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Original Article

Correlation analysis of peripheral blood T cell subgroups, immunoglobulin and prognosis of early hepatocellular carcinoma after hepatectomy

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Abstract: To investigate the prediction value of preoperative changes in the immunological function for the prognosis of hepatocellular carcinoma (HCC) after hepatectomy, 158 cases of HCC patients who received liver resection in our hospital from 2009 to 2010 were enrolled in this study. Immune indices [$CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$ and natural killer (NK), IgA, IgM, IgG], AFP level were calculated. The differences between preoperation and postoperation group, exclude were not both statistically significant (both $P > 0.05$), whereas IgA group was not ($P < 0.05$),The follow-up data showed that the 3-year recurrence rates of high level group on HBsAg, CD3, CD4, CD8, CD19 and IgGwerelarger than that of low level group,while the 3-year recurrence rates of high level group on AFP, NK, IgA and IgM smaller than that of low level group, and we found that $CD3^+$, $CD4^+$, $CD8^+$ and NK^+, $CD19^+$, HBsAg, IgA and IgG above were statistically different ($P < 0.05$), AFP and IgM were no difference with prognosis of HCC ($P > 0.05$).The markers level were not both statistically significant with age, gender, grade, tumor size ($P > 0.05$). Multiple logistic regression analysis indicated that $CD4^+$, $CD8^+$ and NK^+ were closely correlated with HCC postoperative survival rate ($P < 0.05$) ($Y = 1.262 \times CD4^+ + 1.448 \times CD8^+ - 0.646 \times NK^+$). Construction of *Cox*'s proportional hazards regression model showed IgA and IgG were correlated with HCC postoperative survival rate at a trend ($P = 0.079$) ($Y = 127.9 \times IgG - 28.7 \times IgM$). Preoperative Immune indices ($CD4^+$, $CD8^+$, NK^+, IgA, IgG) were closely correlated with HCC survival rate and these were considerable predictive value for the malignantfeature and prognosis of HCC.

Keywords: Subgroups, immunoglobulin, hepatocellular carcinoma, hepatectomy, Prognosis

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and a major health problem in parts of Asia and Africa. HCC has a very poor prognosis, and the majority of cases are detected at advanced stages. It has been demonstrated that the immune system is able to induce responses against tumors and these responses can be enhanced using a number of strategies. T cells or T lymphocytes are a group of immune system cells that play a central role in cell-mediated immunity. Cytotoxic T cells ($CD8^+$ T cells) and helper T cells ($CD4^+$ T cells) recognize tumor associated antigens presented on MHC class I or II of antigen presenting cells via their T cell receptors.

Host immunity can either protect or promote tumor growth by the predominance and activation of certain subsets of immune cells. Cytotoxic T-cell infiltration of the tumors is indicative of a better survival, whereas the predominance of suppressor cells is associated with a worse outcome and lower survival rates. Certain therapeutic strategies can enhance the release and exposure of tumor antigens, which might help to overcome the immune tolerance towards the tumor. Therefore, such immune-stimulating therapeutic interventions in combination with immunotherapy strategies represent a promising future approach for HCC treatment [1]. We has been recently demonstrated that T, B cell-sandimmunoglobulin cooperative responses in HCC patients.

AFP is indicated as an immunosuppressant in HCC patients. Equally well known is AFP as biomarker for hepatocellular carcinoma and some other malignancies. There are at least four neurodegenerative disorders, all inherited as autosomal recessive traits and characterized by the presence of cerebellar ataxia, abnormal ocular movements, and neuropathy, for which an elevated concentration of serum AFP is an important diagnostic biomarker. The availability of a reliable biomarker is not only important during screening or diagnostic processes, but is also relevant for objective follow-up during (future) therapeutic interventions [2].

