumour markers in exhaled breath condensate (EBC) has become an important topic in lung cancer research [6-9].

As a member of the cytokine super family, transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) plays an important role in the occurrence, development and metastasis of tumours [10, 11]. Thrombospondin-1 (TSP-1) is a multifunctional matrix protein that promotes tumour growth by regulating angiogenesis. Studies show that TGF- $\beta$ 1 and EGF promote the secretion of TSP-1. The combined effects of TGF- $\beta$ 1 and EGF promote tumour angiogenesis and stromal hyperplasia [12].

In our study, we detected TGF- $\beta$ 1 levels in EBC and serum by enzyme-linked immunosorbent assay (ELISA) to investigate the clinical significance of EBC- and serum-TGF- $\beta$ 1 levels in the early diagnosis, monitoring and prognosis of

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**Table 1.** General characteristics of the two groups

	NSCLC group	Healthy group	P
N	152	117	>0.05
Male/female	98/54	74/43	>0.05
Age (years)	60.7±11.8	59.1±9.6	>0.05
Smoking (yes/no)	106/46	82/35	>0.05

**Table 2.** Comparison of TGF-β1 levels in EBC and in serum between the two groups ( $\bar{x} \pm s$ )

	N	EBC (ng/ml)	Serum (ng/ml)
NSCLC group	152	20.77±7.62	50.70±23.96
Healthy group	117	9.65±3.38	20.90±6.47
T		14.710	13.086
P		<0.05	<0.05

lung cancer and lung cancer screening in high-risk populations.

### Subjects and methods

#### Study subjects

We designated 152 patients as the NSCLC group. The patients had been diagnosed with squamous cell carcinoma or adenocarcinoma and had undergone lung biopsy, bronchoscopy, or open-chest surgery at Nantong First People's Hospital from October 2011 to June 2016. We excluded patients with severe heart, lung, liver and kidney dysfunction; digestive system diseases; and other chronic diseases. Basing on these criteria, we choosed 95 cases of adenocarcinoma and 57 cases of squamous cell carcinoma. We further categorised NSCLC patients according to the seventh version of the Lung Cancer Tumour-Node-Metastasis (TNM) staging system provided by the Union for International Cancer Control (UICC) in 2009 [13]. We categorised 22 patients as in stage I, 38 patients as in stage II, 62 patients as in stage III and 30 patients as in stage IV. After following up on all patients, we classified the 29 patients who had died within 6 months of specimen collection as the death group. We classified the surviving 123 patients as the survival group. We designated a group of 117 healthy individuals as the healthy group. No significant differences in sex, age and smoking status between the healthy and NSCLC groups (**Table 1**).

#### Sample collection

We collected EBC with an EcoScreen condenser (German Jaeger Corporation). Subjects cleaned their mouths, wore a nose clip and then maintained eupnoea for 20 min by biting mouthparts. We collected 1-3 ml of EBC per subject. We stored the collected EBC in a -70°C refrigerator.

#### Detection method

We determined TGF-β1 levels by a sandwich enzyme-linked immunosorbent assay (ELISA) method. We followed the assay protocol included with the kit. Kits (product number: BMH029, BMH044) were purchased from Shanghai Boatman Biotech Co. Ltd.

#### Statistical analysis

We conducted statistical analysis with SPSS-13.0 statistical software. After subjecting our data to a normal distribution test, we represented normally distributed data as mean ± standard deviation ( $\bar{x} \pm s$ ). Means of two samples were compared by t-test. Measurement data were subjected to  $\chi^2$  test. We determined the relationship between TGF-β1 in EBC and in blood by correlation analysis. The specificity and sensitivity of lung cancer diagnoses were analysed by receiver operating characteristic (ROC) curves. *P* values <0.05 were considered statistically significant.

### Results

*TGF-β1 levels in EBC and in serum were compared between the NSCLC group and the healthy group (Table 2)*

The TGF-β1 levels in EBC and in serum from NSCLC group (20.77±7.62 ng/ml, 50.70±23.96 ng/ml) were higher than from the healthy group (9.65±3.38 ng/ml, 20.90±6.47 ng/ml), *P*<0.05.

