

# Elevated Expression of IL-6 in BALF and Serum - A Diagnostic Tool for Detection of Lung Cancer

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## ABSTRACT

*IL-6 is a prototypical pro-inflammatory cytokine, promoting tumor establishment and progression. The aim of this study was to evaluate the levels of IL-6 in broncho-alveolar lavage fluid (BALF) and serum of patients with different sub-types of lung cancer. The study was performed in a group of samples including 10 Adenocarcinoma, 10 Squamous cell carcinoma, 10 Small cell carcinoma and 7 non-cancerous benign lung diseases. The levels of IL-6 in BALF were significantly higher in patients with all the sub-types of lung cancer compared to patients with benign diseases. However, serum levels of IL-6 were about half as compared to BALF except for adenocarcinoma which showed a nearly equal concentration of IL-6 in BALF and serum. Taken together our results indicate that the high concentration of IL-6 in BALF and serum may serve as a useful indicator to distinguish lung cancer from benign lung diseases and thus may serve as an important screening tool.*

**Key words:** Lung cancer, Non-small cell lung cancer, BALF, IL-6

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## 1. INTRODUCTION

Lung cancer is the most common cause of cancer mortality and constitutes 12.8% of cancer cases worldwide (1). The high mortality rates associated with this cancer is mainly because of the lack of clinically useful tests for early diagnosis and screening (2). Most of the biomarkers used in the clinic lack requisite sensitivity and specificity. The usefulness of cytokines in broncho-alveolar lavage fluid (BALF) for differential diagnosis of lung cancer has been described in various studies (3-8). Changes in cytokine milieu in BALF reflect immunological status of the lung and therefore may present an index of underlying pathology (9). IL-6 is a proinflammatory cytokine with a typical protumorigenic effect. Elevated levels of IL-6 have been detected in the serum of patients with systemic cancers as compared to healthy controls or patients with benign diseases (10). IL-6 plays a key role in promoting proliferation and inhibition of apoptosis. Recently IL-6 has been shown to accentuate tumor development by promoting conversion of non-cancerous cells into tumor stem cells (11). In the context of lung cancers, IL-6 has been demonstrated to be produced by various lung cancer cell lines (12) and lung tumors (12,13). However the utility of BAL fluid as a suitable specimen for evaluating putative markers related to various lung specific pathologies is less investigated (14-15). Based on the above observations, a study was designed to determine the relationship between BAL fluid IL-6 and serum IL - 6 levels in patients with different sub-types of lung cancer.

## 2. MATERIALS AND METHODS

### 2.1 Patients

A total of 37 patients were included in this study. Approval for this study was obtained from the SMHS Hospital ethical committee, and written informed consent was obtained from all the participating subjects. Samples included 10 Adenocarcinoma ( $59.28 \pm 7.28$ ), 10 Squamous cell Carcinoma ( $61.86 \pm 9.35$ ), 10 Small Cell Carcinoma ( $61.66 \pm 12.63$ ) and 7 non-cancerous benign lung diseases ( $49.5 \pm 12.8$  years). All the lung cancer patients were histo-pathologically confirmed. The non-cancerous

(benign) lung diseases included patients with inflammatory diseases, chronic obstructive pulmonary disease, lung fibrosis, and interstitial lung diseases.

