CORRELATION BETWEEN PERIPHERAL BLOOD BIOMARKERS AND CLINICAL EFFICACY OF PD-1/PD-L1 INHIBITORS IN THE TREATMENT OF CHOLANGIOCARCINOMA

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ABSTRACT

Objective: *Investigating the effect of PD-1/PD-L1 inhibitors on peripheral blood biological indexes of cholangiocarcinoma and determining its correlation with the clinical efficacy of cholangiocarcinoma.*

Methods: *A total of 98 cholangiocarcinoma patients (diagnosed by pathological examination from May 2017 to May 2019) were selected as subjects of this study. According to their recovery prognosis, participants were divided into a good prognosis group (Group A) and a poor prognosis group (Group B), with 49 cases in each group. General data for the two groups were compared and the changes of peripheral cytokines (before and after treatment) were determined. In addition, the clinical efficacy of the two groups was established and the correlation between peripheral cytokines and clinical efficacy was analysed.*

Results: *IFN-γ in group B after treatment, TNF- α, The concentrations of IL-6, IL-7, FGF and G-CSF in Group B were significantly lower than that of Group A (P<0.05). The effective rate of clinical efficacy in Group B was 79.59% (39/49), which was significantly better (P<0.05) than the 42.86% (21/49) of Group A. The clinical efficacy of Group B was correlated with IL-6, IL-7, FGF and G-CSF (P<0.05) and the AUC values of IL-6, IL-7, basic FGF and G-CSF were 0.829, 0.815, 0.856 and 0.614 respectively (with the AUC value of basic FGF being the largest). These factors could effectively diagnose the relationship between basic FGF and the clinical efficacy of cholangiocarcinoma.*

Conclusion: *PD-1/PD-L1 inhibitors affect the peripheral cytokines of patients with cholangiocarcinoma. By observing the changes in the concentrations of IL-6, IL-7, FGF and G-CSF, the clinical efficacy of patients could be estimated – providing a reference basis for clinical rational drug use, and helping the patients to receive the best benefit.*

Keywords: *Cholangiocarcinoma, PD-1/PD-L1 inhibitors, peripheral biological indexes, clinical efficacy.*

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Introduction

The incidence in cholangiocarcinoma, a rare malignant tumour caused by bile duct epithelial cells, is increasing annually[1]. According to different anatomical sites, cholangiocarcinoma may be classified as either being intrahepatic, hilar, or distal[2]. Since clinical symptoms appear late, early diagnosis is difficult and this subsequently affects the treatment of cholangiocarcinoma. Due to the special anatomical location of cholangiocarcinoma and its infiltration into surrounding tissues, nerves and blood vessels, its surgical resection rate is generally low, and the prognosis of patients is poor[3]. With the improvement of life quality, the incidence and mortality of cholangiocarcinoma are increasing year by year and have attracted the attention of researchers and medical workers[4]. Programmed Death Factor-1 (PD-1) and its ligand PD-L1 are the main members of the CD28/B7 family. Activation of the PD-1/PD-

L1 pathway may cause a variety of effects on cells, in addition to affecting the immune regulation of tumours and playing an important role in the negative regulation of tumour immune responses[5]. With the progress of medical technology, immunotherapy has gradually become the main anti-tumour treatment method[6]. It has been established that PD-1/PD-L1 inhibitors play an important role in down-regulating inhibitory receptors on the surface of T cells whilst also having a positive effect on restoring the immune synaptic function of T cells[7]. As such, PD-1/PD-L1 inhibitors have been widely used in the treatment of tumours (including lung adenocarcinoma, cervical cancer, colon cancer etc.), although rarely that of a cholangiocarcinoma. This study, therefore, aimed to explore the influence of PD-1/PD-L1 inhibitors on peripheral blood biological indicators of cholangiocarcinoma and their correlation with the clinical efficacy of cholangiocarcinoma.

Methods

General information

A total of 98 patients with cholangiocarcinoma, confirmed by pathological examination in our hospital from May 2017 to May 2019, were selected as the subjects of this study. According to estimated prognosis and recovery, they were divided into a good prognosis group (Group A) and a bad prognosis group (Group B), with 49 cases in each group. There were 52 males and 46 females of which 55 cases were aged > 55 years old and 43 cases were aged < 55 years old. Smoking history had been positive for 62 cases and negative for 36 cases. History of alcoholism had been positive for 48 cases and negative for 50 cases. Regarding the pathological cholangiocarcinoma types: 39 cases were intrahepatic, 28 cases were hilar, and 31 cases were distal in nature. Differentiation degree: low differentiation in 34 cases, medium differentiation in 28 cases, high differentiation in 36 cases (with PD-1 expression being positive in 59 cases and negative in 39 cases). Based on ECOG grading, 25 cases were of grade 0, 26 cases of grade i, 22 cases of grade ii and 25 cases of grade iii.

