EVALUATION OF MEMBRANE AND SOLUBLE PD-L1 EXPRESSION IN ADVANCED-STAGE NON-SMALL CELL LUNG CANCER PATIENTS

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Summary

Objectives: To evaluate membrane and soluble PD-L1 expression in advanced-stage non-small cell lung cancer (NSCLC) patients. ***Subjects and methods:*** A prospective, cross-sectional study on 50 advanced-stage NSCLC patients treated at the Respiratory Center, Military Hospital 103 from January to September 2019; the control group consists of 30 normal people who had personal periodic health examination at the Outpatient Department, Military Hospital 103. Membrane PD-L1 (mPD-L1) expression assay was carried out applying immunohistochemistry on cancer tissue samples, using PD-L1 73 - 10 antibody. The serum level of soluble PD-L1 (sPD-L1) was measured using the ELISA technique. ***Results:*** 62% of lung cancer tissue samples were positive for mPD-L1 (TPS ≥ 1%), of which 51.6% were highly positive and 48.4% low positive. The concentration of sPD-L1 in NSCLC group was significantly higher than that of the control group (2.11 ± 1.48 and 0.73 ± 0.57 ng/mL; $p < 0.001$). sPD-L1 concentration in the mPD-L1 negative group (1.61 ± 1.37 ng/mL) was statistically significantly lower than that in the mPD-L1 positive group (2.42 ± 1.49 ng/mL) ($p = 0.03$). At the cut-off value of 1.815 ng/mL, sPD-L1 had a diagnostic value for mPD-L1 positive with a sensitivity of 65% and specificity of 68%, area under the curve was 0.678 (95% CI: 0.521 - 0.835; $p = 0.036$). ***Conclusion:*** The rate of mPD-L1 positive expression in NSCLC tissue was 62%. The concentration of sPD-L1 in NSCLC patients was significantly higher than that in the control group. No significant relationship was found between the two forms of PD-L1 expression.

** Keywords: Non-small cell lung cancer (NSCLC); Membrane-bound programmed death ligand - 1 (mPD-L1); Soluble programmed death ligand - 1 (sPD-L1).*

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INTRODUCTION

Lung cancer is one of the leading malignant diseases with high incidence, high mortality, and poor prognosis in the world. According to the statistics of the World Health Organization, cancer was the leading cause of death worldwide, accounting for nearly 10 million deaths in 2020, or nearly one in six deaths, in which death from lung cancer ranked first with 1.8 million cases. Many studies showed that more than two-thirds of lung cancer patients were diagnosed when the disease was at the late stage. PD-1/PD-L1 targeted treatment is a novel immunotherapy that has been proven effective in NSCLC patients. PD-L1 (programmed death ligand - 1) is a type 1 transmembrane protein, belonging to the B7 ligand family, that binds to the PD-1 receptor - expressed on the surface of activated T lymphocytes. The interaction of the PD-1 and PD-L1 transmits an immunosuppressive signal to the T cells, resulting in an "inactivated" state of the T cells. Among the ligands of the B7 family, PD-L1 is the most studied in NSCLC. Many studies and clinical trials have demonstrated that PD-L1 expression in tumor tissue was an important key for selecting PD-1/PD-L1 immune checkpoint inhibitor therapy.

There are two forms of PD-L1: Membrane-bound PD-L1 (mPD-L1), mainly determined on tumor cell membranes, and soluble PD-L1 (sPD-L1) found in peripheral blood. Many studies have demonstrated that high mPD-L1 expression in tumor tissue is associated with a short survival time in cancer patients [1, 2]. Similarly, it was suggested that sPD-L1 in peripheral blood could also bind to the PD-1 receptor on T cells, reducing the antitumor activity of T cells. Therefore, our study was conducted with the following objectives: *To evaluate membrane and soluble PD-L1 expression in advanced-stage non-small cell lung cancer patients.*

SUBJECTS AND METHODS

1. Subjects

The patient group was 50 advanced-stage non-small cell lung cancer treated at Military Hospital 103 from January to September 2019.

