Prognostic significance of PD-L1 in advanced non-small cell lung carcinoma patients: a cohort study at a single institute

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Research article

Keywords: non-small cell lung carcinoma, PD-L1, PD-1, prognosis, advanced stage

Posted Date: October 5th, 2019

DOI: <https://doi.org/10.21203/rs.2.15718/v1>

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Abstract

Background This study aimed to investigate the prognostic effect of PD-L1 on Chinese non-small cell lung carcinoma patients. **Methods** A retrospective cohort study was conducted and consecutively recruited 97 patients with non-small cell lung carcinoma. The expression status of PD-1, PD-L1, p53 and Ki-67 was detected by immunohistochemistry. Kaplan-Meier survival curve with log-rank test was used to estimate survival. Cox-Hazard Proportion regression model was used to estimate the hazard ratio and 95% confidence interval. **Results** The median tumor size was 3.5 cm and 2.0 cm among patients with positive and negative PD-L1 expression, respectively ($p < 0.001$). The proportion of patients with positive and negative PD-L1 expression having nerve invasion was 26.3% and 5.0% ($p < 0.01$). 47.4% patients with positive PD-L1 expression had blood vessel invasion compared with 20.0% patients with negative PD-L1 expression ($p < 0.01$). 64.9% patients with positive PD-L1 expression had lymph node metastasis compared with 27.5% patients with negative PD-L1 expression ($p < 0.001$). Patients with positive PD-L1 expression were more likely to be in advanced stage ($p < 0.001$) and had higher Ki-67 index ($p < 0.01$). PD-L1 expression status did not show any significant association with DFS or OS. However among advanced patients, HR of PD-L1 expression was 4.13 (95%CI 1.06, 16.12). **Conclusions** Positive PD-L1 expression is associated with poorer prognosis in advanced stage patients. Compare to patients with negative expression, patients with positive PD-L1 expression had more aggressive pathological features. Further prospective studies are needed to confirm the results.

Background

Lung cancer is the most common cancer and ranks first in both incidence and mortality rates in China [1]. There were 0.77 million new cases in China, accounting for 37% of new cases worldwide in 2018 and the mortality rate was higher in under developed countries than that in developed countries [2]. 80% of lung cancers are non-small cell lung cancer (NSCLC). Chemotherapy and targeted therapy were standard therapies in the past few years. In recent years, with the approval of antibodies against programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1), the survival of NSCLC patients has been greatly improved [3-6]. Patients with positive PD-L1 expression can obtain good effectiveness from immune checkpoint inhibitor therapy [7]. PD-L1 expression was associated with a poor prognosis [8-10], if the patients didn't receive immunotherapy to PD-1 or PD-L1. Though the blocker to PD-1 was approved for NSCLC therapy, the inhibitors to PD-L1 have not been approved in China [8].

In this study, we investigated the association between PD-1/PD-L1 protein expression in NSCLC tumor tissues and the pathological factors, and analyzed the prognostic effect on relapse and survival, providing the reference evidence for immunotherapy of PD-L1 inhibitor to Chinese NSCLC patients.

Methods

All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethical committee of Beijing Shijitan Hospital, Capital Medical University, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: For the retrospective design, the formal consent was not required.

The retrospective cohort design was carried out to explore the association between PD-L1 expression and prognosis. This study retrospectively recruited 97 NSCLC patients receiving surgical treatment at the Department of Thoracic Surgery, Beijing Shijitan Hospital, Capital Medical University and having pathology diagnosis, consecutively from January 1, 2016, to December 31, 2016.

Expression of PD-1, PD-L1, P53 and ki-67 were detected by immunohistochemistry (IHC) on 4µm-thick formalin fixed paraffin-embedded (FFPE) sections. ALL the monoclonal antibodies were purchased from Beijing Zhong Shan Golden Bridge Biotechnology Co. Ltd. Sections were baked for dehydration at 60°C in an oven for 60 min, dewaxed for 20 min, and washed in 100%, 100%, 95% and 75% alcohol for 2 min respectively; washed with PBS by 5 times, 2 min each time. Antigen retrieval were carried out using the EnVision™ FLEX Target Retrieval Solutions for 2 min 30 sec, cooled to room temperature for 20 min; washed with PBS by 5 times, 2 min each time; and then incubated with 3% H₂O₂ at room temperature for 15 min; washed with PBS by 5 times, 2 min each time; sealed with 5% serum at 37°C for 15 min; discarded and added a moderate primary antibody at 4°C for a night; washed with PBS by 5 times, 2 min each time; added DAB for 5-10 min and AP-red for 10-15 min. Slides were counterstained with hematoxylin.