Adoptive cellular immunotherapy has been applied inthe clinical treatment of advanced HCC due to the closerelationship between the pathogenesis of HCC and the autoimmune system. In addition, immunotherapy is regarded as a promising and potentially effective treatment for HCC. Previous studies have shown that immune responses are crucial in HCC and the phenotype and proportion of lymphocytes are informative and valuable to some extent in predicting the prognosis of HCC [3]. For HCC patients undergoing resection, prominent infiltration of inflammatory cells can reduce recurrence and improve survival. The present study was conducted to investigate changes in the peripheral immune cells of HCC patients following treatment with difference.

Materials and methods

Patient

A total of 158 retrospective cases were diagnosed as primary HCC on both imaging and histopathological study from the Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS & PUMC) between 2009 and 2012 were assessed in this study. Patients who underwent hepatectomy for primary HCC (n = 158) were enrolled. From Jan 2009 to July 2013, a total of 158 patients aged 35-73 years (mean 47.6 ± 8.8 years) were included in thisstudy. Written informed consent for each patient was given before participating in the study. All the histopathological diagnoses were confirmed to be HCC with distinctive microscopic features and immunohistochemistry staining results. Patients with serious heart, lung, kidney, or blood diseases, autoimmune liver disease, or the presence of other malignant tumors, were excluded.

Preoperative and postoperative tumor assessed that preoperative tumor staging was performed by dynamic computed tomography (CT), assessing tumor size, tumor number, portal vein involvement and regional tumor invasion. Postoperative tumor assessment was made by pathological study of the degree of tumor differentiation, vascular involvement, nerve involvement, lymphatic involvement and non-cancerous liver tissue fibrosis. Adjacent non-cancerous liver tissue was examined by reticular fiber staining, according to the Ishak scoring system for scoring the degree of fibrosis. Histopathological immunohistochemistry staining of hepatocytes and CK7 were studied by using the EnVision (Zhongshan biotechnology, Beijing) two-step method.

Instrument and reagents

Lymphocyte subset assay

Peripheral blood was collected 1 day before operation and 1and 2 weeks after treatment, and blood samples of healthy subjects served as controls. Blood (2 ml) was anti-coagulated and used for analysis within 6 hours after collection. Samples were detected by a FACS Calibur flow cytometry system EPICS ELITE ESP *(Beckman Coulter*, United States). The percentages of $CD3^+$, $CD4^+$, $CD8^+$ and NKcells were measured by flow cytometry.

AFP and Ig assay

Serum AFP and HBsAg levels were detected by Electrochemiluminescence (Roche cobas 60-00e601, Switzerland). Before and one week after operation and used for the valuation of therapeutic efficacy and liver function. IgA, IgG and IgM were tested immune Turbidimetry (Roche Modulal PPI, Switzerland).

Follow up

According to preoperative serum markers level, the patients were divided into two groups, and the clinicopathological and cytopathological features were compared. Tumor condition was observed by CT scanning every 3-6 months. All the enrolled patients were followed up for 36 months, the postoperative recurrence rates and survival rates were compared and analyzed. Progression-free survival (PFS) of immune indices ($CD3^+$, $CD4^+$, $CD8^+$, NK^+, $CD19^+$), AFP level and immunoglobulin were calculated.

Table 1. HCC preoperation cell immunity indices on recurrence rate and survival time

Marker	*n*	*3-year recurrence rate*	χ^2 *(P)*	*Median survival time (months)*	*Log-rank*	*P*
HBsAg (S/CO)	78		4.154 (0.039)		4.08	0.043
> 2319	48	24 (50%)		32		
≤ 2319	30	8 (27%)		50		
AFP (ng/ml)	75		0.499 (0.494)		0.242	0.622
≥ 25	34	13 (38%)		41		
< 25	41	19 (46%)		36		
$CD3^+$ (%)	111		20.124 (0.000)		4.572	0.033
≥ 67	57	24 (42%)		32		
< 67	54	3 (6%)		48		
$CD4^+$ (%)	99		13.254 (0.001)		4.009	0.045
≥ 47.2	30	14 (47%)		24		
< 47.2	69	9 (13%)		46		
$CD8^+$ (%)	74		4.894 (0.040)		4.122	0.042
≥ 30.8	44	18 (41%)		24		
< 30.8	30	5 (17%)		41		
NK^+ (%)	61		2.634 (0.127)		3.875	0.049
≥ 9.0	33	12 (36%)		46		
< 9.0	28	16 (57%)		32		
$CD19^+$ (%)	27		1.807 (0.257)		3.862	0.049
≥ 8.7	13	8 (62%)		18		
< 8.7	14	5 (36%)				
IgA (g/L)	49		0.980 (0.393)		4.666	0.031
≥ 2.64	21	9 (43%)		48		
< 2.64	28	16 (57%)		24		
IgG (g/L)	52		7.879 (0.011)		7.472	0.006
≥ 10	30	20 (67%)		32		
< 10	22	6 (27%)		46		
IgM (g/L)	63		0.339 (0.616)		0.770	0.380
≥ 0.98	33	13 (39%)		46		
< 0.98	30	14 (47%)		35		