*TGF-β1 levels in EBC and serum samples were compared between the different clinical characteristics (Tables 3, 4)*

The TGF-β1 levels in EBC and in serum in phase III and IV stages of NSCLC group were higher than those in phase I and II stages. The TGF-β1 levels in EBC and in the serum of the death

**Table 3.** Relationship between TGF-β1 levels in EBC and patients' clinical characteristics ( $\bar{x} \pm s$ )

Characteristics		N	EBC (ng/ml)	P
Pathologic Type	Adenocarcinoma	95	21.65±7.43	>0.05
	Squamous carcinoma	57	19.80±7.99	
Pathologic Stage	I+ii	60	14.80±6.31	<0.05
	iii+iv	92	24.66±5.63	
Death or Survival	Survival group	123	19.72±7.59	<0.05
	Death group	29	25.20±6.08	

**Table 4.** Relationship between TGF-β1 levels in serum and patients' clinical characteristics

Characteristics		N	Serum (ng/ml)	P value
Pathologic Type	Adenocarcinoma	95	52.03±40.52	>0.05
	Squamous Carcinoma	57	48.49±21.43	
Pathologic Stage	I+II	60	41.11±23.35	<0.05
	III+IV	92	56.96±22.33	
Death or Survival	Survival Group	123	50.08±24.54	>0.05
	Death Group	29	53.34±21.49	

group were higher than those of the survival group. We found no significant difference between TGF-β1 levels of adenocarcinoma and squamous carcinoma groups.

*Correlation between EBC TGF-β1 levels and serum TGF-β1 levels (Figure 1)*

**Figure 1** shows a positive linear correlation between EBC TGF-β1 and serum TGF-β1. The correlation coefficient was 0.571 ( $P < 0.05$ ).

*Sensitivity and specificity of TGF-β1 in EBC and in serum in NSCLC diagnosis (Figure 2; Table 5)*

In this study, the sensitivity and specificity of TGF-β1 in EBC and in serum were analysed by ROC curve analysis with pathological results as the standard. The sensitivity and specificity of EBC-TGF-β1 test were 80.3% and 94%, respectively. The sensitivity and specificity of serum-TGF-β1 test were 79.6% and 95.7%, respectively. The sensitivity of the combined two-way detection was 82.2%, and its specificity was 99.1%.

**Discussion**

EBC is a new non-invasive method to collect fluids from the lower respiratory tract [14]. Compared with sputum cytology examination, EBC has better diagnostic sensitivity and speci-

ficity. Compared with the induced sputum technique, the EBC collection process does not produce adverse reactions, such as airway reactivity [15]. Current lung cancer diagnostic methods have achieved rapid development, such as PET-CT, bronchoscopy and thoracoscopy, significantly improving the diagnostic efficiency for lung cancer. However, certain restrictions limit their application. EBC, as a new non-invasive detection method, has the advantages of easy operation and good reproducibility. Its scope of application is extremely wide and is not restricted by age, sex and disease severity [16]. Thus, the detection of tumor markers in EBC is a promising method for lung cancer diagnosis [17-20].

Based on the results of this study, a positive linear correlation exists between EBC TGF-β1 levels and serum TGF-β1 levels. The correlation coefficient was 0.571 ( $P < 0.05$ ). This result indicated that the EBC collection method could effectively detect TGF-β1 levels in patients.

In this study, TGF-β1 levels in serum and in EBC were detected by ELISA. The results showed that TGF-β1 levels in EBC and in serum from NSCLC group were higher than those from the healthy control group ( $P < 0.05$ ). The EBC TGF-β1 levels in stages III and IV of NSCLC groups were higher than those of stages I and II NSCLC groups. TGF-β1 is a stable polypeptide growth factor with multiple functions and is an effective inhibitor of cell growth and tumorigenesis. Losing this negative regulation leads to the occurrence and development of tumors [21-23]. In normal cells, TGF-β1 inhibits tumor formation by inhibiting cell proliferation and inducing cell differentiation and apoptosis [24]. In malignant cells, tumor cells and stromal cells show the excessive secretion of TGF-β1 as it has lost its growth inhibitory effect on tumor tissue. As tumor cell growth can no longer be inhibited by TGF-β1, TGF-β1-induced angiogenesis, cell movement, immune suppression, tumor cell invasion and distant metastasis significantly increased, thereby promoting tumor growth [25-28].

