PREDICTION OF PROGNOSIS OF ADVANCED NSCLC PATIENTS AFTER EGFR-TKIS THERAPY BY THE LEVEL OF IL-6 IN PLEURA

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ABSTRACT

Objective: *To evaluate the role of IL-6 level in pleura in indicating the prognosis of patients with advanced non-small cell lung cancer (NSCLC) who accepted the EGFR-TKIs therapy.*

Methods: *In this study, we analyzed the clinical data of 95 advanced NSCLC patients who accepted the EGFR-TKIs therapy to evaluate the relationship between pleura IL-6 and clinical outcome, while the Kaplan-Meier method and Cox proportional risk model were also applied to assess the progression-free survival (PFS) and overall survival (OS) of patients.*

Results: *In comparison with the patients with IL-6 at the normal level in pleura, those with increased IL-6 in pleura would have shorter progression-free survival (PFS) and overall survival (OS) (99 d vs. 123.5 d, P=0.011; 385 d vs. 607 d, P=0.001). Regardless of the mutation of EGFR, any increase in the level of IL-6 would shorten the PFS of patients.*

Conclusion: *IL-6 level may be prognostic factor of NSCLC patients who accept the EGFR-TKIs therapy.*

Keywords: *Non-small cell lung cancer, EGFR-TKIs, IL-6, prognosis.*

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Introduction

Lung cancer is widely recognized as a kind of respiratory malignant tumor threatening the health of human beings. Non-small cell lung cancer, as the major subtype of lung cancer, has taken up nearly 85% of all cases[(1)]. Lung cancer has a quite high prevalence rate and a mortality rate but an extremely low survival rate – 15% in 5-year survival rate according to the available data[(2)]. NSCLC patients are more susceptible to the tumor invasion in their peripheral vessels, which could further give rise to the distant metastasis, affecting the prognosis of patients[(3)]. Thus, increasing the accuracy of early diagnosis has a positive effect on improving the prognosis of patients. Inflammation is pivotal in the development, progression and metastasis of tumor[(4)]. It has been found that several members of interleukin family, especially interleukin 6 (IL-6), are up-regulated evidently in a variety of tumors[(5)]. IL-6, as a kind of cytokine, plays a key role in the response to damage or infection, and, thus, has been taken as a promising biomarkers for predicting the poor prognosis and therapeutic targets of NSCLC[(6)]. Dysfunctional generation of IL-6, or the abnormal activation of IL-6, has been widely reported in a variety of human cancers and the related tumor behaviors, such as proliferation, migration and

adhesion[7]. IL-6-JAK-STAT signal axis has been found to have important roles in multiple tumors, including the breast cancer, colorectal cancer, lung cancer, ovarian cancer, prostatic cancer and multiple myeloma[8-10]. EGF receptor (EGFR), as a kind of membrane protein, is crucial to the proliferation, growth and repair of tumor cells. The mutation rate of EGFR is about 30% to 40%, representing the most frequent mutation type of driver gene in NSCLC[11]. Existence of EGFR mutation, therefore, could provide the scientific evidence for the administration of EGFR-tyrosine kinase inhibitor (TKI) for NSCLC patients at the advanced stage. In the past two decades, numerous Phase II or III clinical trials have been conducted to validate the efficacy of TKI, and as a result, targeted therapy, in comparison with the chemotherapy, could improve the prognosis of NSCLC patients at the advanced stage harboring the EGFR mutation[12, 13].

Currently, there remain few studies of the role of IL-6 level in pleura in evaluating the prognosis of EGFR-TKIs-treated NSCLC patients. As such, we, in this work, analyzed the expression profile of IL-6 in the pleura of NSCLC patients, aiming to clarify the correlation between IL-6 in pleura and NSCLC prognosis and provide reference for the clinical diagnosis and treatment.

Patients and methods

We retrospectively recruited the NSCLC patients at the advanced stage who received the EGFR-TKI therapy in Cangzhou Central Hospital between 2017 and 2020 into this study. All patients had the NSCLC at Stage IIIB or IV. Medication for patients was performed by gefitinib (250 mg/d), erlotinib (150 mg/d) or afatinib (40 mg/d) until the progression of disease or intolerance of patients to the toxicity. This study had been approved by the Ethical Committee of Cangzhou Central Hospital.

Pleural effusion collected in a heat-free, pathogen-free Falcon tube was centrifuged at 1500 x for 10 min to isolate the supernatant which was then preserved at -80°C. Enzyme-linked immunosorbent assay kits for human IL-6 (BD Biosciences, Rockville, MD) to measure the level of IL-6 in pleura, and for the protein concentrations in each sample, commercial kits (Bio-Rad Laboratories, Hercules, CA) were used. Levels of pleural cytokines were corrected by the level of corresponding protein.

Student's t test was used to analyze the continuous variables, while chi-square test to analyze the categorical variables. PFS interval was counted from the timepoint of first EGFR-TKI therapy to that of disease recurrence, death or last follow-up showing no evidence of recurrence. OS interval was counted from the beginning of EGFR-TKI therapy to death regardless of the causes. Cox proportional risk model was constructed for the univariate or multivariate analysis. $P<0.05$ suggested that the difference had statistical significance.

