TGF-β1, IL-6, and TNF-α in Bronchoalveolar Lavage Fluid: Useful Markers for Lung Cancer?

Zhongbo Chen1*, Zhiwei Xu2*, Shifang Sun1, Yiming Yu1, Dan Lv1, Chao Cao1,3 & Zaichun Deng1

1Department of Respiratory Medicine, Affiliated Hospital of Ningbo University School of Medicine, Ningbo 315020, China, 2Department of Critical Care Medicine, Ningbo Medical Center, Lihuili Hospital, Ningbo University, Ningbo 315041, China, 3Department of Respiratory and Critical Care Medicine, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, China.

Changes of cytokines in bronchoalveolar lavage fluid (BALF) reflect immunologic reactions of the lung in pulmonary malignancies. Detection of biomarkers in BALF might serve as an important method for differential diagnosis of lung cancer. A total of 78 patients admitted into hospital with suspected lung cancer were included in our study. BALF samples were obtained from all patients, and were analyzed for TGF-β1, IL-6, and TNF-α using commercially available sandwich ELISA kits. The levels of TGF-β1 in BALF were significantly higher in patients with lung cancer compared with patients with benign diseases (P = 0.003). However, no significant difference of IL-6 (P = 0.61) or TNF-α (P = 0.72) in BALF was observed between malignant and nonmalignant groups. With a cut-off value of 10.85 pg/ml, TGF-β1 showed a sensitivity of 62.2%, and a specificity of 60.6%, in predicting the malignant nature of pulmonary disease. Our data suggest that TGF-β1 in BALF might be a valuable biomarker for lung cancer. However, measurement of IL-6 or TNF-α in BALF has poor diagnostic value in lung cancer.

A major problem in lung cancer is the lack of clinically useful tests for early diagnosis and screening patients with lung lump by noninvasive diagnostic procedures1. It is reported that approximately two-thirds of lung cancer patients is the presence of metastatic tumors at the time of diagnosis2. Lung cancer screening by chest X-ray and sputum cytology have proven ineffective in improving patient survival rate3,4, leading to the search for more sensitive and specific tests. One promising approach is the identification of lung cancer-specific biomarkers and detection of them at an early stage.

Considering that tumor biomarkers are produced directly by the tumor or by non-tumor cells as a response to the presence of tumor cells, the elevation of tumor biomarkers can be detected earlier than radiographic abnormalities5. Investigating specific molecular markers in airways might serve as an important adjunct to routine examination for lung cancer diagnosis. The utility of cytokines in bronchoalveolar lavage fluid (BALF) for differential diagnosis of lung cancer has been described in several studies6–12. In our previous reports, we also observed that the levels of vascular endothelial growth factor (VEGF) and neuron-specific enolase (NSE) were significantly higher in BALF of lung cancer patients than in that of patients with benign diseases13,14. Recently, transforming growth factor (TGF)-β1, interleukin (IL)-6, and tumor necrosis factor (TNF)-α were suggested in published studies as possible diagnostic biomarkers of lung cancer due to their higher concentrations in serum of lung cancer patients15–17. However, up to data, little study has performed to study these cytokines in BALF of lung cancer. In this study, we performed a prospective study to investigate TGF-β1, IL-6, and TNF-α expression in airways by comparing levels of them in benign diseases and lung cancer.

Results
The BAL fluid of TGF-β1 concentration was significantly higher in patients with lung cancer compared with patients with benign diseases (18.2 [8.4–46.4] pg/ml versus 8.4 [3.5–17.0] pg/ml, P = 0.003; Figure 1A). However, there was no significant difference in BAL fluid IL-6 (4.3 ± 0.4 pg/ml versus 4.1 ± 0.4 pg/ml, respectively, P = 0.61; Figure 1B) or TNF-α (1.4 ± 0.2 pg/ml versus 1.2 ± 0.3 pg/ml, respectively, P = 0.72; Figure 1C) between lung cancer patients and non-cancer controls. However, no significant difference in BALF TGF-β1, IL-6, and TNF-α levels were observed between smokers and nonsmokers in any group (data not shown).
We evaluated the correlation of TGF-β1, IL-6, and TNF-α in BALF by the Pearson correlation analysis. A significant correlation was found between TGF-β1 and IL-6 in BALF (r = 0.337, P = 0.003; Figure 2A). Nevertheless, the levels of TGF-β1 in BALF were not relevant to that of TNF-α (r = 0.121, P = 0.290; Figure 2B). Similarly, there was no significant correlation was observed between IL-6 and TNF-α (r = −0.022, P = 0.847; Figure 2C).

Considering TGF-β1 was statistical differences between lung cancer patients and non-cancer controls, ROC analysis was further conducted to examine the diagnostic ability of the TGF-β1 for predicting lung cancer. As shown in Figure 3, the area under the receiver operating characteristic curve (AUC) was 0.695 (P = 0.003). With a cutoff value of 10.85 pg/ml, TGF-β1 had a sensitivity of 62.2%, a specificity of 60.6%, a positive predictive value of 67.5%, and a negative predictive value of 52.6%, in predicting the malignant nature of pulmonary disease.

