

Positive PD-L1 expression is predictive for patients with advanced EGFR wild-type non-small cell lung cancer treated with gemcitabine and cisplatin

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Abstract. This retrospective study aimed to investigate the association between programmed death ligand-1 (PD-L1) expression and the clinicopathological characteristics of patients with advanced epidermal growth factor receptor (EGFR) wild-type non-small cell lung cancer (NSCLC). The predictive role and cut-off value of PD-L1 expression was subsequently investigated. A total of 172 patients with advanced EGFR wild-type NSCLC were enrolled. All patients received platinum-based doublet chemotherapy (gemcitabine plus cisplatin). PD-L1 expression in lung tissues was assessed using immunohistochemical methods. The χ^2 test was used to analyze the association between PD-L1 expression and clinicopathological characteristics. Survival time analysis was performed using the Kaplan-Meier method. The two groups, positive PD-L1 expression and negative PD-L1 expression, were compared using the log-rank test. Multivariate analysis using the Cox proportional hazard regression model was conducted to determine prognostic factors for overall survival (OS) and progression-free survival (PFS) times. Positive PD-L1 expression was observed in 48.3% (84/172), 40.7% (70/172), 21.5% (37/172) and 8.1% (14/172) of patients when using cut-off values of 1, 5, 10 and 50%, respectively. The χ^2 test revealed that elevated pretreatment C-reactive protein (CRP) level and cancer stage IV were significantly associated with positive PD-L1 expression. The OS and PFS of positive PD-L1 (1, 5, 10 and 50% cut-off) expression group were shorter compared with the negative PD-L1 (1, 5, 10 and 50% cut-off) expression group. Multivariate survival analysis revealed that PD-L1 expression $\geq 50\%$ was significantly associated with decreased OS and PFS [OS time, $P=0.001$; hazard ratio (HR), 2.768; 95% confidence interval (CI), 1.551-4.940; PFS time, $P=0.002$;

HR, 2.537; 95% CI, 1.423-4.524]. These results indicated that positive PD-L1 (50% cut-off) expression was an independent predictor of poor prognosis for patients with advanced NSCLC treated with gemcitabine plus cisplatin. PD-L1 expression was associated with CRP level and cancer stage. The results obtained in the present study suggest that positive PD-L1 expression serves a prognostic role in advanced NSCLC and that the optimal cut-off value may be 50%.

Introduction

Lung cancer has been the leading cause of cancer-associated mortality worldwide in males (24%) and females (23%) in 2019 (1). Non-small cell lung cancer (NSCLC) accounts for 85% of all types of lung cancer (2). Platinum-based chemotherapy is the standard treatment for patients with advanced epidermal growth factor receptor (EGFR) wild-type NSCLC (3). Gemcitabine was approved as a first-line treatment for advanced NSCLC (4-6). However, the clinical outcome for patients with advanced stage NSCLC remains poor, and novel effective treatment strategies are required (7).

Immune checkpoint inhibitors have yielded promising results in NSCLC. Programmed death ligand-1 (PD-L1) is an important target for immunotherapy. Previous studies have revealed that PD-L1 expression may be a predictor of treatment response (8,9). High expression of PD-L1 was associated with the presence of EGFR mutations (10-12). Activating mutations of EGFR also induced PD-L1 expression in NSCLC, and EGFR tyrosine kinase inhibitors downregulated PD-L1 expression in EGFR mutation-positive NSCLC (13-15). However, the predictive value of PD-L1 expression in patients with EGFR wild-type NSCLC remains unclear. Furthermore, different chemotherapy regimens may affect the clinical outcome (16,17). Therefore, the aim of the current retrospective study was to analyze PD-L1 expression in patients with advanced EGFR wild-type NSCLC treated with gemcitabine plus cisplatin and to potentially determine the cut-off value of PD-L1 expression.

