Oncology and Translational Medicine
DOI 10.1007/s10330-017-0236-6

December 2017, Vol. 3, No. 6, P245–P248

ORIGINAL ARTICLE

# Clinical predictive values of biomarker levels in non-small cell lung cancer

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## Abstract

**Objective** To evaluate the predictive value of serum levels of PD-1, IL-17, and IL-21 in patients with non-small cell lung cancer.

**Methods** Serum levels of PD-1, IL-17, and IL-21 were analyzed by ELISA in 45 patients with non-small cell lung cancer (NSCLC) and 30 healthy individuals.

**Results** Serum PD-1, IL-17, and IL-21 levels were significantly different between preoperative patients with NSCLC and the control group ($P < 0.01$), while there was no significant differences between the postoperative patients and the control group ($P > 0.05$). Comparison of serum PD-1, IL-17, and IL-21 levels of patients with NSCLC before and after the operation revealed a decrease in PD-1 and IL-17 levels and an increase in IL-21 levels. The serum levels of PD-1 and IL-17 were higher in patients with advanced staged disease than in those with early stage cancer ($P < 0.05$), while IL-21 levels were lower at the advanced stages ($P < 0.05$).

**Conclusion** In patients with NSCLC, serum levels of PD-1, IL-17, and IL-21 changed considering the surgical operation and the course of the disease. Screening these biomarker levels might provide a helpful index for treatment and prognosis

Received: 18 July 2017
Revised: 23 August 2017
Accepted: 27 September 2017

**Keywords:** non-small cell lung cancer; PD-1; IL-17; IL-21

Lung cancer is a malignant disease with rapid recurrence and high mortality rates [1], and is the most common cause of death among patients with cancer in China. More than 85% of patients with lung cancer present with non-small cell lung cancer (NSCLC), which has a 5-year survival rate of only about 15% [2]. Studies have identified a variety of immune molecules involved in the occurrence and development of NSCLC, and detecting the related immune molecules might be helpful for early diagnosis, effective treatment, and improving the prognosis of the disease. The programmed cell death protein 1 (PD-1) was isolated in 1992 and belongs to the B7 family of immunoglobulins. PD-1 is an important negative costimulatory molecule that, upon binding to its ligand, inhibits T cell activation, proliferation, participation in immune tolerance, and immune evasion, which promote tumor development and progression. Interleukin-21 (IL-21) is a type I cytokine that was discovered by Parrish-Novak in 2000 and plays an important role in anti-tumor and anti-virus immune functions by promoting the activation, proliferation, and differentiation of NK, B, and T cells. The role of IL-21 in the development, progression, and metastasis of NSCLC has not yet been elucidated [3–4]. Interleukin 17 (IL-17) is a proinflammatory cytokine that stimulates epithelial cells, endothelial cells, and fibroblasts to produce a variety of cytokines that induce inflammation [5–8]. IL-17 has been found to induce tumor angiogenesis and promote tumor growth, invasion, and metastasis in NSCLC animal models. In this study, we investigated PD-1, IL-21, and IL-17 levels in the peripheral blood of patients with NSCLC and healthy individuals to explore the significance of these potential biomarkers in the development and progression of NSCLC.

## Materials and methods

### Materials

From January 2015 to December 2016, 45 inpatients diagnosed with primary non-small cell lung cancer were recruited for our study. This patient cohort included 25 men and 20 women, with an average age of 56.1 years (range, 38 to 70 years). The normal control group

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included 30 healthy volunteers, 14 men and 16 women, and the average age was 53.4 years (range, 36 to 65 years). All patients provided informed consent and reported to the hospital ethics committee for approval. Patient were diagnosed through tissue biopsy, fiberoptic bronchoscopy, and/or chest computed tomography. Diagnostic criteria were categorized in reference to the WHO classification of lung cancer histological criteria [9], and the patients with NSCLC were divided into 21 patients with squamous cell carcinoma and 24 patients with adenocarcinoma. Postoperative pathological stage was determined by TNM standardization (International Union Against Cancer Classification in 2002), and the patients were classified as 7 patients with stage I, 11 patients with stage 2, 17 patients with stage 3, and 10 patients with stage 4 cancer.