## **2.2 Broncho alveolar lavage collection and processing**

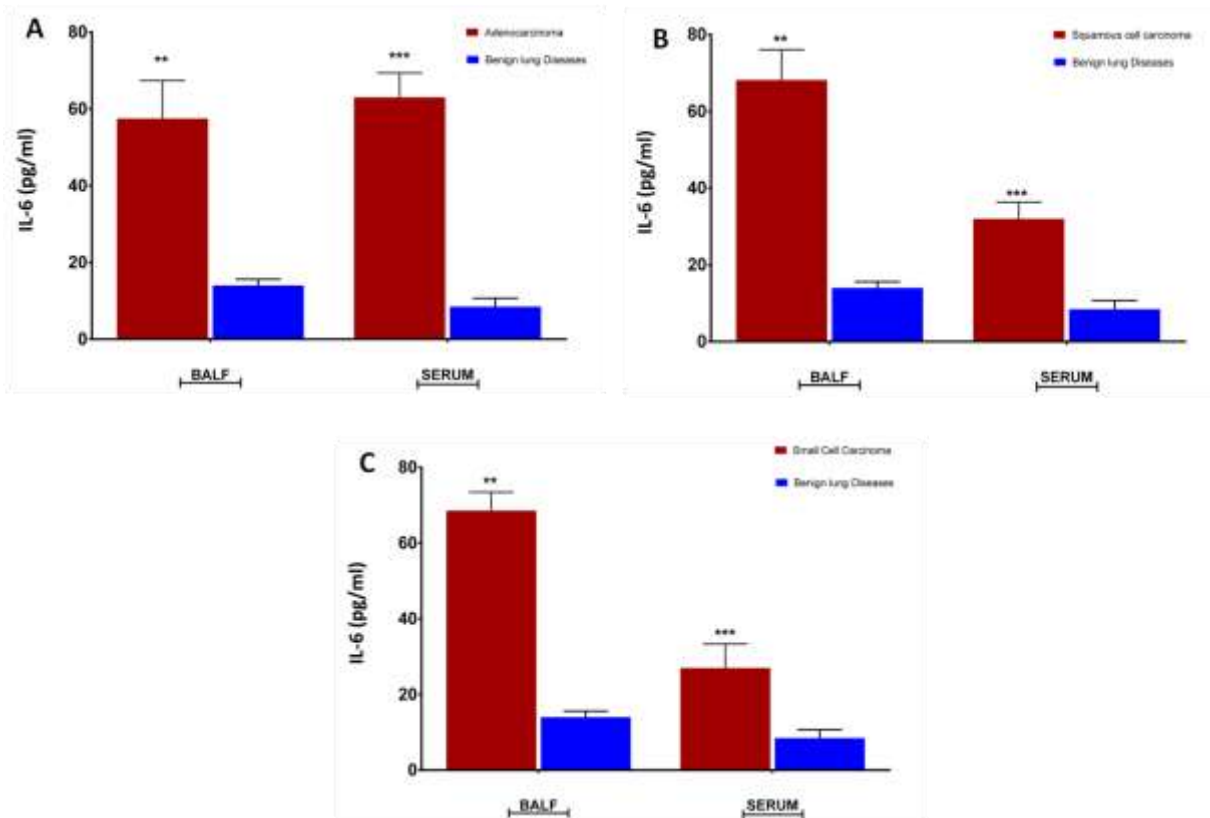
Broncho alveolar lavage (BAL) was performed through a fiberoptic bronchoscope before brushing or biopsies. The bronchoscope was advanced into the area of clinical interest, which in most cases was the segment thought to be affected by inflammation or tumour. After the local upper airways were anesthetized with 5ml of 2% lidocaine, the bronchus on the disease side was washed with 0.9% normal saline solution and the fluid was gently withdrawn into a sterilized test tube. The lavage fluid was filtered through a PVDF filter to remove mucus and centrifuged at 6000 rpm for 15-20 minutes, and supernatant aliquots were frozen at -80 for later analysis. Serum was also taken and stored at -80 for further analysis. Processing of each sample included removal of salts and depletion of abundant interfering proteins. Desalting was performed by ultra-filtration with spin concentrators (Corning, USA) (MW cutoff of 5,000 Da). The samples in the concentrators were centrifuged (50 min; 5,000 rpm; 4°C) to produce 1ml of retentate. After the first concentration step, tris 0.5M (10 mL) was added to the retentate and the concentration step was repeated for a total of three times.

## **2.3 Measurements of IL-6**

IL-6 was quantified in BAL fluid and serum by sandwich ELISA assays using commercially available kits (BD Biosciences, USA) following manufacturer's instructions. Briefly, a 96-well microplate (Maxisorp, Genetix) was coated with 50 µl capture antibody (diluted 1: 250 in 100mM carbonate buffer, pH 9.5) and kept overnight at 4°C. Then plate was washed 3 times with PBS-Tween (PBST) and blocked with PBS-BSA-1% (100µl/well) for 1h at 37°C. After washing, samples were added to each well and the plate was incubated for 1h at 37°C. Subsequently, the plate was washed and incubated with detection reagent mix (detection antibody + avidin-HRP) diluted 1: 250 in PBS-BSA 1%. After 1h of incubation, the plate was washed and the enzyme activity determined by adding freshly prepared substrate solution containing TMB/TBABH/H<sub>2</sub>O<sub>2</sub> (50 µl/well). The reaction was stopped with 50 µl of 2N H<sub>2</sub>SO<sub>4</sub> and the absorbance was read at 450 nm. All the assays were performed in triplicate.