Materials and methods

Patients. A total of 172 eligible patients were enrolled in the current study between August 2011 and December 2017 at The

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First Affiliated Hospital of Zhengzhou University (Zhengzhou, China). The inclusion criteria were as follows: i) Histologically confirmed diagnosis of NSCLC based on the WHO classification (18); ii) newly diagnosed with cancer stage IIIB or IV; iii) ≥ 18 and ≤ 80 years of age; iv) European Cooperative Oncology Group (ECOG) performance status (PS) 0-2; v) EGFR wild-type; vi) measurable disease according to revised Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (19); vii) adequate hematological, hepatic and organ function; and viii) adequate clinicopathological information and follow-up data. The exclusion criteria were as follows: i) Uncontrolled brain metastases; ii) autoimmune disease; iii) previous malignant tumor or second primary tumor; and iv) prior treatment with chemotherapy, radiotherapy or immunotherapy. Clinicopathological characteristics were recorded for each patient, including patient demographics, histology, EGFR status, pretreatment serum C-reactive protein (CRP) level, date of diagnosis, imaging of the involved region, cancer stage, ECOG PS, smoking status, chemotherapy schedule, treatment response, PD-L1 expression, overall survival (OS) and progression-free survival (PFS) times. OS was measured from the date of the initial therapy until the last follow-up. PFS was measured from the date of the initial treatment until the date of disease progression or death from any cause. Patients were followed up at a median duration of 9 months (range, 2-25 months). The current study was approved by The Ethics Committee of The First Affiliated Hospital of Zhengzhou University, and written informed consent was obtained from all enrolled patients. All experiments were performed in accordance with approved guidelines and regulations (20,21).

PD-L1 expression. Pretreatment lung cancer tumor tissue was collected for PD-L1 analyses. PD-L1 expression was retrospectively assessed in tumor biopsies using immunohistochemical methods. Sections (4- μ m-thick) from each formalin-fixed (10% formaldehyde at 20°C for 24 h) paraffin-embedded tissue were used, followed by the modified avidin-biotin complex method (Envision method) using an automated immunostainer (model no. 314683; Ventana Medical Systems, Inc., Tucson, AZ, USA) (22). A rabbit antihuman PD-L1 antibody (ready-to-use; cat. no. ZA-0629; OriGene Technologies Inc., Beijing, China) was used to detect PD-L1, and was incubated for 40 min at 37°C. The tissues were then incubated with the horseradish peroxidase-anti-rat IgG secondary antibody (ready-to-use; cat. no. 760-500; Roche, Basel, Switzerland) for 8 min at 37°C. The sections were counterstained with hematoxylin at 37°C for 4 min and then mounted. Images were taken using a light microscope and analyzed using HistoQuest software (version 6.0; TissueGnostics, Vienna, Austria) for an automated measurement. PD-L1 expression was defined by tumor cell membrane expression levels, and classified according to prespecified levels (≥ 1 , ≥ 5 , ≥ 10 and $\geq 50\%$) (23-25).

Treatment and response. Eligible patients received platinum-based doublet chemotherapy (gemcitabine 1,000 mg/m² on days 1 and 8; cisplatin 25 mg/m² on days 1, 2 and 3, repeated every 3 weeks; both from Hanson Pharma, Lianyungang, China). This treatment is the standard of care for managing patients with advanced NSCLC in China (7). Assessment of

treatment response was based on the RECIST version 1.1 guidelines (19).

Statistical analysis. The association between PD-L1 expression and clinicopathological characteristics was analyzed using the χ^2 test. Survival curves and rates were estimated using the Kaplan-Meier method and groups were compared using the log-rank test. Multivariate analysis using Cox proportional hazard regression model was performed to evaluate the prognostic and predictive role of PD-L1 expression. The hazard ratio (HR) and 95% confidence interval (CI) were estimated using a stratified Cox proportional hazards model. Statistical analyses were performed using GraphPad Prism software (version 6; GraphPad Software Inc., La Jolla CA, USA) and SPSS software (version 21.0; IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. The clinicopathological characteristics of the enrolled patients are presented in Table I. Among 172 patients, positive PD-L1 expression was observed in 48.3% (84/172), 40.7% (70/172), 21.5% (37/172) and 8.1% (14/172) of patients when using cut-off values of 1, 5, 10 and 50%, respectively.