### Instruments and reagents

IL-17 and IL-21 ELISA kits were purchased from Beijing Jingmei Bioengineering Co., Ltd. The PD-1 ELISA kit was purchased from R&D Systems, USA.

### PD-1, IL-17, and IL-21 quantification with ELISA

Venous blood (4 mL) from patients was drawn 3 days before and 7 days after the operation. The blood was made to stand for 1 h at room temperature and was centrifuged at 2500 r/min for 15 min. PD-1, IL-17, and IL-21 were measured by using double antibody sandwich ELISA. According to manufacturer instructions, each sample and standard was repeated three times.

### Statistical analysis

The data were expressed as mean ± standard deviation (mean ± SD). Statistical comparisons, using analysis of variance, were performed with SPSS 13.0 software. $P$ values less than 0.05 were considered statistically significant.

## Results

### The levels of serum PD-1, IL-17, and IL-21 in patients with NSCLC and healthy subjects, determined by ELISA

Serum levels of PD-1, IL-17, and IL-21 in 45 patients with NSCLC were significantly different from those of healthy volunteers ($P < 0.01$) (Table 1). Serum IL-21 levels were significantly greater ($P < 0.05$), and serum PD-1 and IL-17 levels were significantly lower, than those observed in the postoperative group ($P < 0.05$).

### The relationship between the serum levels of PD-1, IL-17, and IL-21 in patients with NSCLC and corresponding clinicopathological features

The levels of serum PD-1 and IL-17 gradually increased with TNM stage progression, and the differences were significant ($P = 0.031$, $P = 0.037$). IL-21 levels decreased gradually as the TNM stage progressed, and this difference was significant ($P = 0.025$). The changes in serum PD-1, IL-17, and IL-21 levels did not correlate with the pathological type of NSCLC. There were no significant differences in the levels of squamous cell carcinoma and adenocarcinoma between the two groups (P = 0.249, P = 0.251, P = 0.305; Table 2).

### The relationship between PD-1, IL-17, and IL-21 levels in patients with NSCLC

There was a negative correlation between PD-1 and IL-21 levels in the peripheral blood of patients with NSCLC ($r = 0.031$, $P = 0.532$) and between IL-17 and IL-21 levels ($r = 0.012$, $P = 0.325$). There was a positive correlation between PD-1 and IL-21 levels in the peripheral blood of patients with NSCLC ($r = 0.534$, $P = 0.265$).

## Discussion

As NSCLC does not usually show early clinical manifestations, most patients with NSCLC are diagnosed in the advanced stage. At the time of NSCLC diagnosis, tumor cells already exhibit invasion and metastasis, which affect disease treatment and prognosis, thereby underscoring the need to monitor and control tumor cell

**Table 1** The serum levels of IL-17 ,IL-21 and PD-1 of the preoperative and postoperative NSCLC patients vs controls ($\chi \pm s$)

| Group | *n* | IL-17 (pg/mL) | | IL-21 (pg/mL) | | PD-1 (pg/mL) | |
|---|---|---|---|---|---|---|---|
| | | Pre-operate | Post-operate | Pre-operate | Post-operate | Pre-operate | Post-operate |
| NSCLC | 45 | 46.21 ± 13.42 | 26.89 ± 8.13 | 63.21 ± 12.21 | 82.46 ± 10.87 | 110.34 ± 20.13 | 55.27 ± 14.76 |
| Control | 30 | 20.08±7.93 | – | 86.93 ± 10.62 | – | 51.22 ± 15.12 | – |
| *t* | | 3.679 | 1.693 | 3.435 | 1.652 | 4.009 | 1.730 |
| P | | 0.031 | 0.093 | 0.042 | 0.102 | 0.020 | 0.134 |