Statistical analysis

Statistical analysis was done with MedCalc statistical software (version 12.7.8). Quantitative data such as percentage of lymphocyte subsets, AFP and Ig levels were presented as mean ± standard deviation (SD). Three-year recurrence rates were detected by *Pearson*'s Chi-Square Statistic and Fisher's exact test. Comparisons of pre and post therapy between and within groups were analyzed by using repeated measures, and qualitative data were compared between groups with the t-test. Multiple logistic regression analyses were performed to ascertain the independent risk factors for HCC postoperative prognosis. The survival curves were calculated using the Kaplan-Meier method. A value of $P < 0.05$ was considered statistically significant.

Results

Comparison before and after treatment

In a subsequent cohort of 158 additional HCC patients, prospective flow cytometric immune-monitoring analysis was performed to identify specific changes on distinct lymphocyte subsets (i.e., CD3, CD4, CD8 T, and CD19 B lymphocytes) and NK cells opposite numbers. The $CD4^+/CD8^+$ ratio, $CD3^+$ cells and NK^+ cells in the HCC group were significantly lower than in the healthy controls, but no significant differences were found in the other variables of lymphocyte

Table 2. Immunity indices before and after treatment (mean ± SD)

Group	$\bar{x}$±s		t	P
	preoperation	postoperation		
$CD3^+$	71.94±12.54	74.06±6.53	-0.940	0.358
$CD4^+$	41.82±10.83	44.83±8.17	-1.293	0.210
$CD8^+$	30.30±6.35	30.15±7.51	0.125	0.902
NK^+	9.57±4.49	9.21±5.64	0.986	0.336
$CD19^+$	13.13±7.23	11.00±5.33	1.573	0.132
IgA	2.59±1.22	3.16±1.94	-2.731	0.018
IgG	10.26±2.39	9.18±2.03	1.415	0.182
IgM	0.97±0.77	1.16±0.42	-0.333	0.745

subset. The markers level were not both statistically significant with age, gender, grade, tumor size (P > 0.05).

Through paired comparison before and after treatment, the differences between preoperation and postoperation group, exclude were not both statistically significant (both P > 0.05), whereas IgA group was not (P < 0.05), as shown in **Table 2**.

We found that lack of recovery of CD19, CD3, CD8, and especially CD4 T cells was linked to poor patient survival. The percentages of $CD3^+$, $CD4^+$, IgA and IgM were increased from (71.94 ± 12.54)%, (41.82 ± 10.83)%, (2.59 ± 1.22)%, and(0.97 ± 0.77)% to (74.06 ± 6.53)% (P = 0.358), (44.83 ± 8.17)% (P = 0.210), (3.16 ± 1.94)% (P = 0.018), and (1.16 ± 0.42)% (P = 0.745) respectively after hepatectomy; while the percentages of $CD8^+$, NK^+, $CD19^+$ and IgG were decreased from (30.30 ± 6.35)%, (9.57 ± 4.49)%, (13.13 ± 7.23)% and (10.26 ± 2.39)% to (30.15 ± 7.51)% (P = 0.902), (9.21 ± 5.64)% (P = 0.336), (11.00 ± 5.33)% (P = 0.132)and (9.18 ± 2.03)% (P = 0.182). The 3-year recurrence rates of high level group on HBsAg, CD3, CD4, CD8, CD19 and IgG were larger than that of low level group, while the 3-year recurrence rates of high level group on AFP, NK, IgA and IgM smaller than that of low level group (**Table 1**) in study group.

