Significance of PD-L1 in the diagnosis and treatment of B-cell malignant lymphoma

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Abstract. The present study explored the significance of programmed death-ligand 1 (PD-L1) molecules in the diagnosis and treatment of B-cell malignant lymphoma. A total of 92 patients with B-cell malignant lymphoma (experimental group), admitted to the Quanzhou First Hospital Affiliated to Fujian Medical University from February 2014 to May 2017, and 60 healthy subjects (control group) were enrolled in this study and their clinical data were retrospectively analyzed. Plasma levels of PD-L1 before treatment and at 5, 10, and 15 days after treatment were measured by ELISA. Correlation between PD-L1 expression levels and treatment time was analyzed. Levels of PD-L1 in different pathological types were compared. ROC curve was used to analyze the efficacy of PD-L1 in the treatment of B-cell lymphoma. The expression level of PD-L1 in experimental group was 272.86±48.21 pg/ml, significantly higher than that in control group (18.24±3.62 pg/ml) (P<0.01). In patients with B-cell lymphoma, PD-L1 expression was highest in diffuse large B-cell lymphoma, followed by small lymphocyte lymphoma, mucosa-associated lymphoid tissue lymphoma, mantle cell lymphoma, and the lowest PD-L1 expression level was observed in follicular lymphoma (P<0.05). Linear correlation analysis showed that the expression level of PD-L1 was negatively correlated with treatment time (r=-0.683, P<0.01). The highest Youden index (51.24) was set as cut-off score, the sensitivity of the diagnosis of B-cell lymphoma was 81.66%, and the specificity was 90.24%. PD-L1 is highly expressed in B-cell malignant lymphomas and negatively correlated with treatment time. It has high diagnostic efficiency for B-cell lymphoma and is expected to be an effective immunotherapeutic target for B-cell lymphoma.

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Introduction

Cancer is the most harmful malignancy among humans in the world, and hematologic malignancy is one of the most dangerous cancers (1). Malignant lymphoma is the most common type of blood system disease (2). Malignant lymphomas include Hodgkin lymphoma (HL) and Non-Hodgkin lymphoma (NHL) types, of which NHL accounts for ~62.28% (3). In NHL, a heterogeneous proliferative disease originating from B lymphocytes is referred to as B-cell lymphoma (4). B-cell lymphoma accounts for ~85% of NHL (5). Tilly *et al* (6) have reported that ~1.2 million new B-lymphoma patients were diagnosed in 2016 worldwide, and the incidence rate of this disease has increased year by year. Scott *et al* (7) have shown that the prevalence of B-cell lymphomas ranks sixth among all malignancies, and its mortality rate is as high as ~53.82%. In view of the rising incidence and mortality of B-cell lymphoma, the treatment of this disease has attracted increasing attention. At present, the treatment of B-cell lymphoma is still dominated by chemotherapy or various targeted therapies, but the therapeutic effect is not significant, and the negative impact on patients is obvious (8). In recent years, the study of immunodetection inhibitors that block the immunosuppressive signals that tumor cells present to immune cells in order to promote the cytotoxicity of B cells has become a research hotspot (9). Immune checkpoints induce a relatively inactivated immune state to avoid the occurrence of autoimmune reactions. This regulation not only maintains the state of immune activation, but also plays a certain role in reconciling the dynamic balance of autoimmunity. In tumors, changes in the microenvironment of tumor cells lead to the activation of immune checkpoint signals in the microenvironment, resulting in the occurrence and development of tumors (10). Programmed death-ligand 1 (PD-L1) is an important immune checkpoint-related molecule. A number of previous studies (11-13) have demonstrated that PD-L1 is involved in the activation of multiple tumors and the progression of the cell cycle. However, the significance of PD-L1 in B-cell malignant lymphoma is not yet clear. The present study explored the expression of PD-L1 in B-cell malignant lymphomas and analyzed the significance of PD-L1 in the diagnosis and treatment of B-cell malignant lymphomas, so as to provide reference and guidance for the treatment of B-cell lymphoma.

Patients and methods

General information. B-cell lymphoma patients who were admitted to the Quanzhou First Hospital Affiliated to Fujian Medical University (Quanzhou, China) from February 2014 to May 2017 were selected as the study subjects and their clinical data were retrospectively analyzed. Inclusion criteria: 20-60 years of age; clinical manifestation consistent with the 2012 B-cell lymphoma diagnosis guidelines (14); B-cell lymphoma diagnosed by pathology biopsy in the above hospital; no treatment before diagnosis. A total of 162 cases were included in the study based on inclusion criteria. Exclusion criteria: combination with critical organ failure; combination with other malignancies; suffering from immune system diseases; suffering from nervous system diseases; surgery and chemotherapy tolerant patients; physical disability; long-term bedridden; transferred to hospital during treatment; not willing to cooperate with researchers. Only 92 patients were finally included based on exclusion criteria (experimental group) and the mean age was 42.52±9.82 years (Table I). In the same period, 60 patients with no physical disability were selected as control group. Control group included 38 males and 22 females, with a mean age of 41.81±8.76 years. There was no significant difference in gender, age, and other clinical data between the two groups (P>0.05).