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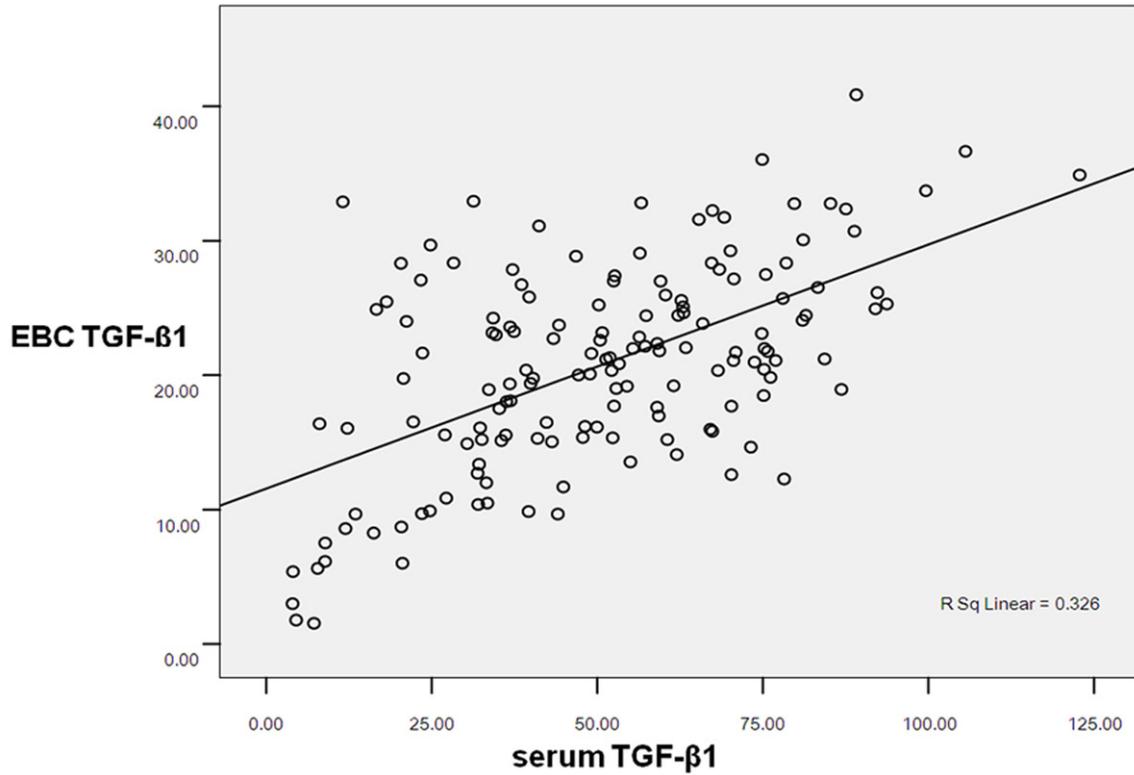


Figure 1. Correlation scatter diagram of TGF-β1 levels in EBC and in serum (ng/ml).