Changes in cell immunity indices

The immunologic markers examined included the plasma levels of $CD3CD4^+$, $CD8^+$, NK^+, $CD19^+$, AFP, HBsAg, IgA, IgG, IgM. The $CD3^+$ cells, $CD4^+$ cells, IgA and IgM slightly increased after operation (P > 0.05). Preoperative clinicopathologic variables, including $CD3^+$, $CD4^+$, $CD8^+$ and NK^+, $CD19^+$, AFP, HBsAg, IgA, IgG, IgM were analyzed by statistical software. $CD3^+$, $CD4^+$, $CD8^+$ and NK^+, $CD19^+$, HBsAg, IgA, IgG above were statistically different (both P < 0.05), AFP and HBsAg were no difference (P > 0.05), as shown in the **Table 1**.

Three-year recurrence rates of HBsAg on high level group were higher than that of low level group (50% vs 27%). According to preoperative serum AFP level, the patients were divided into AFP < 25 ng/mL group and

AFP ≥ 25 ng/mL group, 3-year recurrence rates were compared (38% vs 46%) those were not statistically significant (P = 0.494). The patients were divided into $CD3^+ \geq 67\%$ group and $CD3^+ <$ 67% group, 3-year recurrence rates were compared (42% vs 6%) those were statistically significant (P = 0.000).

Follow up

All patients received consecutive follow up for 3 years. Three-year recurrence rates between the two groups of HBsAg, $CD3^+$, $CD4^+$, $CD8^+$ and IgG showed statistically differences (P < 0.05), however, 3-year recurrence rates between the two groups of AFP, NK^+, $CD19^+$, IgA and IgM showed no statistical difference (P > 0.05), as shown in **Table 1**.

The Kaplan-Meier method was employed to analyze the correlation between preoperative serum AFP, HBsAg, IgA, IgG, IgM levels and the prognosis of HCC. The relation of plasma $CD3^+$, $CD4^+$, $CD8^+$, NK^+, $CD19^+$ and the prognosis of HCC were tested by the Log-rank method and the survival curves in **Figure 1**. Our results show that AFP and IgM levels was not correlated with prognosis. Among them, $CD3^+$, $CD4^+$, $CD8^+$, NK^+, $CD19^+$, HBsAg, IgA, IgG were correlated with prognosis.

We investigated the relationships between hepatocellular carcinomaprognosis and changes in not only T-lymphocyte subtypes but also immunoglobulin (Ig) and alpha-fetoprotein (AFP) levels. We performed relative cell counts of T and B cells from 158 hepatocellular carcinoma patients and examined the AFP and IgG levels of these patients. Patients whose $CD3^+$ T cell counts decreased after surgery had a lower 3-year progression-free survival rate than patients whose $CD3^+$ T cell counts increased after surgery. Patients whose $CD8^+$ T cell counts