The present study was approved by the Ethics Committee of Quanzhou First Hospital Affiliated to Fujian Medical University. Patients who participated in this research, or their guardians, signed an informed consent and had complete clinical data.

Method. All patients with B-cell lymphoma were treated with rituximab and methotrexate, after diagnosis in the Quanzhou First Hospital Affiliated to Fujian Medical University, in strict accordance with the principles of chemotherapy. Methotrexate was administered to patients intravenously at a dose of 3 g/m^2 and rituximab at a dose of 375 mg/m^2 (Shanghai Roche Pharmaceutical Co., Ltd., Shanghai, China) for chemotherapy. The total dose was strictly controlled at 36 Gy, 5 times/week, 2.0 Gy/time. In case of residual lesions, 10.0 Gy of radiation was locally used and the treatment period was 1 month. Detoxification of calcium tetrahydrofolate was performed 12 h after each treatment. A treatment cycle was 1 month and 4 cycles were performed. Patient blood samples were collected and plasma levels of PD-L1 were measured using ELISA (cat. no. DB7H10; R&D Systems, Inc., Minneapolis, MN, USA). Then, 50 μl Assay Diluent RD1-41 (R&D Systems, Inc.) was added to 100 μl of samples per well. The mixture was covered with adhesive tape and incubated at room temperature on a horizontal rail microplate oscillator (Bio-Rad Laboratories, Inc., Hercules, CA, USA) for 2 h (400 x g). The wells were washed 4 times, followed by the addition of 200 μl Hine/Cynomolgus Monkey B7-H1 Conjugate (R&D Systems, Inc.) to each well, and covered with new tape. The mixture was again incubated at room temperature on an oscillator at 400 x g for 2 h and the wells were washed 4 times. Substrate solution (20 μl) was added and incubated for 30 min in the dark. Subsequently, 200 μl of color reagent were added into each well, and incubated at room temperature for 30 min. Then, 50 μl termination solution was added and the absorbance (OD) of each well was measured using a microplate reader (wavelength, 450 nm; Bio-Rad Laboratories, Inc.).

Table I. General information.

Variables	Cases (n=92)	%
Sex		
Male	58	63.04
Female	34	36.96
Pathological stage		
I-II	8	8.70
III-IV	84	91.30
Pathological type		
Diffuse large B-cell lymphoma	28	30.43
Follicular lymphoma	15	16.30
Mucosa-associated lymphoid tissue lymphoma	17	18.48
Small lymphocyte lymphoma	20	21.74
Mantle cell lymphoma	12	13.04
Place of residence		
Urban area	56	60.87
Rural area	36	39.13

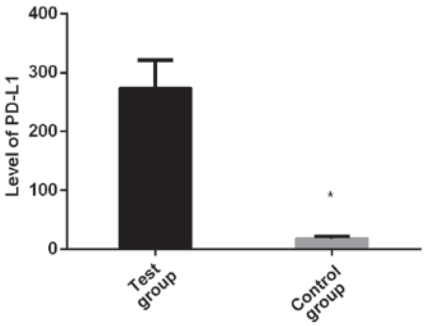

Figure 1. Level of PD-L1 expression in experimental and control group. PD-L1 expression level was significantly higher in experimental group than that in control group. *P<0.01, compared with experimental group. PD-L1, programmed death-ligand 1.

Observation indicators. Patients' clinical information (such as, age, sex and pathological stage); PD-L1 expression levels in the blood samples of the two groups of patients; PD-L1 expression levels in experimental group before and at 5, 10 and 15 days after the beginning of treatment; diagnostic efficacy of PD-L1 for B-cell lymphoma.

Statistical analysis. SPSS v.22.0 statistical software (IBM Corp., Armonk, NY, USA) was used to analyze and process the data. Count data were expressed as rates and their comparison between two groups was performed using Chi-square test. Measurement data were expressed as mean ± standard deviation and t-test was used for their comparison between groups. One way analysis of variance was used for multiple comparisons and LSD test was the post hoc test used. The diagnostic value was analyzed by ROC curve analysis.

