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Original Article

Association between PD-L1 and Ki-67 expression and clinicopathologic features in NSCLC patients

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Abstract: Objective: To investigate the relationship between PD-L1, Ki-67 and the association between PD-L1, Ki-67, and clinicopathologic features and laboratory parameters in the peripheral blood of non-small cell lung cancer (NSCLC) patients. Methods: The clinical records of 213 NSCLC patients were retrospectively reviewed. The patients were divided into high or low expression groups by cut-off values of Ki-67 and PD-L1. Correlation of PD-L1 and Ki-67 expression were analyzed by linear regression analysis. The data were tested by Mann-Whitney test. Relationship of PD-L1 and Ki-67 was tested by chi-square test. Results: Linear regression analysis revealed a positive association between the expression of PD-L1 and Ki-67 (R^2=0.26, P<0.001). The clinicopathologic features and laboratory parameters such as gender, smoking history, histological types, TNM stage, tumor size, ALB, and FIB were all significantly associated with PD-L1 and Ki-67 expression (all P<0.05). Conclusions: The expression of PD-L1 and Ki-67 is related to some clinicopathologic features and inflammatory factors, which brings new sight for exploiting combination biomarkers and therapeutic strategies.

Keywords: Non-small cell lung cancer, PD-L1, Ki-67, inflammation, proliferation

Introduction

Lung cancer is a leading cause of cancer-associated mortality globally [1, 2]. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases [3]. NSCLC is divided into squamous cell carcinoma and non-squamous cell carcinoma, and lung adenocarcinoma is the primary histologic type in non-squamous cell carcinoma and the most prevalent type of lung cancer [4]. Conventional treatments, including radiotherapy, platinum-based chemotherapy, and surgical resection, have limited therapeutic effects [5]. However, with increased understanding of immune checkpoint inhibitors, antibodies against PD-1/PD-L1 in combination with or without platinum-based chemotherapy have been conducted to treat patients with lung cancer at advanced stages [6]. While most studies suggest that PD-1/PD-L1 inhibitors can benefit patients with advanced lung cancer [7], immune-related adverse events [8], drug resistance [9], and poor prognosis have led to an ongoing search for effective PD-1/PD-L1 inhibitors. Several factors, including PD-L1 expression and smoking status, have been suggested to be associated with efficacy.

PD-L1, a type I transmembrane protein, is expressed not only on cancer cells but also on various types of cells present in the tumor microenvironment (TME), including macrophages, dendritic cells (DCs), activated T cells, and cancer-related fibroblasts [10]. It is widely known that PD-L1 plays a significant role in helping tumor cells evade immune surveillance by binding to its receptor PD-1 on activated T cells, thus inhibiting the T cell-based immune system, which recognizes and eliminates abnormal cells [11]. The FDA has approved PD-L1 testing for several tumors, including NSCLC [12]. PD-L1 is valuable in selecting patients suitable for immunotherapy, providing prognostic information, and assessing the efficacy of immunotherapy, although the reliability of the provided information remains controversial [13]. Ki-67, a non-histone DNA-binding nuclear protein, which is expressed in all cell cycle phases except the G_0 phase [14], has become a common proliferative marker in clinical practice [15].

The interaction between tumor cells and the immune system plays a crucial role in tumor development, with chronic inflammation triggered by the immune system driving tumor initiation, promotion, metastasis, and angiogenesis. Therefore, it is essential to assess the inflammatory status of patients with tumors [16]. In addition to inflammation in the tumor microenvironment, systemic inflammation in peripheral circulation is also a critical element of cancer-associated inflammation [17]. Peripheral blood biomarkers have become an attractive research focus given their convenience, simplicity, affordability, and ease of collection via routine blood draws [18]. Some studies have suggested that peripheral blood inflammatory markers have diagnostic or prognostic value in certain types of cancer [19]. PD-L1 expression is regulated by multiple inflammatory pathways [20], but few studies have examined the association between PD-L1 expression and systemic inflammatory markers in NSCLC until recently.