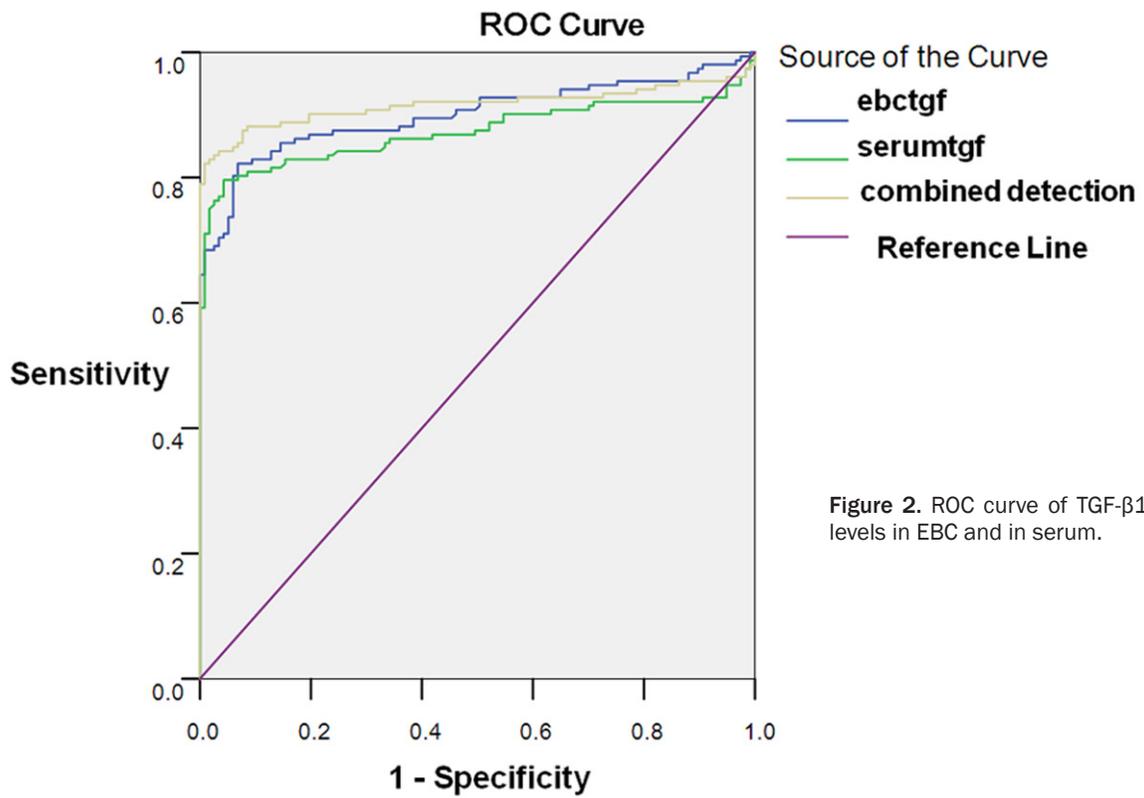


Figure 2. ROC curve of TGF-β1 levels in EBC and in serum.

Diagonal segments are produced by ties.

## TGF- $\beta$ 1 in exhaled breath condensate of NSCLC

**Table 5.** Specificity and sensitivity of TGF- $\beta$ 1 in EBC and in serum in NSCLC diagnosis

	Area under the ROC curve	Sensitivity	Specificity
EBC	0.900	80.3%	94.0%
Serum	0.873	79.6%	95.7%
Combined detection	0.916	82.2%	99.1%

We divided NSCLC patients into survival group or death group according to prognosis. The results showed that the EBC TGF- $\beta$ 1 levels of the death group were higher than those in the survival group, whereas no significant difference was found in the serum TGF- $\beta$ 1 levels. These results indicate that the EBC TGF- $\beta$ 1 levels could be used to validate the prognosis of NSCLC patients [29]. We found no significant difference between TGF- $\beta$ 1 levels of adenocarcinoma and squamous carcinoma groups. This result suggested that TGF- $\beta$ 1 levels are not closely linked with the pathological type of lung cancer [30].

In this study, the sensitivity and specificity of TGF- $\beta$ 1 in EBC and in serum were analyzed by ROC curve analysis with pathological results as the standard. The sensitivity and specificity of EBC-TGF- $\beta$ 1 test were 80.3% and 94%, respectively. The sensitivity and specificity of serum-TGF- $\beta$ 1 test were 79.6% and 95.7%, respectively. The sensitivity of the combined two-way detection was 82.2%, and its specificity was 99.1%. Thus, the sensitivity and specificity of the combined detection of the two pathways are higher. This finding showed that the combined detection of TGF- $\beta$ 1 in EBC and serum could improve the detection rate of NSCLC and gain valuable time for early diagnosis and early treatment for NSCLC. At the same time, the specificity of joint detection was 99.1%, which could reduce the possibility of misdiagnosis.

To summarize, we confirmed that TGF- $\beta$ 1 detection in EBC of NSCLC patients is feasible. TGF- $\beta$ 1 levels in EBC were positively correlated with TGF- $\beta$ 1 levels in serum. The detection of TGF- $\beta$ 1 levels in EBC has a valuable application in the diagnosis, monitoring and prognosis of NSCLC. The combined detection of EBC TGF- $\beta$ 1 and serum TGF- $\beta$ 1 is invaluable in the diagnosis of NSCLC and is worth adopting as a diagnostic method for NSCLC.

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### Disclosure of conflict of interest

None.

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