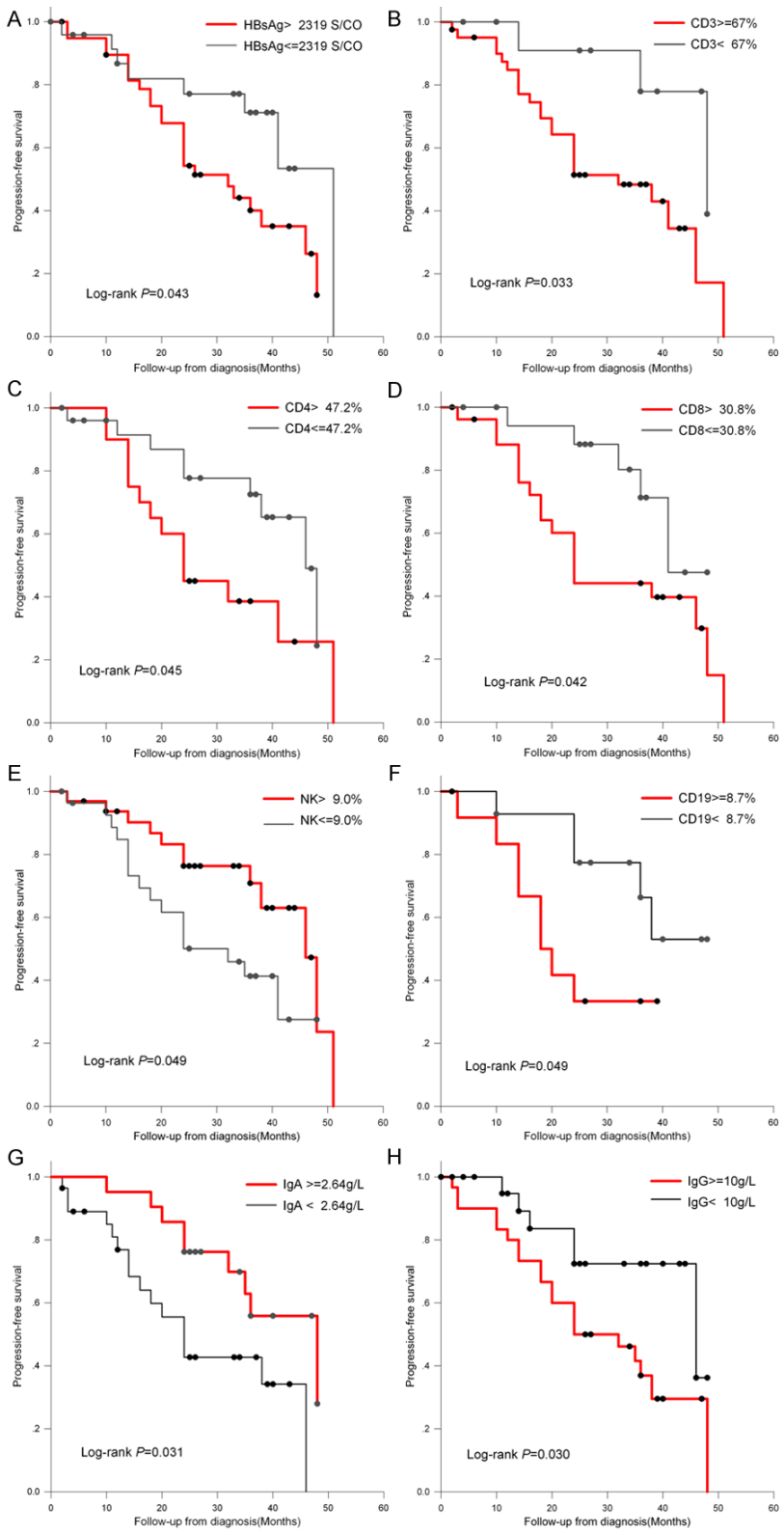

Figure 1. Preoperation HBsAg, $CD3^+$, $CD4^+$, $CD8^+$, NK^+, $CD19^+$, IgA, IgG progression-free survival curve for patients with hepatocellular carcinoma. A. HBsAg; B. $CD3^+$; C. $CD4^+$; D. $CD8^+$; E. NK^+; F. $CD19^+$; G. IgA; H. IgG.

increased after surgery had a lower 3-year progression-free survival rate than patients whose $CD8^+$ T cell counts decreased after surgery. There was no correlation between changes in the populations of T-lymphocyte subtypes and changes in AFP levels. Absolute T-lymphocyte subtype counts and IgG levels reflected changes in immune function; in addition, this information and AFP data can be used in evaluations of the prognoses of hepatocellular carcinomapatients. Notably, the relative abundance of $CD3^+$, $CD4^+$, $CD8^+$, NK^+ cells appeared to be correlated with patient survival.