On the basis of the abovementioned research, this study is conducted to investigate the correlation between PD-L1, ki-67 expression and clinicopathological factors, peripheral blood inflammatory markers, and the relationship between PD-L1 and ki-67 through data collection and retrospective analysis.

Materials and methods

Study population

This study was approved by the ethical committee of First Affiliated Hospital of Nanchang University (Jiangxi Province, China), and data of 213 NSCLC patients were collected from the hospital between April 2016 and October 2021 upon confirmation of their histological or cytological diagnosis.

Data collection

Medical records were used to obtain laboratory parameters, including White blood cell (WBC), Hemoglobin (HGB), Lymphocyte, Monocyte, Neutrophil, Fibrinogen (FIB), Albumin (ALB), Total cholesterol (TC) and Total bilirubin (T-BIL), as well as patient characteristics, such as gender, age, smoking history, pathologic types, lymph node metastasis, and organ metastasis, for this retrospective analysis. Tumor stage was determined based on the 8th edition of the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) classification system. The PD-L1 and Ki-67 expression in paraffin-embedded NSCLC tissues stored in Department of Pathology, First Affiliated Hospital of Nanchang University, were detected by immunohistochemistry. Hot-spot area was determined under low-power field for Ki-67 and PD-L1 assessment. Then 1000 cells were counted under high-power field and the percentage of nuclear-positive cells was calculated.

Statistical methods

Continuous variables with non-normal distribution were performed as the median (IQR), and counted variables were expressed as percentages. The Mann-Whitney U test was utilized to examine the connection between laboratory parameters and the expression of PD-L1 and KI-67 proteins. Spearman's rank correlation was conducted to analyze the correlation of continuous variables, such as laboratory parameters, and ordinal categorical variables. The Chi-square test was utilized to evaluate the independence between clinicopathologic factors and PD-L1 and KI-67 expressions. PD-L1 and KI-67 expressions were analyzed as categorical variables with cut-off values of 50% and 25%, respectively [21, 22]. The statistical analysis was performed by using SPSS software version 26.0 (SPSS Inc., Chicago, IL, USA), and Graph Pad Prism 9.0 was used to create the graphs. Two-tailed P-value ≤ 0.05 was considered significant.

Result

Our study evaluated a total of 213 NSCLC patients, consisting of 146 males and 67 females, with a mean age of 63.84 years old. All tissue types were NSCLC, with adenocarcinoma accounting for 60.6%, squamous carcinoma for 28.2%, and the rest with no specific type indicated. Organ metastases accounted for 1.4%, and lymph node metastases for 54.0% of the total sample. The median PD-L1 expression was 40%, whereas median Ki-67 expression was 60%. Baseline patient characteristics are described in **Table 1**. Of the total samples, 100 cases were assessed as PD-L1-high expression, and 171 were Ki-67 high expression. Compared to the PD-L1 low expres-

Table 1. Baseline patient characteristics

Factor	Value
Age (years)	
Range	37-90
Mean	63.84
Gender	
Male	146 (68.5%)
Female	67 (31.5%)
Lymph node metastasis	
Yes	115 (54.0%)
No	98 (46.0%)
Tumor size (cm)	
≤3	99 (46.5%)
3-5	63 (29.6%)
5-7	34 (16.0%)
>7	17 (7.9%)
Tumor node metastasis staging	
I	71 (33.3%)
II-IV	142 (66.7%)
Histology	
Squamous carcinoma	60 (28.2%)
Adenocarcinoma	129 (60.6%)
Others	24 (11.2%)
Smoking status	
Current	68 (32.0%)
Never	116 (54.5%)
Former	29 (13.5%)
Organ metastases	
Yes	3 (1.4%)
No	210 (98.6%)
PD-L1 Median (%)	40 (10-80)
Ki-67 Median (%)	60 (30-70)

sion group, the PD-L1 high expression group showed significant differences in their circulating median concentrations, with higher FIB (3.84 vs 3.10) (P=0.003), lower ALB (40.35 vs 41.9) (P=0.003), and lower T-BIL (7.95 vs 9.70) (P=0.001), respectively. Similarly, compared to the Ki-67 low expression group, the Ki-67 high expression group showed significant differences in their circulating median concentrations, with higher FIB (3.59 vs 2.96) (P=0.001), lower ALB (40.7 vs 42.10) (P=0.02), and lower T-BIL (8.60 vs 9.70) (P=0.027), respectively, as shown in **Table 2**.