Inclusion criteria:

• Cholangiocarcinoma had been confirmed by abdominal CT;

• No relevant immunotherapy had been used before treatment;

• Participation of patients and their families had been voluntary. In addition, a signed consent form, approved by the Ethics Committee of our hospital, had been obtained from all participants of the experiment.

Exclusion criteria:

• Patients without a clear diagnosis;

• The presence of other malignant tumours;

• Long-term use of glucocorticoids;

• Patients with mental disorders and/or exhibiting a poor mental condition;

• The presence of congenital immune diseases;

• Participants of other experiments;

• Patients of intermediate transfer and discharge.

Treatment plan

Group A was treated with chemotherapy whereas Group B was treated with PD-1 /PD-L1 inhibitors in combination with chemotherapy. The main chemotherapy drugs used for the treatment of cholangiocarcinoma are vincristine, methotrexate, and mitomycin. The PD-1/PD-L1 inhibitors dosage (3 mg/kg) was given intravenously every two weeks, and the clinical effect was evaluated after 12 weeks of medication. Before and after each treatment cycle, 3 mL of peripheral venous blood had been collected from all patients under sterile conditions (until the end of the PD-1/PD-L1 inhibitor treatment period).

Experimental instruments and reagents

A high-speed centrifuge (Shanghai Hetian Scientific Instrument Co., Ltd); EP tube (Zhejiang Runlan Technology Co., Ltd); mixing oscillator (Hangzhou Runcheng Instrument Co., Ltd); pipette gun (Nanjing Beideng Electronic Commerce Co., Ltd); liquid chip instrument (Huajin Technology Co., Ltd); magnetic plate washing machine (Jinan Junchi Biotechnology Co., Ltd); fluorescence quantitative detector (Jinan Haolaibao Medical Equipment Co., Ltd).

Experimental methods

Specimen collection

Extracted peripheral blood was centrifuged for 30 min at 3000 rpm for 10 min after which the supernatant was extracted with a pipette gun and stored in an EP tube (marked with the collection time).

Specimen inspection

A sandwich ELISA method was used where diluent was added, shaken, oscillated, cooled, and incubated under dark conditions. After cleaning, a fluorescence antibody was supplemented and

biomarkers were detected via a fluorescence quantitative detector.

Detection index

Interferon-γ (IFN-γ), tumour necrosis factor-α (IFN-α), interleukin-6 (IL-6), interleukin-7 (IL-7), basic fibroblast growth factor (basic FGF), and Granulocyte-colony factor (G-CSF).

Evaluation criteria

Immune-Related Response Criteria (irRC) [8] were used to evaluate the efficacy. It was divided into four parts, i.e. complete response (CR), partial response (PR), stable (SD) and progressive (PD). Patients with an efficacy evaluation of CR, PR, and SD could continue with treatment, whereas patients with PD efficacy had to be evaluated again using irRC standards (determining whether they could be treated according to the original plan).

Statistics

Data in this study were analysed using the SPSS21.0 software package. Measurements of data were expressed as ($\bar{x}\pm s$) and a t-test was used to compare data between groups.

All count data were expressed as [n (%)] and a χ^2 test was used to compare data between two groups. The correlation between clinical efficacy and peripheral cytokines were studied using logistic multifactor regression analysis and a ROC curve. $P<0.05$ was used as the statistical standard to indicate significance.

Results

Comparison of general data

There were no significant differences ($P>0.05$) in gender, age, histories of smoking or alcoholism, pathological carcinoma type, degree of differentiation, PD-1 expression or ECOG grades between the two groups of patients, which were comparable as illustrated in Table 1.

Comparison of peripheral cytokines before and after treatment

There were no significant differences in IFN-γ, TNF-α, IL-6, IL-7, Basic FGF and G-CSF between the two patient groups before treatment ($P>0.05$). However, after treatment, the concentration of these factors in Group B had been significantly lower than that of Group A. As shown in Table 2, these differences had been statistically significant ($P<0.05$).