Hot-spot area was determined under low-power field for Ki-67 assessment. Then 1000 cells were counted under high-power field and the percentage of nuclear-positive cells was calculated. The p53 gene mutation was defined as nuclear-positive cells more than 70%. More than 1% lymphocytes with positive staining on cytoplasm/membrane was diagnosed as positive expression of PD-1. More than 1% tumor cells with brown staining on cytoplasm/membrane was determined as positive expression of PD-L1.

EGFR Mutation Test

Tumor DNA was extracted from FFPE tissue according to the instructions of DNA extraction kit. EGFR Master Mix containing EGFR Enzyme Mix and Reaction Mix was prepared in separate sterile centrifuge tube, pipetted gently more than 10 times and centrifuged briefly. 35.3µL of each EGFR Master Mix and 4.7µL NTC, PC or sample DNA were added in PCR tube respectively, and centrifuged to the bottom of the tube.

PCR protocol was set according to the cycling parameters recommended.

TNM stage is performed in accordance with eighth edition of the TNM classification of Lung Cancer [11].

Statistical Analysis.

All data was analyzed by SPSS 19.0 version. Age, tumor size, Ki-67 index, p53 status and AJCC stage were analyzed by Wilcoxon rank-sum test between PD-L1 groups. Pathological type, nerve invasion, blood vessel invasion EGFR mutation status and lymph node metastasis were analyzed by Chi-square test between PD-L1 expression statuses. PD-1 expression status was analyzed by McNemar test with PD-L1 expression status. Kaplan-Meier survival curve with Log-rank test was used to estimate the effects of PD-L1 expression on DFS and OS. The HR and 95% confidence interval (95%CI) were estimated by COX-Hazard Proportion Model with further adjustment of age, sex, pathology type, stage, blood vessel invasion and nerve invasion. All analyses were two-sided test with significant level of 0.05.

Results

58.8% NSCLC patients had positive PD-L1 expression and had no relationship with age (Table 1). NSCLC patients with positive PD-L1 expression were more likely to be diagnosed as squamous cell carcinoma than 38.6% patients with positive PD-L1 expression compared with 12.5% patients with negative expression classified as squamous cell carcinoma ($p < 0.01$, Table 1). The median tumor size was 3.5 cm and 2.0 cm among patients with positive and negative PD-L1 expression, respectively ($p < 0.001$, Table 1). 26.3% NSCLC patients with positive PD-L1 expression had nerve invasion and 5.0% patients with negative PD-L1 expression had nerve invasion ($p < 0.01$, Table 1). 47.4% patients with positive PD-L1 expression had blood vessel invasion compared with 20.0% patients with negative PD-L1 expression ($p < 0.01$, Table 1). 64.9% patients with positive PD-L1 expression had lymph node metastasis compared with 27.5% patients with negative PD-L1 expression ($p < 0.001$, Table 1). NSCLC patients with positive PD-L1 expression were more likely to be in advanced stage than 61.4% patients were diagnosed in stage more than II, in contrast to 20% patients with negative PD-L1 expression ($p < 0.001$, Table 1).

PD-L1 expression had a significant association with PD-1 expression that 36.8% patients with PD-L1 expression had PD-1 expression and 22.5% patients with negative PD-L1 had PD-1 expression ($p < 0.001$, Table 2). The median Ki-67 index was 50% and 20% among patients with positive and negative PD-L1 expression ($p < 0.01$, Table 2). p53 status and EGFR mutation status had no significant correlation with PD-L1 expression (Table 2).