To further explore the potential relationship between laboratory parameters and PD-L1 and Ki-67 levels, we used PD-L1 as a categorical variable and laboratory parameters as continuous variables to perform the Spearman correlation analysis. We found positive correlations between PD-L1 expression and FIB (r=0.202; P=0.003), D-D (r=0.137; P=0.045), but negative correlations with ALB (r=-0.204; P=0.003) and T-BIL (r=-0.221; P=0.001), and positive correlation between Ki-67 expression and FIB (r=0.235; P=0.001), whereas negative correlations with ALB (r=-0.161; P=0.019) and T-BIL (r=-0.151; P=0.027), as shown in **Table 3**.

Table 4 displays the significant correlations between higher expression of PD-L1 with male gender, higher stage (stage II-IV), the histological type of squamous cell carcinoma, and smoking, but no obvious relationship was found between age, lymph node status, previous hypertension, or diabetes. Higher expression of Ki-67 was significantly associated with male gender, larger tumor (≥5 cm) diameter, higher stage (stage II-IV), and histological type of squamous, but no association was found between age, lymph node status, previous hypertension or diabetes. Furthermore, in **Figure 1**, we analyzed the relationship between PD-L1 and Ki-67 by linear regression and found a significant correlation (R^2=0.26, P<0.001).

Disscusion

PD-1/PD-L1 inhibitors have revolutionized the treatment of lung cancer, a disease that poses a significant threat to human health worldwide. However, the differential response of patients receiving PD-1/PD-L1 inhibitors has prompted the development of relevant biomarker studies. Among these, the study of PD-L1 expression based on the interaction between PD-L1 and PD-1 has gained significant attention [23]. The level of PD-L1 expression is dynamic, and several mechanisms influence its expression, including oncogenic activating mutations (e.g., KRAS), activation of receptor complex kinases (e.g., EGFR, ALK), important signaling pathways (e.g., PI3K/ALK/mTOR), abnormal tumor suppressors (e.g., P53), some abnormal epigenetic alterations, and inflammatory signals, cytokines (e.g., IFN-γ), growth factors (e.g., EGFR, TGF-β), hypoxia, and post-translational modifications [24].

In addition to the mechanisms mentioned above, the differences in PD-L1 IHC expression rates arise from the use of various antibody clones (22C3, 22-8, SP142, and SP263), different evaluation criteria, cut-off values, and spe-

Table 2. Ki-67 and PD-L1 and laboratory values

	Ki-67		P	PD-L1		P
	≤25%	>25%		<50%	≥50%	
WBC	6.07 (5.44-7.85)	6.49 (5.19-8.04)	0.403	6.21 (5.05-7.49)	6.89 (5.30-8.81)	0.138
HGB	129.0 (118.3-143.0)	132.0 (117.0-140.5)	0.997	131.0 (112.0-140.5)	132.0 (118.3-141.0)	0.373
Lymphocyte	1.65 (1.20-1.99)	1.57 (1.25-1.90)	0.644	1.54 (1.26-1.92)	1.59 (1.24-1.90)	0.937
Monocyte	0.41 (0.34-0.63)	0.50 (0.37-0.68)	0.061	0.46 (0.35-0.61)	0.55 (0.37-0.72)	0.081
Neutrophil	3.88 (2.99-5.27)	4.25 (3.14-5.41)	0.315	3.10 (2.67-3.84)	4.29 (3.40-6.19)	0.084
FIB	2.96 (2.59-3.43)	3.59 (2.78-4.70)	0.001	3.10 (2.67-3.84)	3.84 (2.71-4.83)	0.003
ALB	42.10 (40.40-44.10)	40.7 (37.00-43.90)	0.02	41.9 (38.9-44.4)	40.35 (35.98-42.80)	0.003
T-BIL	9.70 (8.40-13.08)	8.60 (6.15-12.90)	0.027	9.70 (7.50-13.40)	7.95 (5.70-11.40)	0.001
TC	4.65 (4.10-5.05)	4.47 (3.85-5.13)	0.186	4.54 (4.01-5.06)	4.44 (3.76-5.14)	0.247

Abbreviations: WBC, White blood cell; HGB, Hemoglobin; FIB, Fibrinogen; ALB, Albumin; T-BIL, Total bilirubin; TC, Total cholesterol.