Information	A group (n=49)	B group (n=49)	χ^2	*P*
Gender			1.474	0.224
Man	29 (55.77%)	23 (44.23%)		
Woman	20 (43.48%)	26 (56.52%)		
Age			2.03	0.154
>55 year	31 (56.36%)	24 (43.64%)		
<55 year	18 (41.86%)	25 (58.13%)		
Smoking history			2.81	0.093
With	35 (56.45%)	27 (43.55%)		
Without	14 (38.89%)	22 (61.11%)		
Alcoholism history			0.653	0.418
With	22 (45.83%)	26 (54.17%)		
Without	27 (54%)	23 (46%)		
Pathological type			0.458	0.795
Intrahepatic bile duct carcinoma	19 (48.71%)	20 (51.28%)		
Hilar cholangiocarcinoma	13 (46.42%)	15 (53.57%)		
Distal cholangiocarcinoma	17 (54.84%)	14 (45.16%)		
Degree of differentiation			4.512	0.104
Poorly	22 (46.71%)	12 (35.29%)		
Moderately	12 (42.86%)	16 (57.14%)		
High	15 (41.67%)	21 (58.33%)		
Expression of PD-1			0.042	0.836
Positive	29 (49.15%)	30 (50.85%)		
Negative	20 (51.28%)	19 (48.71%)		
ECOG grade			2.797	0.423
0 grade	16 (64%)	9 (36%)		
I grade	11 (42.30%)	15 (57.69%)		
II grade	10 (45.45%)	12 (54.55%)		
III grade	12 (48%)	13 (52%)		

Table 1: Comparison of general data between two groups of patients.

Cytokines		A group (n=49)	B group (n=49)	*t*	*P*
IFN-γ	Before treatment	36.48±5.69	36.51±4.95	0.027	0.977
	After treatment	25.35±6.45*	17.33±5.94*	6.402	<0.001
TNF-α	Before treatment	51.36±6.48	52.33±5.31	0.81	0.419
	After treatment	27.66±5.67*	19.37±4.92*	15.239	<0.001
IL-6	Before treatment	21.33±4.37	21.41±3.99	7.73	0.839
	After treatment	15.99±2.37*	9.27±1.33*	17.308	<0.001
IL-7	Before treatment	16.38±1.35	16.42±1.22	0.153	0.878
	After treatment	8.55±1.07*	6.84±1.02*	8.097	<0.001
Basic FGF	Before treatment	45.67±1.68	45.71±1.20	0.135	0.892
	After treatment	15.25±2.17*	11.64±2.07*	8.426	<0.001
G-CSF	Before treatment	67.11±9.79	67.18±9.35	0.036	0.971
	After treatment	28.78±5.33*	21.38±5.68*	6.65	<0.001

Table 2: Comparison of peripheral cytokines between two patient groups before and after treatment ($\bar{x}\pm s$).
**P<0.05 (in comparison with the 'before treatment').*

Comparison of clinical efficacy

As indicated in Table 3, the effective rate of Group B at 79.59% (39/49) was significantly higher and statistically significant (P<0.05), when compared to that of Group A at 42.86% (21/49).

Group	CR	PR	SD	PD	Effective rate
A group (n=49)	0	5 (10.20%)	16 (32.65%)	27 (55.10%)	21 (42.86%)
B group (n=49)	2 (4.09%)	15 (30.61%)	22 (44.90%)	10 (20.41%)	39 (79.59%)
χ^2					14.615
P					0.002

Table 3: Comparison of clinical efficacy between two patient groups.

Logistic multifactor regression analysis of the correlation between peripheral cytokines and clinical efficacy

For Group B, there was no significant difference between clinical efficacy and IFN-γ and IFN-α concentrations (P>0.05).

The clinical efficacy was, however, correlated with IL-6, IL-7, Basic FGF and G-CSF values of Group B. These differences were statistically significant (P<0.05), as shown in Table 4.

Peripheral cytokines	Clinical efficacy	HR	95%CI	*P*
IFN-γ	CR/ PR/ SD	0.62	0.312~0.967	0.751
TNF-α	CR/ PR/ SD	1.15	0.781~1.525	0.411
IL-6	CR/ PR/ SD	1.96	1.286~3.123	0.001
IL-7	CR/ PR/ SD	1.92	1.029~3.158	0.008
Basic FGF	CR/ PR/ SD	1.99	1.163~3.451	0.015
G-CSF	CR/ PR/ SD	1.54	1.081~2.311	0.041

Table 4: Logistic multifactor regression analysis of the correlation between peripheral cytokines and clinical efficacy.