Multivariate analysis

We performed univariate analysis of risk factors for HCC postoperative recurrence and survival, including age, gender, HBsAg-positive status, family history of liver cancer, tumor volume, tumor diameter, and AFP level were not statistically significant ($P > 0.05$). Moreover, multiple logistic regression analysis indicated that $CD4^+$, $CD8^+$ and NK^+ were closely correlated with HCC postoperative survival rate ($P = 0.00329$) ($Y = 1.262 \times CD4^+ + 1.448 \times CD8^+ - 0.646 \times NK^+$). Construction of *Cox*'s proportional hazards regression model showed IgA and IgG were correlated with HCC postoperative survival rate at a trend ($P = 0.079$) ($Y = 127.9 \times IgG - 28.7 \times IgM$) (**Figure 2**). However, AFP and IgM were not associated with the out-

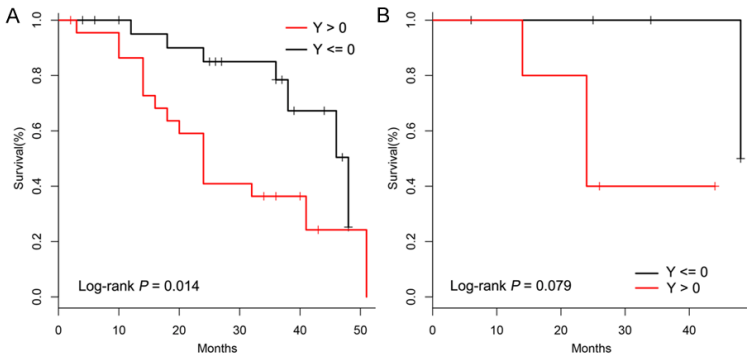

Figure 2. Construction of Cox's proportional hazards regression model on immunity indices. A. $CD4^+$, $CD8^+$, NK^+ mod prediction; B. IgA, IgG mod prediction.

come of HCC. In summary, our results suggest that IgM and IgG- two-component-determined circulating immune complex (TCIC) may play an important role in immune regulation during the course of malignancies and may be a hallmark for cancer pathogenesis. Our findings thus provide new insights into immune regulation in patients with HCC.

Construction of Cox's proportional hazards regression model

Coxph (formula = Surv (Survival, Events)~CD4 + CD8 + NK, data = cox. data)

	Coef	Exp (coef)	Se (coef)	Z	P
CD4	1.262	3.534	0.467	2.270	0.0068
CD8	1.448	4.255	0.542	2.67	0.0075
NK	-0.646	0.524	0.461	-1.40	0.1600

Likelihood ratio test = 13.4 on 3 df, p = 0.00386, n = 46, number of events = 23 (16 observations deleted due to missingness).

$Y = 1.262 \times CD4^+ + 1.448 \times CD8^+ - 0.646 \times NK^+$

Coxph (formula = Surv (Survival, Events)~IgG + IgM, data = cox.data)

	Coef	Exp (coef)	Se (coef)	Z	P
IgG	127.9	3.57e + 55	26.38	4.85	1.2e-06
IgM	-28.7	3.29e-13	5.98	-4.81	1.5e-06

Likelihood ratio test =11.4 on 2 df, p = 0.00329, n = 10, number of events = 4.

$Y = 127.9 \times IgG - 28.7 \times IgM$

Discussion

T cell-mediated immune responses represent the main cellular antitumor immunity in cancer patients. The purpose of current study is to evaluate whether surgical procedure might change a systemic antitumor immunity, particularly T lymphocyte-mediated immunity in cancer patients. Correlation analyses of immunoglobulin and T-lymphocyte subtypes in hepatocellular carcinomapatients.

The presence of $CD8^+$ T cell responses to tumor associated antigens have been reported in patients with different malignancies. The kinetic and the pattern of T helper 1 and cytotoxic T lymphocyte responses to AFP, a tumor rejection antigen in HCC [4].