Association of PD-L1 expression with clinicopathological characteristics. The χ^2 test revealed that elevated pretreatment serum CRP (≥ 10 mg/l) was significantly associated with positive PD-L1 expression for 1, 5, 10 and 50% cut-off values ($P = 0.001$, 0.001, 0.001 and 0.008, respectively). Similarly, stage IV cancer was significantly associated with positive PD-L1 expression for 1, 5, 10 and 50% cut-off values ($P = 0.001$, 0.001, 0.001 and 0.018, respectively; Table I). These data suggested that PD-L1 expression was associated with pretreatment serum CRP level and cancer stage.

Survival time. To illustrate the prognostic value of PD-L1, the association between PD-L1 expression and OS and PFS times was determined. At the median follow-up duration of 9 months, the one-year OS and PFS times of all patients were 43.3 and 22.0% respectively. Positive PD-L1 expression group was significantly associated with shorter OS and PFS times compared with negative PD-L1 expression group (Fig. 1). The one-year OS time for positive PD-L1 expression group were shorter than negative PD-L1 expression group (36.4 vs. 50% at 1% cut-off; 35.4 vs. 49.0% at 5% cut-off; 33.2 vs. 46.5% at 10% cut-off; and 7.1 vs. 46.2% at 50% cut-off, respectively). The one-year PFS time for positive PD-L1 expression group were also shorter than negative PD-L1 expression group (16.4 vs. 27.6% at 1% cut-off; 16.0 vs. 25.8% at 5% cut-off; 11.5 vs. 24.6% at 10% cut-off; and 7.0 vs. 23.3% at 50% cut-off, respectively; Fig. 2). Representative immunohistochemical staining images of tumor biopsies with PD-L1 were shown in Figure 3. Univariate survival analysis revealed that cancer stage IV, positive PD-L1 (1% cut-off) expression, ECOG PS 2 and age ≥ 60 were significantly associated with shorter OS and PFS times ($P < 0.0001$, 0.0481, 0.0050 and < 0.0001 for OS time; $P < 0.0001$, 0.0035, 0.0278 and 0.0010 for PFS time, respectively; Table II).

Table I. Association of PD-L1 expression and clinicopathological characteristics (n=number of patients).

Variables	No. of patients (%)	PD-L1 (1% cut-off) (n)			PD-L1 (5% cut-off) (n)			PD-L1 (10% cut-off) (n)			PD-L1 (50% cut-off) (n)		
		Positive (84)	Negative (88)	P-value	Positive (70)	Negative (102)	P-value	Positive (37)	Negative (135)	P-value	Positive (14)	Negative (158)	P-value
Gender				0.304			0.645			0.947			0.776
Female	55 (32.0)	30	25		21	34		12	43		4	51	
Male	117 (68.0)	54	63		49	68		25	92		10	107	
Age (years)				0.281			0.035			0.071			0.124
<60	83 (48.3)	37	46		27	56		13	70		4	79	
≥60	89 (51.7)	47	42		43	46		24	65		10	79	
Histology				0.634			0.905			0.757			0.568
Adenocarcinoma	122 (70.9)	61	61		50	72		27	95		9	113	
Non-adenocarcinoma	50 (29.1)	23	27		20	30		10	40		5	45	
Stage				0.001			0.001			0.001			0.018
IIIB	89 (51.7)	32	57		22	67		12	77		3	86	
IV	83 (48.3)	52	31		48	35		25	58		11	72	
ECOG PS				0.706			0.291			0.368			0.160
0-1	104 (60.5)	52	52		39	65		20	84		6	198	
2	68 (39.5)	32	36		31	37		17	51		8	60	
Smoking status				0.551			0.908			0.812			0.856
Former or current smoker	82 (47.7)	42	40		33	49		17	65		7	75	
Non-smoker	90 (52.3)	42	48		37	53		20	70		7	83	
CRP				0.001			0.001			0.001			0.008
Elevated	66 (38.4)	43	23		37	29		23	43		10	56	
Normal	106 (61.7)	41	65		33	73		14	92		4	102	

PD-L1, programmed death ligand-1; ECOG, European Cooperative Oncology Group; PS, performance status; CRP, C-reactive protein.