Table II. Diagnostic efficacy of PD-L1 for B-cell lymphoma.

Items	Values
AUC	0.9082
Cut-off	51.24
OR	1.51
95% CI	1.04-1.77
Sensitivity	81.66%
Specificity	90.24%
P-value	0.02

PD-L1, programmed death-ligand 1.

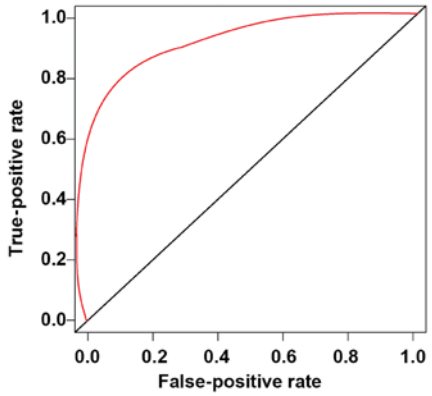

Figure 2. ROC curve analysis of the diagnostic value of PD-L1 for B-cell lymphoma. PD-L1, programmed death-ligand 1.

Spearman's correlation analysis was performed using linear correlation analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

PD-L1 level. Expression level of PD-L1 in experimental group was 272.86±48.21 pg/ml, and in control group was 18.24±3.62 pg/ml. There was a significant difference between the groups. PD-L1 expression level in experimental group was significantly higher than that in control group (P<0.01; Fig. 1).

Diagnostic efficacy of PD-L1 for B-cell lymphoma. ROC curve analysis showed that AUC of PD-L1 in peripheral blood was 0.9082, with the cut-off value of 51.24 being the most approximate index. The sensitivity for diagnosis of B-cell lymphoma was 81.66% and the specificity was 90.24% (Table II and Fig. 2).

Expression of PD-L1 in different pathological types. The expression levels of PD-L1 in diffuse large B-cell lymphoma, follicular lymphoma, mucosa-associated lymphoid tissue lymphoma, small lymphocyte lymphoma, and mantle cell lymphoma were 265.42±36.04, 142.77±21.88, 167.56±32.61, 246.82±46.25, and 159.55±26.84 pg/ml, respectively. There were statistically significant differences in the expression levels of PD-L1 among all five pathological types (P<0.01).

Table III. Expression of PD-L1 in different pathological types.

Types	PD-L1 (pg/ml)
Diffuse large B-cell lymphoma (n=28)	265.42±36.04
Follicular lymphoma (n=15)	142.77±21.88[a]
Mucosa-associated lymphoid tissue lymphoma (n=17)	167.56±32.61[a,b]
Small lymphocyte lymphoma (n=20)	246.82±46.25[a-c]
Mantle cell lymphoma (n=12)	159.55±26.84[a-d]
F	49.04
P-value	<0.01

[a]P<0.05, compared with diffuse large B-cell lymphoma; [b]P<0.05, compared with follicular lymphoma; [c]P<0.05, compared with mucosa-associated lymphoid tissue lymphoma; [d]P<0.05, compared with small lymphocyte lymphoma. PD-L1, programmed death-ligand 1.

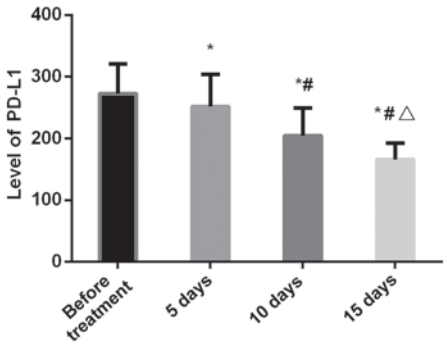

Figure 3. Changes in PD-L1 expression level during treatment in experimental group. The level of PD-L1 in patients gradually decreased with prolonged treatment. *P<0.05, compared with the pre-treatment level; #P<0.05, compared with the level at 5 days after the beginning of treatment; △P<0.05, compared with the level at 10 days after the beginning of treatment. PD-L1, programmed death-ligand 1.

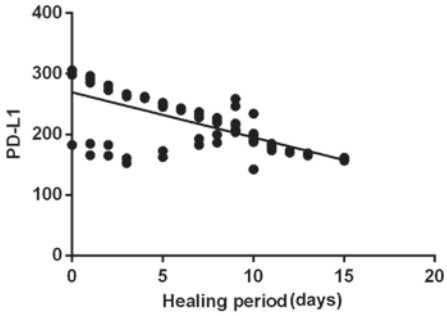

Figure 4. Linear correlation analysis of PD-L1 and treatment time. Linear correlation analysis showed that the expression level of PD-L1 was negatively correlated with treatment time (r=-0.683, P<0.01). PD-L1, programmed death-ligand 1.