One potential underlying cause of this finding is that patients with AFP levels that decreased to normal levels had highly necrotic tumors that were relatively incapable of promoting immunosuppression in the body. The resulting preservation of higher levels of immune function was associated with greater anti-tumor capabilities and increased tumor necrosis, which contributed to decreases in AFP levels. AFP indirectly promotes tumor growth by producing a broad range of immunosuppressive effects; in particular, phenomena suppressed by AFP include macrophage function, mitogen-induced T cell proliferation, theallogeneic mixed lymphocyte reaction, natural killer (NK^+) cell cytotoxicity, and interferon (IFN) and tumor necrosis factor (TNF) activity. Decreases in AFP levels directly contribute to enhanced immune function.

Prior to surgery, $CD4^+$ cell count is not correlated with AFP level but is correlated with tumor capsule condition, status with respect to the invasion of the outer hepatic membrane, and the level of hepatitis B virus (HBV) DNA.

The quantity and cytotoxic activity of $CD8^+$ T cells could decrease in response to increases in the quantity of $CD4^+$ T cells. This decrease could lead to reductions in immune surveillance and immunoprotective functions and the indirect induction of tumorigenesis and cancer progression. In addition, studies have demonstrated that tumor cells can secrete Fas ligand (FasL) and that cancer patients may exhibit elevated soluble FasL (sFasL) levels. These

phenomena could explain why apoptosis is induced in $CD8^+$ T cells. By defending against $CD8^+$ T lymphocytes via FasL, tumor cells can evade immune surveillance, leading to tumor immune escape. This mechanism is considered to be another route through which a tumor can induce local immunosuppression. From the twoaforementioned mechanisms, we conclude that multiple inhibitory factors in the tumor microenvironment induce decreases in the quantity of $CD8^+$ T cells or promote apoptosis among these cells. These effects decrease anti-tumor immune activity and provide the means by which tumor cells escape immune surveillance.

$CD8^+$ T cells could kill tumor cells and inhibit the metastasis of liver cancer cells. Decreased $CD8^+$ T cell counts might be a risk factor indicating tumor metastasis and could therefore be associated with poor post-surgical prognosis.

The $CD4^+/CD8^+$ T cell ratio was decreased and even inverted in certain patients. This result is consistent with the data presented in other literature reports. A decreased $CD4^+/CD8^+$ ratio indicates a trend of immune suppression and abnormalities in immune regulatory mechanisms. A comparison of positivity rates for AFP and T-lymphocyte subtypes in HCC patients revealed that positivity rates were significantly greater for T-lymphocyte subtypes than for AFP. This finding indicates that in the human body, the immune system produces the first response to attacks by viruses and other pathogens. AFP could alter the $CD4^+/CD8^+$ ratio and induce lymphocyte death; moreover, certain domains of AFP could inhibit the T cell immune response.

Currently, two primary molecular mechanisms have been shown to play a role in CTL induced target cell death: the Fas ligand/Fas pathway and the perforin/Granzyme B pathway [5]. Both induce cell death directly in target cells via caspase activation. Hayashida et al [6] reported that perforin/Granzyme B played a dominant role on CTL-induced hepatocyte apoptosis. Moreover, there was increased IL-2 production in the experimental groups including $CD4^+$ T cells, indicating that inclusion of the $CD4^+$ T cells have a helperrole for the anti-tumor activity.

In this study, hepatocellular carcinoma patients are immunosuppressed due to interactions among multiple factors. In particular, T cells may be inhibited not only by complexes formed by tumor antigens and target-specific antibodies but also by immune suppressors released by tumor cells. Hepatocellular carcinoma metastasis and relapse are multistep pathological processes that result from the interplay between genetic mutations in liver carcinomas and patients' microenvironments. Investigations of the biological characteristics of liver carcinomas and factors relevant to the host's immune system could not only facilitate a better understanding of the underlying mechanisms of HCC relapse and metastasis but also contribute to improving the accuracy of prognoses. This research sought to predict HCC metastasis and relapse from a new perspective by examining changes in the quantities of $CD4^+$, $CD8^+$ and NK cells in early-stage hepatocellular carcinoma patients and assessing how trends in these changes influence HCC prognoses.