Table 3. Correlation between laboratory parameters and PD-L1 and Ki-67

Analyte	Ki-67		PDL-1	
	Spearman's P	*P*-value	Spearman's P	*P*-value
WBC	0.058	0.404	0.102	0.139
HGB	0.000	0.997	0.061	0.374
Lymphocyte	-0.032	0.646	0.005	0.937
Monocyte	0.129	0.061	0.120	0.081
Neutrophil	0.069	0.316	0.119	0.084
FIB	0.235	0.001	0.202	0.003
ALB	-0.161	0.019	-0.204	0.003
T-BIL	-0.151	0.027	-0.221	0.001
TC	-0.091	0.187	-0.080	0.248

Abbreviations: WBC, White blood cell; HGB, Hemoglobin; FIB, Fibrinogen; ALB, Albumin; T-BIL, Total bilirubin; TC, Total cholesterol.

cific biopsy samples. According to the tumor proportion score (TPS) classification criteria used in this study, the rate of PD-L1 high expression (≥50%) was 46.9%, which is slightly higher than the rates reported in other studies [25, 26]. This finding might be due to ethnicity [27] or the lack of negative cases.

Our study revealed that high PD-L1 expression was significantly associated with squamous cell carcinoma (SCC), male gender, smoking history, high stage (II-IV), and larger tumor size. These results are consistent with previous research on gender, habitus, and tumor stage [28]. However, there is a contradiction in the histological type, as some studies suggest high expression on squamous carcinomas, while others indicate high expression on adenocarcinomas [29, 30]. Furthermore, most of the articles reported significantly higher PD-L1 expression increased tumor proliferation and aggressiveness as well as shorter patient survival in NSCLC [31].

Our study revealed a positive correlation between PD-L1 expression and fibrinogen (FIB) and a negative correlation between PD-L1 expression and albumin (ALB) in the peripheral blood. Tumor microenvironments consist of immune cells, cancer cells, and cytokines that contribute to cancer development and progression. In this context, immune cells, such as T-cells and NK cells, release IFN-γ, which activates the JAK-STAT pathway to promote PD-L1 expression [32]. Meanwhile, pro-inflammatory cytokines such as TNF/IL-6 are released as a part of tumor-associated systemic inflammation [33]. TNF-α increases capillary permeability, leading to decreased plasma albumin levels [34], while IL-6 is an inflammatory mediator that promotes tumor growth and affects albumin synthesis by hepatocytes. As a result, low plasma albumin levels ensue, and the liver produces fibrinogen, an acute-phase protein, which increases plasma FIB levels [35, 36]. The IL-6-MEK/ERK signaling pathway also promotes the upregulation of PD-L1 expression [37]. It is well-established that FIB is an important determinant of metastatic potential, and low serum albumin reflects poor nutritional status [38, 39]. The fibrinogen-albumin ratio (FAR) has been proposed as a new prognostic factor in advanced NSCLC patients [40]. The laboratory values are closely associated with elevated PD-L1 expression of NSCLC.

High Ki-67 levels have been associated with a poor clinical course, including invasion, infiltra-