**Discussion**

Distinguishing benign lung diseases from lung cancer through non-invasive approaches such as useful tumor markers is very important.
observed between lung cancer patients and non-cancer controls. Tobacco smoking is an established risk factor for lung cancers and may have an effect on human immune response. Although no differences in cytokine levels between smokers and non-smokers were detected in our study, environmental factors should also be considered in the development of lung cancer. Second, the prognostic value of TGF-β1, IL-6, and TNF-α in BALF were not described in our study. Some cytokines may provide information about the clinical outcome of lung cancer patients. On the other hand, biomarkers can also help clinicians in selecting the most effective anticancer treatments for each patient. Future studies with well-matched controls are needed to detect these cytokines and their prognostic values, which might lead to better understanding biological characteristics of them in lung cancer.

In summary, our study showed that the levels of TGF-β1 were significantly higher in BALF in lung cancer patients than in that of patients with benign diseases. This work may only suggest that TGF-β1 in BALF is a valuable biomarker for lung cancer and further investigation is needed. However, measurement of TNF-α or IL-6 in BALF has poor diagnostic value in lung cancer.

**Methods**

**Study subjects.** A prospective study was conducted in Affiliated Hospital of Ningbo University in China from February 2011 to July 2013. The study protocol was approved by the Institutional Review Board for Human Studies of Affiliated Hospital, School of Medicine, Ningbo University (Ningbo, China). Written informed consent was obtained from all participants. The experiments were performed in accordance with American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. 78 patients admitted into this hospital with suspected lung cancer were included. Clinical information regarding patient characteristics was based on patient records and registries. All patients had histological confirmed and were excluded if they had received preoperative chemotherapy or radiotherapy. Basic characteristics for patients are summarized in Table 1. There were 45 lung cancer patients (60.8 ± 1.2 years) and 33 patients with noncancerous diseases (58.2 ± 1.7 years). The pathologic types included 18 squamous cell carcinomas, 11 adenocarcinomas, 10 small cell carcinomas, and 6 of other cell types. There were 31 patients with pneumonia, 2 with tuberculosis, and 1 with pulmonary sarcoidosis in the control group. Smoking habits were defined at 1 year prior to diagnosis for cases or 1 year prior to interview for controls.

**Bronchoalveolar lavage.** BALF samples were collected and the methods were referred to previous studies. The lavage was done prior to brushing or biopsies to avoid contamination with blood. The bronchus on the disease side was washed with 50-ml aliquots sterile physiological saline. The fluid was gently withdrawn into a

| Table 1 | The characteristics of the patients |
|---------|-----------------------------------|
|         | Lung cancer | Benign group | P value |
| Age, years | 60.8 ± 1.2 | 58.2 ± 1.7 | 0.20 |
| Gender | | | 0.68 |
| Male | 28 | 19 | |
| Female | 17 | 14 | |
| Smoking status | | | 0.55 |
| Smokers | 20 | 12 | |
| Non-smokers | 25 | 21 | |
| Pack/years | 66.1 ± 9.3 | 29.6 ± 5.3 | 0.001 |
| Histological type | | | |
| Squamous cell carcinoma | 18 | | |
| Adenocarcinoma | 11 | | |
| Small-cell lung cancer | 10 | | |
| Others | 6 | | |
siliconized container placed in iced water. The recovered volume of BALF > 60 ml was considered to be an acceptable level of quality. The chilled lavage fluid was filtered through a nylon filter to remove mucus and centrifuged at 3,000 rpm for 10 min. The cell pellets were separated from the supernatants and stored at ~80°C.

Measurements of TGF-β1, IL-6, and TNF-α. The levels of TGF-β1 (pg/ml), IL-6 (pg/ml), and TNF-α (pg/ml) were measured using sandwich enzyme linked immunosorbent assays (ELISA). All reagents used for the experiments were standard high-quality chemicals from international companies (TGF-β1: R&D systems, Minneapolis, MN, USA; IL-6 and TNF-α: eBioscience, San Diego, CA, USA). The assays were conducted according to the manufacturer’s guidelines. The samples were analyzed in batches to minimize interassay variability.

Statistical analysis. Data was presented as means ± standard error of the mean (SEM). TGF-β1 levels were not normally distributed and were expressed as medians and interquartile ranges (IQR). If the data distribution was normal, comparison between different groups was done using the Student’s t-test; otherwise, the nonparametric Mann-Whitney U-test was applied. The relationships between different markers were determined using Pearson correlation. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated to evaluate diagnostic performance for TGF-β1. Receiver operating characteristic (ROC) analysis was conducted to examine the diagnostic ability of the TGF-β1 for predicting lung cancer. All hypothesis tests were 2-sided, with statistical significance defined as having a P value of less than 0.05. Statistical analyses were conducted using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA) or SPSS for windows (version 13; SPSS, Chicago, IL).

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Author contributions
C.C., Z.D., Z.C. and Z.X. designed the experiments. C.C., Z.D., Z.C., Z.X., S.S., Y.Y. and D.L. carried out the experiments and calculations. C.C., Z.D., Z.C. and Z.X. wrote and edited the paper.

Additional information
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