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ORIGINAL ARTICLE

Effect of RFA and TACE combined with postoperative cytokine-induced killer cell immunotherapy in primary hepatocellular carcinoma

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Summary

Purpose: *To evaluate the efficacy and safety of radiofrequency ablation (RFA) and transcatheter arterial chemoembolization (TACE) combined with postoperative cytokine-induced killer (CIK) cell immunotherapy in the treatment of primary hepatocellular carcinoma (HCC).*

Methods: *The clinical data of 116 patients with primary HCC were collected. 58 patients were treated with RFA+TACE (RFA+TACE group), and the other 58 patients underwent RFA+TACE+CIK cell immunotherapy (RFA+TACE+CIK group). Before and after treatment, the proportions of cluster of differentiation 3$^+$ ($CD3^+$), $CD3^+CD4^+$, and $CD3^+CD8^+$ T cells, regulatory T cells (Tregs) and natural killer (NK) cells and the $CD4^+/CD8^+$ ratio were detected via flow cytometry, and the levels of serum interferon-γ (IFN-γ), interleukin-2 (IL-2) and IL-6 were detected via enzyme-linked immunosorbent assay (ELISA). The incidence of adverse reactions and the quality of life score of patients after treatment were compared between the two groups, and the patient's survival status was recorded through follow-up.*

Results: *After treatment, the levels of $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD4^+/CD8^+$ ratio, Tregs and NK cells were significantly higher, while the level of $CD3^+CD8^+$ T cells was significantly lower in RFA+TACE+CIK group than those in RFA+TACE group. After treatment, the level of alpha fetoprotein (AFP) obviously declined in both groups compared with that before treatment, and it was significantly lower in RFA+TACE+CIK group than that in RFA+TACE group. After treatment, the scores of the QLQ-C30 questionnaire were all significantly higher in RFA+TACE+CIK group than those in RFA+TACE group. After treatment, the general functioning score rose from (58.55±11.82) and (59.39±10.97) points to (74.74±15.58) and (68.42±14.85) points, respectively, in RFA+TACE+CIK group and RFA+TACE group, and it was significantly higher in RFA+TACE+CIK group than that in RFA+TACE group. According to the follow-up results, the mean overall survival (OS) of patients was (42.1±5.6) months and (37.8±4.8) months, and the 5-year OS rate was 29.3% (17/58) and 13.8% (8/58), respectively, in RFA+TACE+CIK group and RFA+TACE group. The results of log-rank test showed that the OS in RFA+TACE+CIK group was significantly superior to that in RFA+TACE group.*

Conclusions: *RFA and TACE combined with postoperative autologous CIK cell reinfusion have significant efficacy in the treatment of primary HCC, which can enhance the immune function, improve the postoperative quality of life and raise the survival rate of patients, with tolerable adverse reactions.*

Key words: *radiofrequency ablation, transcatheter arterial chemoembolization, cytokine-induced killer cells, primary hepatocellular carcinoma*

Introduction

Hepatocellular carcinoma (HCC) is one of the common malignant tumors, and it is mainly treated with surgery-based comprehensive treatment supplemented by interventional therapy, local radiotherapy and chemotherapy currently [1, 2]. Transcatheter arterial chemoembolization (TACE)

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has the dual functions of diagnosis and treatment, and it is not restricted by the location and size of tumors, so it can help find and label micro-lesions in the liver [3]. The combination of TACE and RFA can reduce the times of TACE and effectively protect the liver function. Therefore, TACE combined with RFA has been gradually recognized in the treatment of HCC [4, 5]. However, HCC is prone to recurrence in most patients after treatment, and the long-term survival rate remains unsatisfactory.

HCC mostly occurs based on chronic hepatitis, and there are multiple immunosuppressive mechanisms in its microenvironment, interfering with the body's normal immune response, which is one of the important causes of tumor growth, recurrence and metastasis. Immunotherapy has gradually become an important adjuvant therapy for HCC [6, 7]. Cytokine-induced killer (CIK) cells refer to cytotoxic lymphocytes obtained by *in vitro* co-culture of human peripheral blood mononuclear cells and multiple cytokines, and they are not restricted by the major histocompatibility complex (MHC). CIK cells are characterized by simultaneous expression of cluster of differentiation 3 (CD3) and CD56, strong proliferation ability, great lethality, and small toxic and side effects [8]. Studies have shown that CIK cell reinfusion combined with TACE and RFA is safe and feasible in the treatment of HCC, and it can reduce the tumor recurrence rate, prolong the progression-free survival, and improve the immune status and quality of life [9, 10]. In this study, the clinical data of primary HCC patients treated with CIK cell reinfusion combined with TACE and RFA were retrospectively analyzed, and the clinical efficacy and safety of the treatment were explored.