Due to the presence of multiple and complex factors influencing the immunologic function, it is difficult to balance all the known and unknown factors among groups. In order to minimize the influence of the former factors on the results, HCCs who were naïve to any treatment were recruited to evaluate the short term influence (within 1 month) of different interventional treatments on the immune function, tumor marker and liver function. This was associated with the higher numbers of $CD4^+$ T cells producing TNF-α, IFN-γ and IL-17, IFN-γ producing NK cells, IL-4 and IL-17 producing NKT cells [7].

Recently, targeted therapy and palliative chemotherapy have been the main treatment approaches for patients with advanced HCC. New targeted drugs such as sorafenib and sunitinib have improved clinical efficacy. However, the serious side effects and high costs of these drugs make it impossible for many patients to complete the entire treatment course [8, 9]. Thus, it is necessary to investigate alternative therapies for patients with advanced HCC. The special biological behavior of primary HCC, such as multiple lesions, existing hepatitis and cirrhosis, continuously suppresses cellular immunity and results in immunedys function. Autoimmune disorders in patients with HCC is manifested by lower levels of $CD4^+$, $CD3^+$ and NK^+ cells and significantly higher levels of $CD8^+$ cells [10, 11].

Under the electron microscope, AFP negative HCC cells show simple organelles and rich free ribosomes; in AFP-positive ones, rich organelles, particularly the rough endoplasmic reticulum, and mitochondria and Golgi complex can be clearly observed in the cytoplasm. Whether these features are associated with AFP expression in HCC needs further study [12].

Naturalkiller (NK) cells represent the main effector population of the innate immune system and are abundant in the human liver. Recently, it has been demonstrated that NK cells not only exhibit antiviral functions but may also regulate adaptive immune responses by deletion of HBV-specific $CD8^+$ T cells [13]. The decrease of NK cell activity in patients with primary hepatocellular carcinoma is closely related to their lower expression of natural killer group 2D (NKG2D). Liver function affects the expression of NKG2D and the activity of NK cells [14]. NK cells, the main innate immune cells that target viral infections, play important roles in the eradication of HBV from hepatocytes. NK cells carry several stimulatory and inhibitor receptors, and binding of receptors with their ligands results in activation and suppression of NK cells, respectively. There is the potential to reduce the risk of HCC or cirrhosis of the liver by targeting NK cells [15].

Cytotoxic T-cell infiltration of the tumors is indicative of a better survival, whereas the predominance of suppressor cells is associated with a worse outcome and lower survival rates. Finally, certain therapeutic strategies, including radiofrequency ablation and chemoembolization, can enhance the release and exposure of tumor antigens, which might help to overcome the immune tolerance towards the tumor. Therefore, such immune-stimulating therapeutic interventions in combination with immunotherapy strategies represent a promising future approach for HCC treatment [16]. Our results do not square with other report [17], our results showed that patients who had higher preoperative $CD4^+$, $CD8^+$, NK^+ levels were more likely to experience recurrence on follow up. We need longer a follow-up period to confirm the long-term prognosis.

Apart from this, a long follow-up period was in our study for evaluating the relationship between immune indicator and recurrence as well as survival. More prospective studies with better design and larger sample size are needed to further confirm our findings. Better understanding of the changes in the immune system in HCC patients following treatment will be beneficial for the selection of drug, determination of the time for treatment and the prevention of complications on the basis of further research.

Conclusions

Results showed the $CD3^+$, $CD4^+$, $CD8^+$, NK^+, $CD19^+$, IgA, IgG level were some important prediction factor for the recurrence and prognosis of HCC after resection, and it could be used to evaluate the prognosis of HCC as an independent influential factor.

HCC prognosis is affected not only by metastasis-related genes but also by host genes related to inflammatory and immune responses. Immunity indices can efficiently improve immunological status, and may prolong survival in HCC patients. This opinion provides important insights regarding improvements in future treatment plans and studies of prediction models.

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Disclosure of conflict of interest

None.

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