* *Selection criteria:* Patients who were confirmed diagnosed with NSCLC by histopathology and immunohistochemistry assay agreed to participate in the study.

* *Exclusion criteria:* Patients who had been treated, had autoimmune diseases, immunodeficiency, co-morbidity with other organ cancers, and used immunosuppressive drugs or corticosteroids within 1 month.

The control group consists of 30 normal people who had personal periodic health examinations at the Outpatient Department, Military Hospital 103.

2. Methods

* *Study design:* A prospective, descriptive study.

Membrane PD-L1 assay was conducted on cancer tissue samples applying immunohistochemistry technique using PD-L1 antibody 73 - 10. The results were calculated by the Tumor Proportion Score (TPS), which is the percentage of tumor cells that picked up color after being stained. mPD-L1 was positive if TPS ≥ 1%, negative if TPS < 1%, high postive if TPS ≥ 50% and low postivive if TPS < 50%.

Soluble PD-L1 expression was measured on serum samples separated from peripheral blood samples, based on the ELISA method, using SEA788HU Enzyme-Linked Immunosorbent Assay (ELISA) Kit For Programmed Cell Death Protein 1 Ligand (PDL1), Lot L190410338 and Lot L210517534, from Cloud-Clone Corp. The measurement unit is ng/mL.

Using the 7th TNM classification for lung cancer staging.

* *Data processing:* The data were analyzed using IBM SPSS Statistics 28.0.

RESULTS

* *General characteristics of the study group:*

Our study was conducted on 50 NSCLC patients and 30 normal people with average ages of 67.14 ± 13.08 and 61.23 ± 15.85, respectively; the male and female ratio is 38/12 and 14/16, respectively.

There were 39/50 cancer patients (78%) diagnosed with stage IV according to the 7th TNM classification. The predominant histopathological type was adenocarcinoma (68%). EGFR mutation test was performed in 29/50 samples (58%). The mutation rate was 9/29 (31.03%), of which 3 mutation sites were exon 18 (1/9), 19 (6/9), and exon 21 (2/9).

Table 1: Characteristics of disease stage, histopathology, and EGFR mutation.

Characteristics	n (%)
Stage:	
IIIB	11 (22)
IV	39 (78)
Histopathology:	
Adenocarcinoma	34 (68)
Squamous cell	12 (24)
Adenosquamous cell	4 (8)
EGFR mutation	9/29 (31.03)

** Membrane PD-L1 expression:*

An immunohistochemistry assay was performed on the tumor tissues of 50 NSCLC patients to determine the level of membrane PD-L1 expression. The result showed that 19/50 samples (38%) were negative with TPS < 1%; 31/50 samples (62%) were positive (TPS ≥ 1%). The degree of positivity was calculated by TPS, of which 16/31 (51.6%) showed high positivity (≥ 50%) and 15/31 (48.4%) showed low positivity (< 50 %).

** Soluble PD-L1 expression:*

Table 2: Comparison of mean sPD-L1 concentration between 2 study groups.

	NSCLC	**Control group**
Number (n)	50	30
X ± SD (ng/mL)	2.11 ± 1.48	0.73 ± 0.57
Min - max	0.24 - 5.56	0.25 - 2.48
p	p < 0.001	

sPD-L1 concentration in NSCLC group was 2.11 ± 1.48 ng/mL (minimum 0.24; maximum 5.56 ng/mL), significantly higher than that in the control group (0.73 ± 0.57 ng/mL) (p < 0.001).