Median follow-up time was 32 months and lost of follow-up rate was 9.3%. PD-L1 expression status did not show any significant association with DFS or OS (Figure 1). Three-year DFS rate was 54.7% and 65.8% among patients with positive and negative PD-L1 expression. The HR of PD-L1 expression for DFS was 1.70 (95%CI 0.86, 3.34, Table 3). Three-year OS rate was 60.8% and 78.9% among patients with positive and negative PD-L1 expression. The HR of PD-L1 expression for OS was 2.01 (95%CI 0.89, 4.58, Table 3). In early stage NSCLC patients, the HRs of PD-L1 expression were not significant for DFS and OS (Table 3). However, in advanced stage of NSCLC patients, HR of PD-L1 expression was 4.13 (95%CI 1.06, 16.12, Table 3).

Discussion

Lung cancer is the most common malignant cancer worldwide and threatens human health seriously. The crude mortality rate was 43.41/10⁵ (57.64/10⁵ in males and 28.45/10⁵ in females), and the cumulative incidence rate (0-74 years old) was 3.34% in 2013 [1]. Immunotherapy is an alternative approach to treat patients with NSCLC [12] and has forwarded to the central stage. With the widespread use of immune checkpoint inhibitors, the prognosis of many malignant tumors has been significantly improved. On June 15, 2018, China Food and Drug Administration (CFDA) approved Opdivo anti-PD-1 for the second line treatment of NSCLC. On September 12 and October 22, CFDA approved Keytruda anti-PD-1 for the first line treatment of non-squamous NSCLC and squamous NSCLC. PD-L1 expression not only can predict the efficacy of checkpoint inhibitors in many tumors, but also can be seen as a negative prognostic factor of many malignant tumors.

PD-1 is a checkpoint receptor on T lymphocytes, plays an important role in limiting adaptive immune responses and prevents auto-inflammatory and autoimmune reactivity [13]. In cancer patients, PD-1 expression is higher on T tumor-infiltrating lymphocytes, PD-1 transmits inhibitory signals into T cells after ligation with PD-1 ligands, PD-L1 [14] and PD-L2 [15] on neoplastic cells. PD-L1, the primary ligand of PD-1, is variably expressed on cancer cells and antigen-presenting cells within tumors tissues, providing a potent inhibitory influence within the tumor microenvironment [16]. Our study reveals that PD-L1 expression on tumor cells had a significant association with PD-1 expression on T cells. PD-1 expression is higher in those patients with positive expression of PD-L1 than that of patients with negative expression (36.8% vs 22.5%). Yayi He also reported a comparable result of positive correlation between PD-1 expression on TILs and PD-L1 expression on tumor cells [17].

Positive expression of PD-L1 leads to poor prognosis in cancer patients [18]. Our study revealed that compare to negative expression of PD-L1, patients with positive expression of had a bigger tumor size, higher risk of nerve invasion, higher risk of blood vessel invasion and higher risk of lymph node metastasis. Also, NSCLC patients with positive expression of PD-L1 were more likely to be in advanced stage. A meta-analysis [19] also showed that PD-L1 expression was associated with gender, histology, tumor size, lymph nodal metastasis, TNM stage and EGFR mutation.

Ki-67 was first identified as a nuclear non-histone protein 30 years ago [20]. Because it is expressed during all phases of the cell cycle except the resting stage (G₀), it has been used as a marker to evaluate proliferation in NSCLC [21], as well as in other tumors, such as lymphoma [22], oral carcinoma [23] and breast cancer [24]. Our previous study also indicated that Ki-67 as a negative prognostic and predictive marker for BC patients, and a high Ki-67 index implied an exhausted status of tumor microenvironment [25]. Nonetheless, studies examining the relationship between Ki-67 expression and NSCLC prognosis were inconsistent [26-28]. Our study indicated that the median Ki-67 index was higher among patients with high expression of PD-L1.

PD-L1 expression on tumor cells correlates with poor clinical prognosis in many cancers, such as renal cancer, ovarian cancer, lung cancer, and breast cancer [29-32]. Some researches considered that high PD-L1 expression in NSCLC was an independent predictor of poor prognosis [33]. Although considerable research links PD-L1 expression in tumors to a shorter survival in advanced NSCLC, its use as a prognostic factor requires more studies. And the prognostic function of PD-L1 still remains controversial. Cooper et al. considered that high PD-L1 expression is independently associated with longer OS [34]. Our study showed that PD-L1 expression status did not show any significant association with DFS or OS. However, hierarchical analysis revealed that among advanced NSCLC patients, high expression of PD-L1 increased the risk of death to 4.13 times (95%CI 1.06, 16.12).