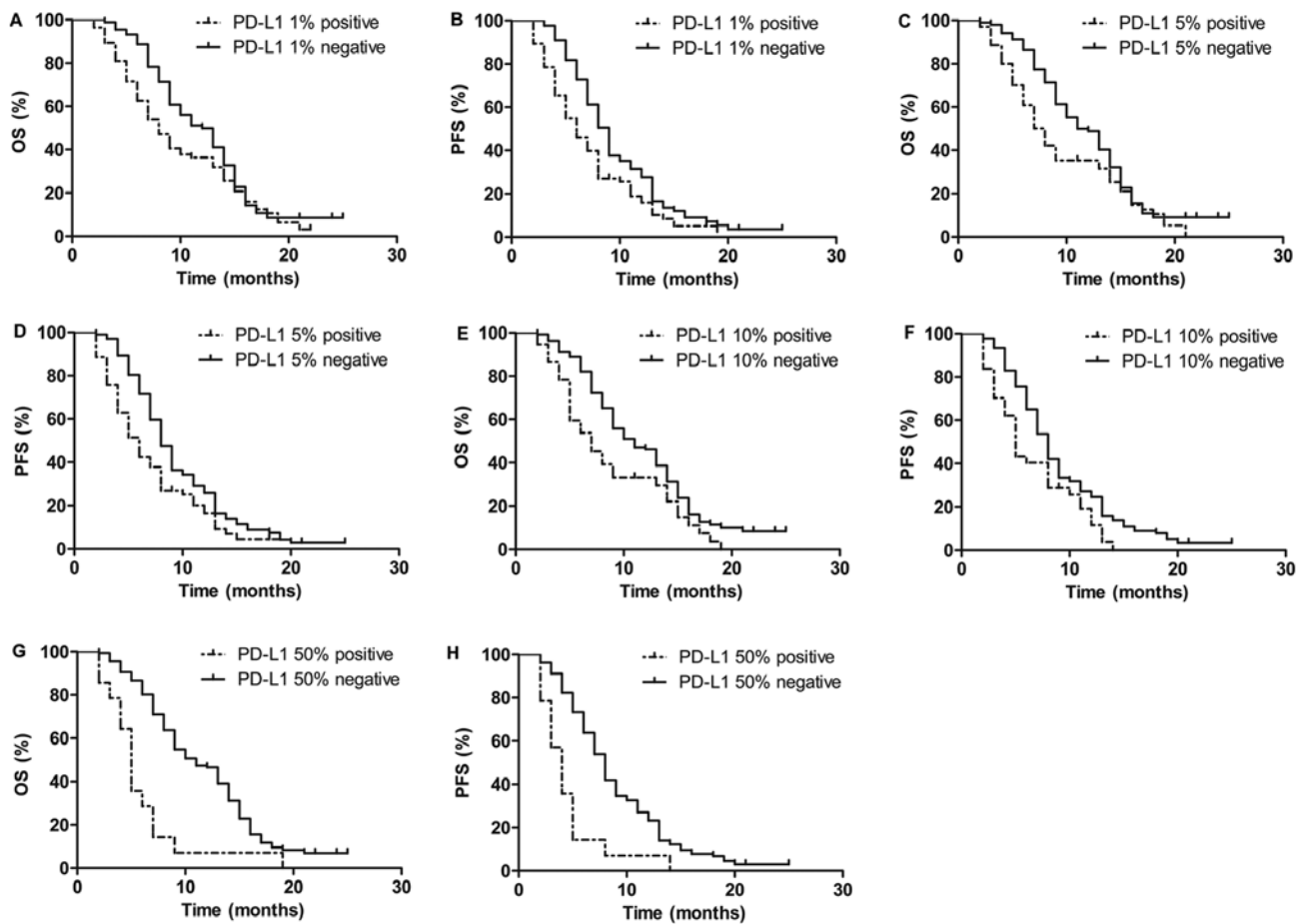


Figure 1. Kaplan-Meier survival curves of OS and PFS times according to PD-L1 expression in patients with advanced non-small cell lung cancer. OS time for the (A) 1%, (C) 5%, (E) 10% and (G) 50% cut-off. PFS time for the (B) 1%, (D) 5%, (F) 10% and (H) 50% cut-off. OS, overall survival; PFS, progression-free survival; PD-L1, programmed death ligand-1.

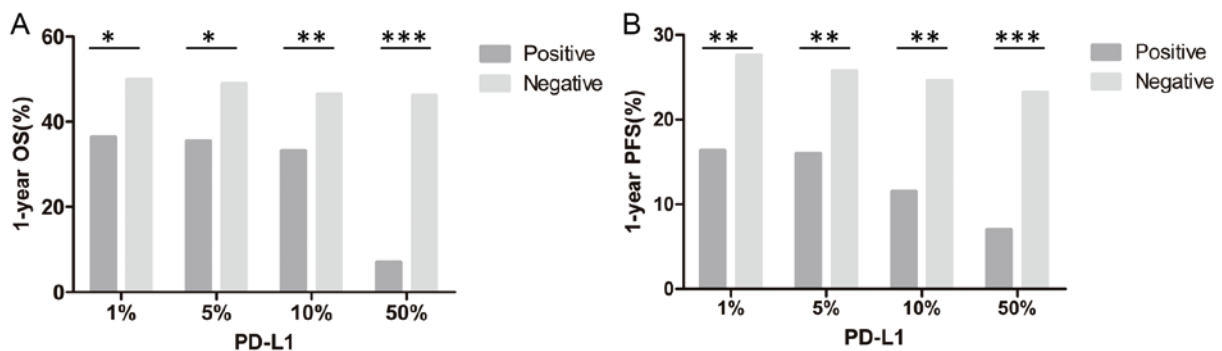


Figure 2. Comparison of one-year OS and PFS times according to PD-L1 expression. (A) Comparison of one-year OS time between patients with positive PD-L1 expression and patients with negative PD-L1 expression (defined as positive, \geq cut-off; and negative, $<$ cut-off). (B) Comparison of one-year PFS time between patients with positive PD-L1 expression and patients with negative PD-L1 expression. * $P<0.05$; ** $P<0.01$; *** $P<0.001$. OS, overall survival; PFS, progression-free survival; PD-L1, programmed death ligand-1.

These results indicated that positive PD-L1 (1% cut-off) expression predicted a shorter survival time.

Prognostic significance of PD-L1 expression cut-off values. To investigate the cut-off value of PD-L1 expression, the association between survival and PD-L1 expression at 1, 5, 10 and 50% levels was investigated. Univariate survival analysis revealed that positive PD-L1 expression for 1, 5, 10

and 50% cut-off values was significantly associated with shorter OS and PFS times (OS time: $P=0.0481$, 0.0212 , 0.0068 and <0.0001 , respectively; PFS time: $P=0.0035$, 0.0044 , 0.0051 and <0.0001 , respectively; Fig. 1 and Table III). All parameters that were statistically significant according to the univariate analysis were included in the multivariate analysis, which demonstrated that positive PD-L1 (50% cut-off) expression was significantly associated with shorter survival time

Table II. Univariate and multivariate analysis of the association between clinicopathological characteristics and survival.