ROC curve of cytokines and clinical efficacy

The AUC values of IL-6, IL-7, Basic FGF and G-CSF were 0.829, 0.815, 0.856 and 0.614, respectively (Table 5).

Cytokines	AUC	Sensibility	Specificity	95%CI	*P*
IL-6	0.829	85.42	81.78	0.678~0.975	0.001
IL-7	0.815	66.15	86.15	0.698~0.926	<0.001
Basic FGF	0.856	88.02	80.11	0.745~0.996	<0.001
G-CSF	0.614	52.35	80.16	1.621~11.264	0.011

Table 5: ROC curves of cytokines and clinical efficacy.

Discussion

Cholangiocarcinoma is one of the gastrointestinal malignancies that have a very high mortality rate. Since pathogenesis and pathological features of cholangiocarcinoma are not obvious, most patients lose the opportunity of surgical treatment when diagnosed[9]. As cholangiocarcinoma is less sensitive to chemotherapy drugs, its main treatment is that of surgical resection which results in a poor prognosis because of the complicated metastasis commonly seen in patients[10]. Since effective early diagnosis and defined clinical symptoms of the opposite sex are lacking, patients are usually already in the middle or advanced stages of cholangiocarcinoma when diagnosed (with an average life expectancy of less than one year[11]). It is therefore particularly important to select the most appropriate and effective treatment(s) for cholangiocarcinoma.

In recent years, immunotherapy has gradually entered public awareness, and PD-1/PD-L1 inhibitors have become widely used in clinical treatments. The PD-1 /PD-L1 immune checkpoint can transmit inhibitory signals, which can subsequently inhibit T cells and lead to tumour immune tolerance. Therefore, T-cell activation can be stimulated by blocking the PD-1/PD-L1 signalling pathway and the tumour cells can be killed[12]. PD-1, present in the peripheral effector of T cells, inhibits a class of immune checkpoints (needed for T-cell activation) and causes cellular immune tolerance of PD-L1 and PD-L2[13]. In this study, the concentrations of IFN-γ, TNF-α, IL-6, IL-7, Basic FGF and G-CSF in Group B (after treatment) were significantly lower (P<0.05) than that of Group A (after treatment). This suggested that PD-1/PD-L1 inhibitors can effectively change the concentration of cytokines in patients and thus regulate immune disorders in patients. From a molecular structure perspective, PD-1 only has biological activity when it is expressed in the cell membrane and can change the expression of IFN-γ and activate oncogenes to achieve biological effects[14]. The up-regulation of PD-1 expression in cancer cells has been shown to promote metastasis, allowing tumour cells to escape the attack of CD8+T cells and thus achieving the purpose of escaping immune surveillance[15].

Tumour formation and development is a relatively complex process that involves many aspects and factors, including immune escape which may be the most important mechanism in the occurrence, development, invasion and metastasis of tumours[16].

Multiple studies have reported overexpression of PD-1/PD-L1 in malignant tumours, whereas some have detected abnormal expression levels in non-small cell carcinoma, breast cancer and ovarian cancer[17]. In this study, the effective rate of Group B at 79.59% (39/49) was significantly higher than that of Group A at 42.86% (21/49) and this difference was statistically significant ($P<0.05$). These results suggested that PD-1/PD-L1 inhibitors may have a good clinical effect in the treatment of cholangiocarcinoma and may improve the patient's condition. Previous studies have proven that the immune system participates in tumour cell generation and development (in the states of clearance, confrontation, and escape), and that the application of PD-1/PD-L1 inhibitors in tumours may also form part of a complex process, with different cytokines changes at different stages[18]. In this study, there had been a statistically significant correlation between the clinical efficacy of Group B and IL-6, IL-7, Basic FGF and G-CSF concentrations which suggested that patient condition may deteriorate when concentrations of these factors are increased.

Furthermore, the AUC values of IL-6 (0.829), IL-7 (0.815), Basic FGF (0.856) and G-CSF (0.614) (Basic FGF being the largest) could effectively indicate the relationship with the clinical efficacy of cholangiocarcinoma. These factors may thus be closely related to the clinical efficacy of cholangiocarcinoma and, as such, the clinical efficacy of cholangiocarcinoma could potentially be diagnosed by monitoring changes in the concentration of these factors. In conclusion, PD-1/PD-L1 inhibitors affect peripheral cytokines in patients with cholangiocarcinoma, and the observation of changes in the concentrations of IL-6, IL-7, Basic FGF and G-CSF may help suggest poor clinical efficacy in patients – providing a reference for rational clinical drug use and enabling patients to obtain the best benefits.

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