Table 4. Expression of PD-L1 according to clinical data

Clinicopathologic Data	NSCLC					
	Ki-67 Expression		*P*-Value	PDL-1 Expression		*P*-Value
	≤25%	>25%		<50%	≥50%	
Age			0.671			0.315
≤60	15	57		43	29	
>60	33	108		74	67	
Sex			0.001			<0.001
Male	23	123		68	79	
Female	25	42		49	18	
Lymph node			0.179			0.182
N0	30	85		49	49	
N1-N3	18	80		68	47	
Tumor size			<0.001			0.002
T1	36	63		68	31	
T2	9	54		27	36	
T3	3	31		30	18	
T4	0	17		6	11	
Stage			0.001			<0.001
I	26	45		51	20	
II-IV	22	120		66	76	
Histology type			<0.001			0.002
AC	43	88		24	36	
SCC	4	56		84	46	
Other and Unclassified carcinomas	1	21		9	14	
Smoking status			<0.001			0.002
Yes	10	87		42	55	
No	38	78		75	41	
Drinking status			0.173			0.079
Yes	5	37		18	24	
No	43	128		99	72	
Hypertension			0.929			0.359
Yes	9	30		24	15	
No	39	135		93	81	
Diabetes			1.000			0.135
Yes	3	12		5	9	
No	45	153		112	87	
Ki-67						<0.001
high				76	90	
low				41	6	
PDL-1			<0.001			
high	10	89				
low	38	76				

NSCLC, Non-small cell lung cancer; SCC, squamous cell carcinoma.

tion, and metastasis [15], PD-L1 provides tumor cells with resistance by aiding in their escape from host immune surveillance, leading to tumor angiogenesis, proliferation, and invasion [41]. It has been revealed that cell proliferation is one of the direct factors in PD-L1 expression in breast cancer patients [42].

It has been reported that the prognostic value of PD-L1 expression may be impacted by NLR,

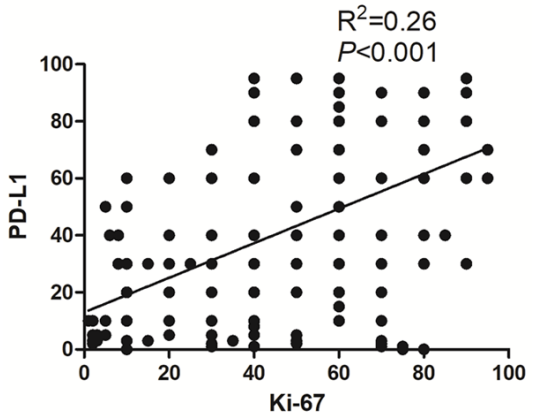

Figure 1. PD-L1 and Ki-67 correlation.

a biomarker that reflects systemic inflammatory state [43]. The influence of T cells with PD-L1 expression can be divided into two types: first, as the receptor of PD-L1, PD-1 is expressed on T cells and establishes a direct link with tumor cells through the PD-1/PD-L1 axis; second, as immune cells, T cells release inflammatory factors to regulate PD-L1 expression [44]. Furthermore, the consistency between local and systemic markers of inflammation has been demonstrated to some extent in previous studies [45]. Our experiment, consistent with the results of a study in lung cancer [46] did not find a relationship between PD-L1 expression and immune cells such as neutrophils and lymphocytes in peripheral blood. However, the association between PD-L1 and NLR has been reported in other tumors such as esophageal, liver, and bile duct cancers. Therefore, further clinical trials are necessary to explore the relationship between PD-L1 and peripheral blood immune cells.

There are several limitations of this study. As the data was retrospectively collected from a single clinical center, there may be inherent bias in our observations, particularly in the enrolled patients. Furthermore, our collected data lacks samples from small cell and large cell lung cancer subtypes, negative samples in terms of PD-L1 expression, and the detection of tumor driver mutations such as KRAS, EGFR, ALK, or TP53. It is also challenging to distinguish between active infection and chronic tumor inflammation and determine the reasons for the statistical significance of laboratory data reflecting inflammation in different groups. There is currently no direct mechanism that can demonstrate the link between PD-L1 expression and inflammatory parameters in peripheral blood. Additionally, PD-L1 expression was measured from surgically resected samples, and some advanced stage patients underwent a period of radiotherapy or chemotherapy before surgery, which could have resulted in altered PD-L1 expression and errors in the analyzed results.

In conclusion, this study has revealed the association between PD-L1 expression and clinicopathological, inflammatory, and proliferative factors and provided new insights into the development of combination markers and better therapeutic strategies for NSCLC patients in the future.

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Informed consent was obtained from all individual participants included in the study.

Disclosure of conflict of interest

None.

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