The expression level of PD-L1 was highest in diffuse large B-cell lymphoma (P<0.05), followed by small lymphocyte lymphoma (P<0.05), mucosa-associated lymphoid tissue lymphoma (P<0.05), mantle cell lymphoma (P<0.05), and PD-L1 expression level in follicular lymphoma was the lowest (P<0.05; Table III).

Changes in PD-L1 during treatment. PD-L1 level in the peripheral blood of patients in experimental group was 272.86±48.21 pg/ml before treatment, 252.17±52.33 pg/ml at 5 days after the beginning of treatment, 204.82±45.16 pg/ml at 10 days after the beginning of treatment, and 166.53±26.18 pg/ml at 15 days after the beginning of treatment. PD-L1 level gradually decreased with prolonged treatment, and PD-L1 expression level was the lowest ($P<0.05$) at 15 days after the beginning of treatment. Linear correlation analysis showed that the expression level of PD-L1 was negatively correlated with treatment time ($r=-0.683$, $P<0.01$; Figs. 3 and 4).

Discussion

The goal of tumor immunotherapy is to activate the killing potential of tumor-specific B cells. In tumor microenvironment, a variety of immunosuppressive factors can initiate activation reaction with B cells, so as to inhibit the immunity of B cells (15). In recent years, an increasing number of studies (16-18) have proven that the main reason for the suppression of B-cell immune function in cancer patients is the abnormal activation of multiple immunosuppressive signals. PD-L1 molecule is an immunonegative regulator that is located on the surface of tumor cells and has been shown to inhibit B cell activation (19). Brahmer *et al* (20), have proposed that PD-L1 antibody treatment can effectively relieve the inhibitory effect of PD-L1 on B cells, so as to achieve autoimmune killing of cancer cells. At present, the mechanism of action of PD-L1 in B cells is not yet clear, and whether there is a significant correlation between the expression of PD-L1 and the development of B-cell lymphoma is still controversial. Therefore, analysis of PD-L1 expression in patients with B-cell lymphoma is of great significance for the diagnosis and treatment of B-cell lymphoma.

The results of the present study revealed that PD-L1 is highly expressed in patients with B-cell lymphoma and is negatively correlated with treatment time. PD-L1 highly sensitive and specific for the diagnosis of B-cell lymphoma. The high expression level of PD-L1 in B-cell lymphoma patients proves that B-cell lymphoma is associated with PD-L1 to a certain extent. The study carried out by Muro *et al* (21), has also demonstrated that PD-L1 is highly expressed in gastric cancer and is negatively correlated with treatment time, supporting the viewpoint of our study. This suggests that PD-L1 may be a predictor of future treatment of B-cell lymphoma. PD-L1 expression level was fould to be highest in diffuse large B-cell lymphoma and lowest in inactive tumor follicular lymphoma, suggesting that PD-L1 may be associated with lymphoma invasiveness. Results of the study carried out by Herbst *et al* (22), have shown that there is no significant correlation between the expression of PD-L1 and treatment efficacy, which may be explained by the different treatment strategies. In this study, all patients with B-cell lymphoma were treated with cyclophosphamide, doxorubicin, vincristine, etoposide, and prednisone, while Herbst *et al* (22), used a traditional cisplatin and paclitaxel chemotherapy. The difference in the use of drugs may have caused different effects on PD-L1 expression.

At present, the use of PD-L1 antibody-targeted inhibition therapy has achieved significant breakthroughs in the treatment of many types of tumors, such as non-small cell lung cancer, and melanoma. PD-L1 inhibitors can be used to remove stubborn residual lesions in patients. The effect is significantly better than the traditional resection surgery, and the safety is higher than that of chemotherapy (20). Results of this study showed that abnormal PD-L1 expression is closely related to B-cell lymphoma, suggesting that PD-L1 antibody is expected to become an effective immunotherapy drug for this disease.

The present study is still limited by the small sample size, while regional differences were not excluded. In future studies we plan to resolve these issues in order to further confirm our findings.

In summary, PD-L1 is highly expressed in B-cell malignant lymphoma and negatively correlated with treatment time. It has high diagnostic efficiency for B-cell lymphoma and is expected to be an effective therapeutic target for B-cell lymphoma.

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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 on reasonable request.

Authors' contributions

JY conceived and designed this study. JY and GH collected and analyzed the general data of patients. GH recorded and analyzed the observation indicators. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Quanzhou First Hospital Affiliated to Fujian Medical University (Quanzhou, China). Patients who participated in this research, or their guardians, signed an informed consent and had complete clinical data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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