**Table 2** The relationship between the serum level of PD-1, IL-17, IL-21 of NSCLC patients and clinical pathological features ($\chi \pm s$)

| Clinical pathological features | *n* | IL-17 | | IL-21 | | PD-1 | |
|---|---|---|---|---|---|---|---|
| | | pg/mL | *P* | pg/mL | *P* | pg/mL | *P* |
| TNM staging | | | | | | | |
| Stage I | 7 | 37.56 ± 4.33 | 0.037 | 78.36 ± 11.49 | 0.025 | 92.14 ± 17.22 | 0.031 |
| Stage II | 11 | 46.64 ± 5.61 | | 67.74 ± 10.02 | | 102.47 ± 22.53 | |
| Stage III | 17 | 54.71 ± 6.19 | | 57.13 ± 10.87 | | 115.35 ± 20.98 | |
| Stage IV | 10 | 62.59 ± 6.85 | | 48.32 ± 9.62 | | 128.31 ± 22.34 | |
| Pathological type | | | | | | | |
| Squamous cell | 21 | 51.81 ± 5.46 | 0.251 | 66.67 ± 12.81 | 0.305 | 109.14 ± 19.73 | 0.249 |
| Carcinoma adenocarcinoma | 24 | 53.34 ± 5.78 | | 61.32 ± 14.34 | | 115.25 ± 21.82 | |

metastasis for patients with NSCLC [10].

The development of NSCLC is closely related to immune status and function. T lymphocytes play an important role in cellular immunity, and CD4+ T cells participate in the immune response at all stages. Th17 cells belong to a CD4+ T cell subset and secrete IL-17 and other cytokines involved in tumor development. In NSCLC mouse models, IL-17 has been found to induce the migration of vascular endothelial cells and formation of endothelial cells, promote the secretion of cytokines such as VEGF, TGF-β1 and PGE2 in tumor or tumor stromal cells, and promote tumor growth, metastasis, and infiltration. IL-21 is a type I cytokine that is secreted by activated CD4+ T cells and NKT cells and contains a chain that is in common with IL-2, IL-4, and IL-15 cytokines. IL-21 can promote lymphocyte proliferation and differentiation and enhance the cytotoxicity of CD8+ T and NK cells. The regulation of IL-21 on immune cells is bidirectional, and depends on microenvironmental factors such as synergistic cytokines [11–13].

The immune escape of tumor cells is closely related to the abnormal expression of synergistic molecules. PD-1/PD-L1 is an important member of the B7/CD28 costimulatory molecule superfamily and has been shown to be responsible for the regulation of T cells by suppressing T cell activation and proliferation, which results in the immune escape of tumor cells. The PD-1 pathway has become a new hotspot for cancer therapy [14–15].

In this study, we measured serum PD-1, IL-17, and IL-21 levels in 45 patients with NSCLC. In comparison with 30 healthy volunteers, the levels of PD-1 and IL-17 in the peripheral blood of patients with NSCLC increased, and IL-21 levels decreased. We hypothesize that these three immune molecules might be involved in the occurrence of NSCLC, and for further investigation, we compared the changes in serum levels of these cytokines in patients before and after surgery. After treatment, the serum levels of IL-17 and PD-1 decreased and IL-21 increased, which are closely associated with tumor load. In order to elucidate whether PD-1, IL-17, and IL-21 levels have a certain relationship with NSCLC pathological stage, serum levels of 45 patients with NSCLC were analyzed according to TNM staging. The results showed that serum PD-1 and IL-17 levels increased with the progression of NSCLC ($P = 0.031$, $P = 0.037$), suggesting that PD-1 and IL-17 were involved in the proliferation, infiltration, and metastasis of NSCLC. The level of IL-21 decreased gradually as TNM stage progressed ($P = 0.025$), suggesting that as the clinical stage of NSCLC progresses, the immune function of gradually reduces and IL-21 level decreases. Our experimental data showed that there were no significant differences in serum PD-1, IL-17, and IL-21 levels between patients with squamous cell carcinoma and those with adenocarcinoma, suggesting that serum levels of PD-1, IL-17, and IL-21 were not related to the pathological subtype of NSCLC.

In summary, the results of our study show that changes in serum PD-1, IL-17, and IL-21 levels in patients with NSCLC are associated with the development, progression, and metastasis of NSCLC. Monitoring the levels of PD-1, IL-17, and IL-21 may be important for guiding the treatment and prognosis of patients with NSCLC.

## Conflict of interest

The authors indicated no potential conflicts of interest.

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**DOI 10.1007/s10330-017-0236-6**
**Cite this article as:** Wei WQ. Clinical predictive values of biomarker levels in non-small cell lung cancer. Oncol Transl Med, 2017, 3: 245–248.