## **3. RESULTS AND DISCUSSION**

In order to establish IL-6 as a putative secretory biomarker in lung cancer, we quantified the levels of IL-6 in BALF and serum of patients suffering from various sub-types of lung cancer including adenocarcinoma, squamous cell carcinoma and small cell carcinoma. As controls, BALF and serum from patients with benign lung diseases were also screened for IL-6. The concentration of IL-6 in BALF and serum was significantly higher in all the lung cancer samples in comparison to benign lung diseases. The average BALF levels of IL-6 were  $57.5 \pm 5.23$  in adenocarcinoma,  $68.2 \pm 4.21$  in squamous cell carcinoma,  $68.75 \pm 3.95$  in small cell carcinoma and  $14 \pm 1.44$  in benign lung diseases (Figure 1). However serum levels of IL-6 were about half as compared to BALF except for adenocarcinoma which showed nearly equal concentration of IL-6 in BALF and serum. The level of IL-6 in serum were  $63 \pm 3.58$  in adenocarcinoma,  $32 \pm 2.66$  in squamous cell carcinoma,  $27.75 \pm 2.87$  in small cell carcinoma and  $8.5 \pm 1.12$  in benign lung diseases (Figure 1)



**Figure 1. Levels of IL-6 in the BALF and serum of (A) Adenocarcinoma (B) Squamous Cell Carcinoma (C) Small Cell Carcinoma**

Figure 1. Levels of IL-6 in the BALF and serum of (A) Adenocarcinoma (B) Squamous Cell Carcinoma (C) Small Cell Carcinoma related to benign lung diseases. The levels of IL-6 were significantly higher in lung cancer patients than those in benign diseases. Data represented as mean  $\pm$  SD of results obtained from at least three independent experiments for both the assays.  $**p < 0.01$ ;  $***p < 0.001$  compared to control.

When these results are analyzed it can be observed that IL-6 is present at a high concentration in BALF than in peripheral blood (Figure 2), pointing at the utility of BALF for screening the lung cancer patients. However, no significant difference between BALF and serum IL-6 levels was observed in adenocarcinoma. The above data indicates that IL-6 serves as a useful indicator for most of the sub-types of lung cancer. It can be argued that expression of IL-6 in BALF and serum may be used to distinguishing benign lung diseases from lung cancer in order to avoid patients undergoing surgery for a benign condition. In summary this finding reaffirms the role of IL-6 in lung cancer progression (16) and thereby impresses upon developing IL-6 based therapies to ablate lung cancer metastasis.

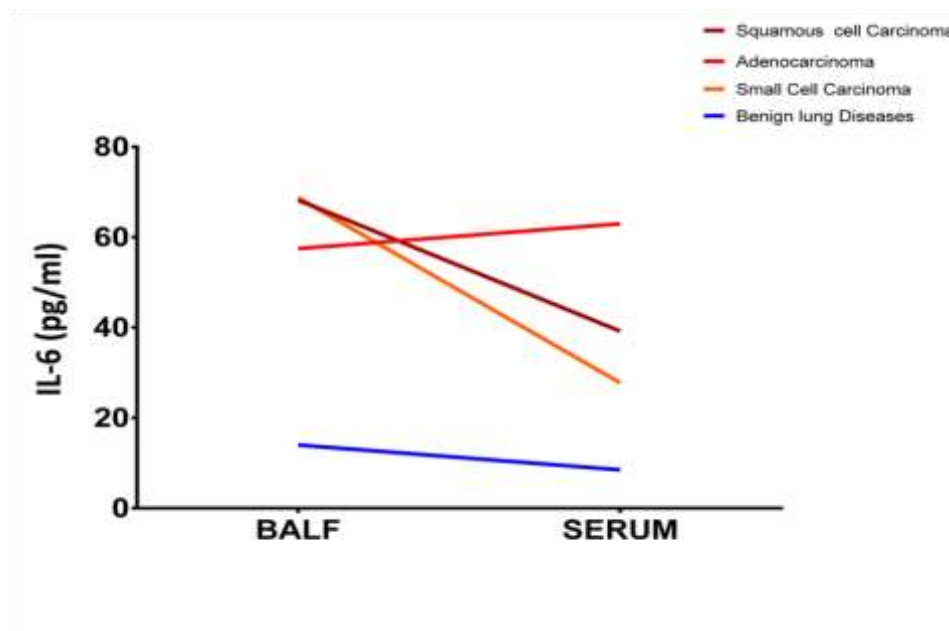


Figure 2 Correlation of IL-6 levels in BALF and serum in different sub-types of lung cancer related to benign lung diseases.

#### 4. CONCLUSION

This study reports the high levels of IL-6 in BALF and serum as a diagnostic tool to differentiate lung cancer from benign lung diseases.

#### Conflict of Interest

The authors declare no conflict of interests

#### Acknowledgement

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