Variable	Category	OS			PFS		
		Univariate analysis P-value	Multivariate analysis		Univariate analysis P-value	Multivariate analysis	
			HR (95% CI)	P-value		HR (95% CI)	P-value
Gender	Male	0.094			0.681		
Age	≥60	<0.0001	1.537 (1.067-2.213)	0.021	0.0010	1.298 (0.915-1.840)	0.144
Histology	Non-adenocarcinoma	0.5878			0.5422		
Stage	IV	<0.0001	1.700 (1.187-2.434)	0.004	<0.0001	1.860 (1.299-2.665)	0.001
ECOG PS	2	0.0050	1.346 (0.937-1.935)	0.108	0.0278	1.390 (0.971-1.988)	0.072
Smoking status	Non-smoker	0.8710			0.7849		
CRP	Elevated	0.6622			0.1117		
PD-L1 (1% cut-off)	Positive	0.0481	1.125 (0.783-1.617)	0.524	0.0035	1.266 (0.884-1.813)	0.199

OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; PD-L1, programmed death ligand-1; ECOG, European Cooperative Oncology Group; PS, performance status; CRP, C-reactive protein.

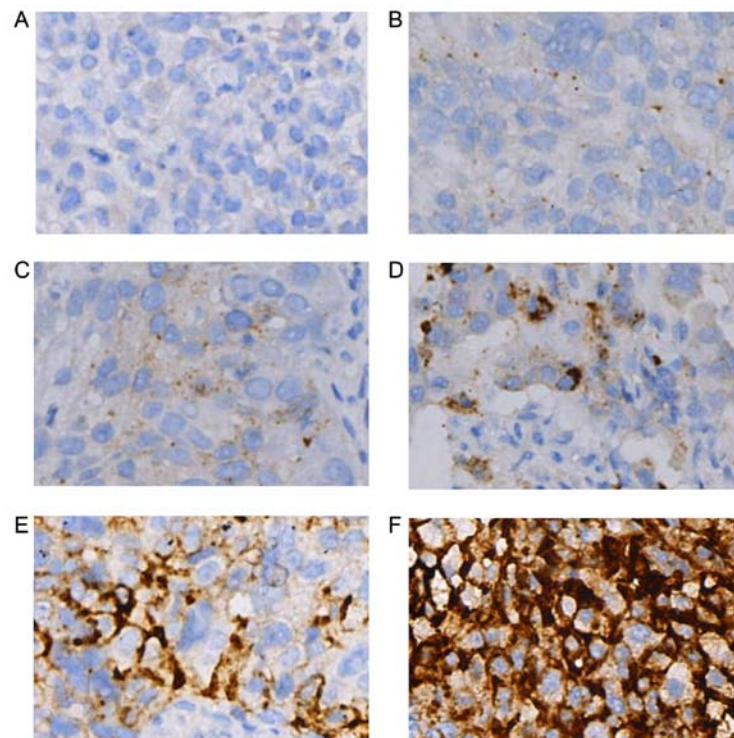


Figure 3. Representative immunohistochemical staining images of tumor biopsies with PD-L1. (A) Negative control. (B) 1% positive. (C) 5% positive. (D) 10% positive. (E) 50% positive. (F) 100% positive. All images were captured at x20 magnification.

(OS time, $P=0.001$; HR=2.768, 95% CI, 1.551-4.940; PFS, $P=0.002$; HR=2.537, 95% CI, 1.423-4.524; Table III). These results suggested that positive PD-L1 (50% cut-off) expression was an independent predictor of poor prognosis for patients with advanced NSCLC treated with gemcitabine plus cisplatin.

Discussion

Increased PD-L1 expression was observed in NSCLC and neuroendocrine tumors of the lung (23,26), suggesting that

patients with NSCLC may benefit from PD-L1 inhibitors. The results obtained in the current study revealed that high PD-L1 expression was observed in patients with advanced NSCLC, compared with normal lung tissue.

There is no universal method for PD-L1 immunostaining and antibodies used in different studies vary (21). The definition of a positive PD-L1 test result differs depending on which biomarker assay is used. Four immunohistochemical assays are approved by the US Food and Drug Administration as diagnostic tests in advanced NSCLC

Table III. Survival and PD-L1 expression level.