** The relationship between the membrane and soluble PD-L1:*

Table 3: Comparison of mean sPD-L1 concentration by subgroup mPD-L1.

mPD-L1	**Number (Rate)**	**Mean sPD-L1 concentration (ng/mL)**	**p**
Positive	31/50 (62%)	2.42 ± 1.49	0.03
Negative	19/50 (38%)	1.61 ± 1.37	
High positive	16/31 (51.61%)	2.23 ± 1.41	0.23
Low positive	15/31 (48.39%)	2.61 ± 1.59	

sPD-L1 concentration in the mPD-L1 positive group (2.42 ± 1.49 ng/mL) was significantly higher than in the mPD-L1 negative group (1.61 ± 1.37 ng/mL) (p = 0.03). However, there was no significant difference in sPD-L1 value between the high and low mPD-L1 positive group (2.23 ± 1.41 vs. 2.61 ± 1.59; p = 0.23).

There was no significant correlation between sPD-L1 and mPD-L1 (r = 0,085; p = 0,559). Figure 1 shows a scatter plot of sPD-L1 and mPD-L1.

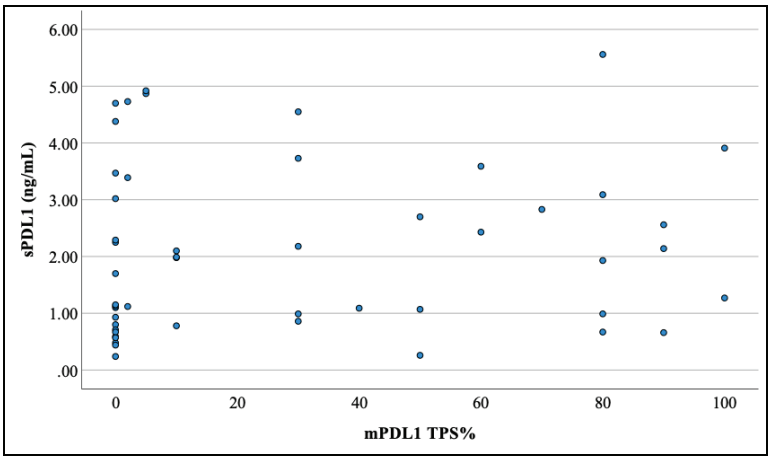

Figure 1: Scatter plot of sPD-L1 and mPD-L1.

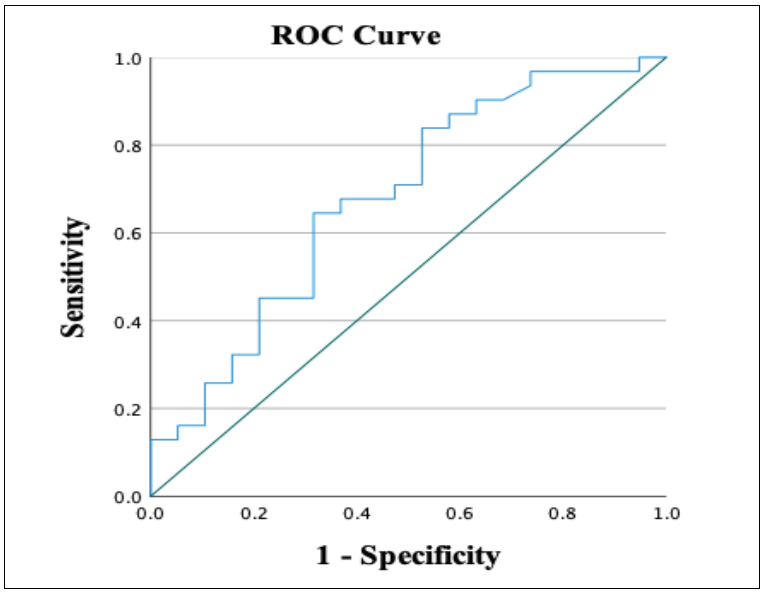

Figure 2: ROC analysis of the diagnostic value of sPD-L1 for mPD-L1 expression status.

ROC analysis showed that: At the cut-off value of 1.815 ng/mL, sPD-L1 had a diagnostic value for mPD-L1 positive with a sensitivity of 65% and specificity of 68%, area under the curve was 0.678 (95% CI: 0.521 - 0.835; p = 0.036). sPD-L1 had poor value in determining mPD-L1 expression status.