Results

Clinicopathological features of patients

In this study, a total of 95 eligible NSCLC patients were enrolled, and the detailed data of patients are listed in Table 1.

The average age of patients at the time of diagnosis was 57.09 years (ranging from 32 to 80 years). Among these patients, there were 43 females and 52 males; 46 patients had the history of smoking; 9 patients were diagnosed at the Stage IIIB and 86 at the Stage IV; 56 patients harbored the EGFR mutation, where 31 patients had the deficiency of exon 19, 23 had the mutation of L858R at exon 21, and 2 had the mutation of L861Q at exon 21, and 39 patients harbored the wild-type EGFR.

Patients' features (n=95)	值
Average age (range), years	59.07 (32-80)
Sex, n	
Male	43
Female	52
Smoking history, n	
Yes	46
No	49
Stage of disease, n	
IIIB	9
IV	86
EGFR mutation, n	
Ex19 Del	31
Ex21 L858R	23
Ex21 L861Q	2
Wild-type EGFR	39
TKI type, n	
Gefitinib	40
Erlotinib	53
Afatinib	2

Table 1: Clinicopathological features of patients.

PFS and OS of EGFR-TKI-treated NSCLC patients at the advanced stage

Prior to the EGFR-TKI treatment, 45 patients had increases in the levels of IL-6 in pleura (>0.46 ng/L), and those patients were enrolled into the elevated group; the PFS and OS of patients in the elevated group were significantly shorter than those in the normal group (PFS: 99 d vs. 123.5 d; OS: 385 d vs. 607 d, P=0.001, Figure 1A and B).

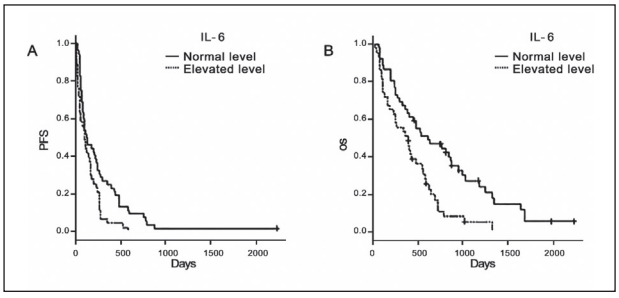

Figure 1: Relationship of IL-6 level in pleura with the PFS (A) and OS (B) by Kaplan-Meier.

At last, 73 patients (76.8%) died, of which 65 had EGFR mutation and 8 had wild-type EGFR, and 22 patients (23.2%) survived at the end of study, of which 6 had eGFR mutation and 16 had wild-type EGFR. The median of OS was 11.7 months (95% CI: 10.521-12.879). In this study, univariate analysis was carried out to analyze the clinicopathological features, especially the age, sex, smoking history, histological types, stage and EGFR status. As a result, IL-6 level and EGFR mutation were the key factors affecting the PFS, while IL-6 level, EGFR mutation and tumor pathological features were the major factors affecting the OS (Table 2).

Factors	Category	n	PFS		OS	
			Average (d)	*P*	Average (d)	*P*
Age	<75	67	105	0.626	471	0.668
	≥75	28	136.5		397	
Sex	Male	43	116	0.475	519	0.054
	Female	52	100.5		329.5	
Smoking history	No	46	123.5	0.283	630	0.291
	Yes	49	99		330	
Pathological type	Non-squamous carcinoma	85	184	0.034	366	<0.001
	Squamous carcinoma	10	105		471	
State of disease	IIIB	9	124	0.225	476	0.325
	IV	86	74		145.5	
Type of EGFR mutation	Mutation	56	214.5	<0.001	777	<0.001
	Wild-type	29	46		228	
Times of previous treatment	0-1	58	174	<0.001	636	<0.001
	2-more	37	46		233.5	
IL-6	≤0.46 ng/L	50	123.5	<0.01	357	<0.001
	>0.46 ng/L	45	99		542	

Table 2: Univariate analysis of PFS and OS.

A multivariate analysis showed that IL-6 level and EGFR mutation were the key factors affecting the PFS [IL-6: hazard ratio (HR)=2.17, P<0.001; EGFR mutation: HR=2.92, P<0.001] and OS (IL-6: HR=2.29, P<0.001; EGFR mutation: HR=4.72, P<0.001) (Table 3 and 4).

Factors	Category	HR (95%CI)	*P*
Stage of disease	IIIB	1.00	0.254
	IV	0.61 (0.31-1.23)	
Type of EGFR mutation	Mutation	1.00	<0.001
	Wild-type	2.92 (1.54-5.52)	
Times of previous treatment	0-1	1.00	0.14
	2-more	1.60 (0.88-2.91)	
IL-6	≤0.46 ng/L	1.00	<0.001
	>0.46 ng/L	2.17 (1.38-3.40)	

Table 3: Multivariate analysis of the PFS by Cox proportional risk regression model.