PD-L1 cut-off	OS				PFS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	P-value	HR	95% CI	P-value	HR	95% CI	P-value	95% CI
≥1%	0.0481	1.434	1.003-2.051	0.524	1.125	0.783-1.617	0.0035	1.188-2.406
≥5%	0.0212	1.558	1.069-2.272	0.807	1.049	0.716-1.537	0.0044	1.185-2.499
≥10%	0.0068	1.952	1.202-3.171	0.084	1.421	0.954-2.117	0.0051	1.232-3.249
≥50%	<0.0001	7.768	3.031-19.91	0.001	2.768	1.551-4.940	<0.0001	3.137-21.04
OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; PD-L1, programmed death ligand-1.								

including PD-L1 IHC 22C3 pharmDx (Dako Omnis), PD-L1 IHC 28-8 pharmDx, VENTANA PD-L1 (SP142) assay and VENTANA PD-L1 (SP263) assay (23). The comparative accuracy and utility between the immunohistochemical assay used in the current study and the aforementioned assays have been verified (27).

Previously published studies reported conflicting results on the association between PD-L1 expression and age, gender, histology, ECOG PS, smoking status and cancer stage (28). The present study revealed a significant association between PD-L1 expression and cancer stage and pretreatment serum CRP level. Expression of the PD-L1 gene may be controlled by inflammatory signaling (29). Expression of the PD-L1 was regulated by interferon- γ through the Janus kinase/signal transducer of activation pathway in NSCLC (30). A recently published study demonstrated that the serum CRP level was associated with PD-L1 expression in patients with NSCLC (29). The inflammatory markers, CRP and neutrophil-lymphocyte ratio, were predictive for the efficacy of nivolumab in patients with NSCLC (29). The results obtained in the current study indicated that pretreatment elevated serum CRP level was associated with positive PD-L1 expression. However, future studies are required to verify this association.

PD-L1 is a co-regulatory molecule that can be expressed on tumor cells and its expression suppress T-cell activity (31). Compared with PD-L2, PD-L1 is the dominant inhibitory ligand of PD-1 on T cells (25). Immune checkpoints inhibitors which target the PD-L1/PD-1 pathway (including pembrolizumab and nivolumab which target PD-1, and atezolizumab and durvalumab which target PD-L1) are a promising treatment method for patients with advanced NSCLC (8,24,32-36). Thus, the identification of potential biomarkers may guide the choice of inhibitor used.

Currently known biomarkers include the ALK receptor tyrosine kinase fusion oncogene, ROS proto-oncogene 1, receptor tyrosine kinase gene rearrangements, sensitizing EGFR gene mutations and B-Raf proto-oncogene, serine/threonine kinase V600E point mutations (37,38). Activation of the immune checkpoint pathways is one of the main mechanisms underlying tumor development (39). PD-L1 expression on tumor cells negatively regulates the immune response and may lead to cancer progression (31). Although PD-L1 expression may not be an optimal biomarker (40,41), PD-L1 expression is currently used to assess whether patients with NSCLC are candidates for treatment with pembrolizumab (42,43). Identification of PD-L1 expression using immunohistochemical methods may aid in treatment selection (21). Previously published studies have reported conflicting results on whether positive PD-L1 expression may be a predictor of treatment response (44,45). The definition of positive PD-L1 expression is variable in different studies and it may impact the results (21). Future studies are required to define the prognostic role and cut-off value of PD-L1. A previous study reported that a PD-L1 expression level of $\geq 50\%$ was a positive test result for first-line pembrolizumab therapy (8). In the present study, PD-L1 expression predicted poor clinical outcome at the prespecified PD-L1 expression levels of 1, 5, 10 and 50% and results obtained suggested that the optimal cut-off value may be 50%. The current study was limited by the small sample size and retrospective analysis. Therefore, a future prospective

study with a larger sample is required to validate the results obtained.

In conclusion, the present study demonstrated that positive PD-L1 expression was associated with poor outcomes in patients with advanced NSCLC treated with gemcitabine plus cisplatin.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

YJQ analyzed and interpreted the data of the study, and wrote the manuscript. MZ, JJ and YRQ participated in the design of this research. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University (Zhengzhou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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