DISCUSSION

** Membrane PD-L1 expression on tumor tissue:*

Among the 50 studied patients, mPD-L1 expression was positive in 31/50 tissue samples (62%). The high and low mPD-L1 expression rates (≥ 50% and < 50%) were 15/31 (48.4%) and 16/31 (51.6%), respectively.

The rate of mPD-L1 positive fluctuated depending on the studies, objectives and the type of PD-L1 antibody used in the immunohistochemistry technique. In a series of CheckMate clinical trials, the PD-L1 antibody was 28-8. Clinical trial CheckMate 057 - phase 3, randomized, open-label, multinational trial in non-squamous NSCLC patients progressed during or after platinum-based chemotherapy, comparing the efficacy of Nivolumab with Docetaxel, the mPD-L1 positive rate was 455/582 (78%) [3]. The CheckMate 017 trial compared the efficacy of Nivolumab with Docetaxel in patients with stage IIIB or IV squamous cell lung cancer progressed after first-line platinum-based chemotherapy, the mPD-L1 positive rate was 225/272 (83%) [4]. This rate was 70% in the CheckMate 012 trial [5].

The TPS was evaluated by consensus with two thresholds, TPS ≥ 1% (positive) and TPS < 1% (negative), despite using different PD-L1 monoclonal antibodies. It was divided into two subgroups in a positive group with cut-off points ≥ 50% and < 50%. In CheckMate 057 and CheckMate 017 trials, with a large number of enrolled patients, the authors also analyzed mPD-L1 TPS with some smaller cut-off points, such as ≥ 5% and < 5%; ≥ 10% and < 10% [3, 4]. Our study used the PD-L1 antibody 73 - 10, which was the same antibody used in the JAVELIN Lung 100 and JAVELIN Lung 200 clinical trials with the mPD-L1 positive rate was 56.4% and 66%, respectively. The rates were also quite similar and tended to be high as approximately 60%.

Currently, immunotherapy targeting PD-1/PD-L1 pathway is one of the new and effective approaches in NSCLC treatment. In Vietnam, the immune checkpoint inhibitor PD-1/PD-L1 has been approved by the Ministry of Health for NSCLC treatment. Therefore, mPD-L1 assay has been deployed in medical facilities with immunohistochemical testing systems. This is an important basis for the indications of PD-1/PD-L1 immune checkpoint inhibitor drugs. However, this is a new assay with limited data and scientific evidence. Our study adds small data on a subgroup of patients with advanced-stage NSCLC.

* *Soluble PD-L1 expression in peripheral blood:*

Up to now, the expression of PD-L1 in tumor tissue has been proven to be an important key in the indication of immune checkpoint inhibitor PD-1/PD-L1 pathway drugs in cancer treatment. It was also considered a prognostic factor for cancer patients in general and lung cancer in particular. The interaction of PD-L1 with PD-1 (negative regulatory receptor) transmits an inhibitory signal to T lymphocytes, resulting in a T-cell inactivation state. With advances in liquid biopsy techniques, the question is whether the interaction between sPD-L1 and PD-1 is similar to that of mPD-L1 with PD-1; whether the inhibitory signal is introduced with T cells and whether the prognostic significance of mPD-L1 expression in tumor tissue is similar to sPD-L1 expression in peripheral blood? It is an issue that is still being studied and discussed.

There are some initial studies demonstrating the role of sPD-L1 quantitative test in lung cancer, such as a study on 109 advanced NSCLC patients and a control group of 65 healthy people at the Beijing Cancer Hospital from January 2012 to March 2014. It showed that the sPD-L1 concentration in the two groups was 0.723 ± 0.081 ng/mL and 0.565 ± 0.048 ng/mL, respectively ($p < 0.001$). The mean overall survival was significantly higher in the low sPD-L1 group than in the high sPD-L1 group (26.8 vs. 18.7 months; $p < 0.001$) [6].