Factors	Category	HR (95%CI)	*P*
Stage of disease	IIIB	1.00	0.254
	IV	0.87 (0.51-1.49)	
Type of EGFR mutation	Mutation	1.00	<0.001
	Wild-type	4.72 (2.09-10.66)	
Times of previous treatment	0-1	1.00	0.14
	2-more	1.07 (0.51-2.23)	
IL-6	≤0.46 ng/L	1.00	<0.001
	>0.46 ng/L	2.29 (1.40-3.74)	
Pathological type	Non-squamous carcinoma	1.00	0.651
	Squamous carcinoma	1.18 (0.53-2.61)	

Table 4: Multivariate analysis of the OS by Cox proportional risk regression model.

In the EGFR-mutated patients, patients with elevation in level of IL-6 had shorter PFS and OS when comparing to those with the normal level of IL-6 (PFS: 168 d vs. 244.5 d, P=0.032); OS: 471 d vs. 1023 d, P<0.001; Figure 2A-B). On the other hand, in the patients harboring the wild-type EGFR, those with elevation in IL-6 level had shorter PFS when comparing to those with normal level of IL-6 (30 d vs. 53.5 d, P=0.027; Figure 2C), and their OS tended to be shortened (106 d vs. 252.5 d, P=0.115, Figure 2D).

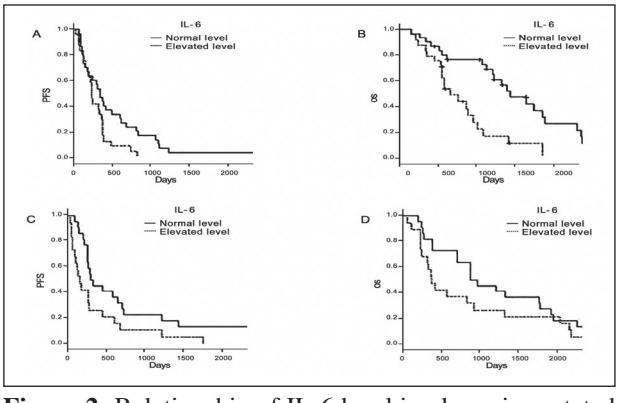

Figure 2: Relationship of IL-6 level in pleura in mutated or wild-type EGFR with PFS (A, C) and OS (B, D) by Kaplan-Meier.

Discussion

In this retrospective study, we found that EGFR-TKI-treated patients with the elevation in IL-6 level had shorter PFS and OS when comparing to those with the normal level of IL-6. The results of multivariate analysis showed that IL-6 level and EGFR mutation are the major factors affecting the PFS and OS of patients. It has been widely recognized that in comparison with the chemotherapy, EGFR-TKI treatment could prolong the PFS of NSCLC patients harboring the EGFR mutation at the advanced stage[14]. Our subgroup analysis has further discovered that patients harboring the EGFR mutation with elevation in level of IL-6 have short PFS and OS, while the PFS of those harboring the wild-type EGFR is much shorter. Thus, the level of IL-6 is the predicting factor for EGFR-TKI-treated patients harboring the mutated or wild-type EGFR.

As a kind of pleiotropic cytokine, IL-6 is associated with the proliferation and survival of tumor cells[15]. IL-6, due to its small molecular weight, could rapidly spread through the cells and tissues and arrive at the microenvironment of tumor[16]. It has been reported that IL-6 is up-regulated in the epithelium and mesenchyme of renal tumors, but poorly expressed in the surrounding normal tissues[17]. Besides, people also have found that IL-6 and other pro-inflammatory cytokines are rich in the tissue microenvironment of pancreatic cancer[18]. A previous meta-analysis reported that the up-regulation of IL-6 is correlated with the shortening of OS of NSCLC patients at advanced stage[19], which coincides with our finding. Moreover, IL-6 has also been taken as a predictor for the PFS of EGFR-TKI-treated NSCLC patients harboring the EGFR mutation[20]. Among the NSCLC patients who received the treatment of EGFR-TKI, the increase in IL-6 is also associated with the poor PFS[21]. Furthermore, the established correlation between the increased level of IL-6 and poor physical status also demonstrates that IL-6 can reflect the volume of tumor. It should be emphasized here that this study may be limited by the following aspects. First, this is only a retrospective study conducted in one agency, which could give rise to the bias in selection of subjects that may affect the result of this study. Besides, the exact molecular basis of IL-6 affecting the growth and invasion of squamous cells in lung cancer remains unknown yet.

In conclusion, the increased IL-6 is associated with the shortening of PFS and OS of EGFR-TKI-treated NSCLC patients. Importantly, IL-6 is related to the much shorter PFS of patients harboring the wild-type EGFR who respond poorly to the current strategy of treatment. As such, IL-6 level, regardless of the mutation status of EGFR, could be used to ascertain whether NSCLC patients are appropriate for the treatment of EGFR-TKI.

Thus, future work should focus on the studies to uncover the underlying mechanism and the prospective clinical studies which could further validate the results above.

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