A small sample size was one limitation. In addition, this was a retrospective study and further prospective studies are necessary to confirm the results.

Conclusions

Positive PD-L1 expression was associated with many aggressive factors in NSCLC patients. PD-L1 expression can raise the risk of death among advanced NSCLC patients. Further prospective studies are warranted.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethical committee of Beijing Shijitan Hospital, Capital Medical University, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication

The contents of this manuscript are our original work and consent to publish in BCM cancer.

Availability of data and materials

All data and materials are from Beijing shijitan Hospital, Capital Medical University.

Competing interests

None.

Funding

This study was financially supported by Beijing Municipal Commission of Health (Q.K.S. grant number 2015-3-057). The supporting organizations had no role in study design, data collection, analysis and interpretation.

Author contributions

Study design: QS and QZ. Data collection: YZ, FS, YL and JW. Data analysis: QS and YZ. Manuscript writing and modification: QS, FS, QZ, YZ, YL and JW. Submission approval: QS, FS, QZ, YZ, YL and JW.

Acknowledgement

This study was financially supported by Beijing Municipal Commission of Health.

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Tables

Table 1. The association between PD-L1 status and clinical-pathological characteristics

	PD-L1		p
	Positive (n=57)	Negative (n=40)	
Age, median (IQR)*	61.0 (14.0)	63.0 (15.0)	0.531
Sex, N (%)			0.116
Male	44 (77.2)	25 (62.5)	
Female	13 (22.8)	15 (37.5)	
Pathological type, N (%)			0.005
Adenocarcinoma	35 (61.4)	35 (87.5)	
Squamous cell carcinoma	22 (38.6)	5 (12.5)	
Tumor size, median (IQR)*	3.5 (4.0)	2.0 (1.9)	<0.001
Nerve invasion			0.007
No	42 (73.7)	38 (95.0)	
Yes	15 (26.3)	2 (5.0)	
Blood vessel invasion			0.006
No	30 (52.6)	32 (80.0)	
Yes	27 (47.4)	8 (20.0)	
Lymph node metastasis			<0.001
No	20 (35.1)	29 (72.5)	
Yes	37 (64.9)	11 (27.5)	
Stage*			<0.001
I	14 (24.6)	22 (55.0)	
II	8 (14.0)	10 (25.0)	
III	35 (61.4)	8 (20)	

* Wilcoxon rank-sum test

Table 2. Relationship between PD-L1 expression and other markers

	PD-L1		p
	Positive (n=57)	Negative (n=40)	
PD-1 status, n (%) [*]			<0.001
Negative	36 (63.2)	31 (77.5)	
Positive	21 (36.8)	9 (22.5)	
Ki-67 index, median (IQR) ^{**}	50% (35%)	20% (48%)	0.001
P53 status, n (%) ^{**}			0.078
Negative	8 (14.0)	7 (17.5)	
Weak	5 (8.0)	8 (20.0)	
Normal	38 (66.7)	24 (60.0)	
Strong	6 (10.5)	1 (2.5)	
EGFR mutation, n (%)			0.395
Negative	10 (55.6)	7 (41.2)	
Positive	8 (44.4)	10 (58.8)	

* McNemar test, ** Wilcoxon rank-sum test

Table 3. HR and 95%CI of PD-L1 expression on prognosis

	Disease-free survival		Overall survival
	HR	95%CI	HR
Univariate analysis	1.70	0.86, 3.35	2.01
Multivariate analysis [*]	1.04	0.48, 2.24	1.60
Multivariate analysis in stage I and II [*]	1.53	0.41, 5.78	0.48
Multivariate analysis in stage more than II [*]	1.45	0.43, 4.88	4.13

* adjusting age, sex, stage, pathology type, blood vessel invasion and nerve invasion

Figures

Figure 1