Soluble PD-L1 is also expressed in healthy people's serum and tends to increase with age. The lowest sPD-L1 concentrations were observed in children from 3 - 10 years old (0.725 ± 0.181 ng/mL) and the highest in adults from 51 - 70 years old (1.04 ± 0.681 ng/mL) [7]. In our study, the mean value of sPD-L1 concentration of advanced-stage NSCLC patients was 2.11 ± 1.48 ng/mL ($n = 50$), significantly higher than that in the control group (0.73 ± 0.57 ng/mL) ($p < 0.001$). It was also higher than the average value in healthy adults observed in previous studies [6, 8]. These results were similar to some studies, such as a study conducted by Cheng et al. on 288 NSCLC patients and a control group of 300 people, showing that the concentration of sPD-L1 in the NSCLC group was higher than that in the control group (1.92 ng/mL vs. 0.91 ng/mL; $p < 0.001$) [8]. Studies on sPD-L1 levels in NSCLC patients suggested that sPD-L1 could be used as a tumor marker with increasing sPD-L1 concentration compared with controls.

** The relationship between membrane and soluble PD-L1 expression:*

The sPD-L1 concentration in the mPD-L1 negative group (1.61 ± 1.37 ng/mL) was statistically significantly lower than in the mPD-L1 positive group (2.42 ± 1.49 ng/mL) (p = 0.03). In the mPD-L1 positive expression group, when taking the cut-off point of the 50% to divide the high (TPS ≥ 50%) and low (TPS < 50%) expression levels, the sPD-L1 concentrations in the two subgroups was non-significantly different (2.23 ± 1.41 ng/mL vs. 2.61 ± 1.59 ng/mL, p = 0.23).

Shuji Murakami et al. conducted a study to evaluate the relationship between sPD-L1 and prognosis in 223 patients with advanced or recurrent metastatic NSCLC who were treated with anti-PD-1 immunotherapy from first-line to third-line with pembrolizumab or nivolumab, at National Cancer Center Hospital, Tokyo, Japan from December 1, 2015 to August 31, 2018, showed sPD-L1 levels in 3 mPD-L1 TPS subgroups < 1%; 1 - 49%; ≥ 50% were 64.4 ± 17.5 pg/mL, 70.6 ± 18.0 pg/mL, and 77.7 ± 28.9 pg/mL, respectively (p = 0.0101), implying that higher TPS corresponds to higher sPD-L1 concentration [9]. In this study, mPD-L1 assay was carried out applying immunohistochemistry technique, using PD-L1 antibody 22C3, and sPD-L1 assay was conducted based on ELISA principle; the patients were also similar to our study.

Adrien Costantini et al. carried out a study to assess the prognostic value of biomarkers on 43 NSCLC patients treated with Nivolumab and found no relationship between sPD-L1 levels and mPD-L1 expression levels. There was no significant difference between sPD-L1 levels in patients with mPD-L1 positive and negative (30.86 pg/mL (IQR: 22.13 - 48.23) versus 36.68 pg/mL (IQR: 26.10-55.34); p = 0.604) [10].

There was a limited number of simultaneous studies of both mPD-L1 and sPD-L1. The initial result showed a trend towards higher sPD-L1 concentrations in the group with high mPD-L1 expression.

CONCLUSION

62% of lung cancer tissue samples were positive for mPD-L1 (TPS ≥ 1%), of which 51.6% were highly positive and 48.4% low positive. The concentration of sPD-L1 in the NSCLC group was statistically significantly higher than that of the control group (2.11 ± 1.48 versus 0.73 ± 0.57 ng/mL; p < 0.001). sPD-L1 concentration in the mPD-L1 negative group (1.61 ± 1.37 ng/mL) was statistically significantly lower than in the mPD-L1 positive group (2.42 ± 1.49 ng/mL) (p = 0.03).

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