Methods

General data

The clinical data of 116 patients diagnosed with HCC clinically or pathologically were collected. Inclusion criteria: 1) patients diagnosed with HCC clinically or pathologically according to the diagnostic criteria of the American Society of Hepatology, 2) those aged >18 years old, 3) those with the diameter of a single tumor ≤5 cm or the maximum diameter ≤3 cm of ≤3 tumors, 4) those treated with TACE + RFA for the first time, 5) those with Child-Pugh class A or B of liver function, and 6) those with an expected survival time ≥3 months. Exclusion criteria: 1) patients with severe liver dysfunction (Child-Pugh class C), 2) those suffering from extrahepatic metastasis or portal vein tumor thrombosis at the

Table 1. Demographics and general clinical data of all studied patients

Parameters	*RFA+TACE+CIK group (n=58)* *n (%)*	*RFA+TACE group (n=58)* *n (%)*	*p value*
Gender (Male/Female)	41/17	36/22	0.432
Age (years), mean±SD	57.64±9.51	58.88±10.03	0.496
Cell morphological classification			0.208
Massive type	46 (79.3)	39 (67.2)	
Multiple nodules type	12 (20.7)	19 (32.8)	
Number of tumor lesions			0.426
1	37 (63.8)	42 (72.4)	
≥2	21 (36.2)	16 (27.6)	
Largest tumor diameter (cm), mean±SD	3.28±0.65	3.11±0.59	0.143
HBsAg (+)	50 (86.2)	54 (93.1)	0.361
Child-Pugh class			0.452
A	31 (53.4)	36 (62.1)	
B	27 (46.6)	22 (37.9)	
AFP (μg/L)			0.187
≥400	30 (51.7)	38 (65.5)	
<400	28 (48.3)	20 (34.5)	
Blood vessel tumor thrombus	36 (62.1)	42 (72.4)	0.323
KPS score			0.456
80-90	34 (58.6)	29 (50.0)	
70-80	24 (41.4)	29 (50.0)	

TACE: Transcatheter arterial chemoembolization; RFA: Radiofrequency ablation; CIK: Cytokine-induced killer; AFP: Alpha fetoprotein; KPS: Karnofsky Performance Status.

time of initial treatment, 3) those who used to undergo surgical resection, liver transplantation, chemotherapy, radiotherapy or other antitumor therapies, 4) those with severe disease in the heart, lungs, kidney or blood system, or 5) those accompanied by other primary malignant tumors. According to different therapeutic regimens, the patients were divided into RFA+TACE group (treated with RFA+TACE, n=58) and RFA+TACE+CIK group (treated with RFA+TACE+CIK, n=58). There were 77 males and 39 females aged 23-79 years old, with an average of 58.4 years old. The baseline data had no statistically significant differences between the two groups (p>0.05), and they were comparable (Table 1). All patients enrolled signed the informed consent in accordance with the *Declaration of Helsinki*. This study was approved by the Ethics Committee of Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine.

Treatment methods

RFA + TACE: The B-mode ultrasound-guided puncture biopsy of liver tumor was first performed for all patients. According to the size and location of the tumor, multi-point multi-needle RFA was conducted (about 12 min/time), and the ablation area should be 1 cm beyond the tumor as far as possible. As for TACE, the right femoral artery was punctured using Seldinger technique with the support of digital subtraction angiography equipment. Then arteriography was performed for the celiac trunk and superior mesenteric artery to determine the blood supply of intrahepatic lesions, followed by chemoembolization (40-60 mg of pirarubicin, 6-12 mg of mitomycin, 20-60 mg of cisplatin, and an appropriate amount of iodized oil based on the condition of lesions).

Culture and reinfusion of CIK cells: 50 mL of peripheral blood was taken, cultured in a culture flask containing interferon-γ (IFN-γ) (1000 U/mL) for 24 h, and then cultured with interleukin-2 (IL-2) (1000 U/mL) and IL-1α (1000 U/mL). 2 weeks later, CIK cells (over 1×10^{10}) were collected, centrifuged, washed and resuspended with 100 mL of normal saline containing 3-5 mL of 20% human serum albumin. If the CIK cell preparations met the safety standards for clinical use (cell survival rate ≥95%), the results of flow cytometry met the "CIK" criteria ($CD3^+$ ≥70%, $CD3^+/CD56^+$ ≥30%, $CD8^+$ ≥40%), and the samples were qualified in the sterility test on the 1st and 5th d of preparation and at 30 h before sampling, CIK cells could be intravenously reinfused within 30 min ($1.0\text{-}1.3\times10^{10}$/time) for a total of 6 cycles. Whether adverse reactions such as fever and allergy occurred was closely observed during reinfusion and within 2-3 h after reinfusion.

Observation indexes

The changes in levels of liver function indexes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL), and serum alpha fetoprotein (AFP) were compared between the two groups before and after treatment. The proportions of peripheral blood lymphocyte subsets [$CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, $CD4^+/CD8^+$ ratio, and regulatory T cells (Tregs)] and natural killer (NK) cells were detected *via* flow cytometry before and after treatment. The peripheral venous blood was collected and centrifuged in both groups before and after treatment, and the separated serum was harvested to determine the levels of IL-2, IL-6 and IFN-γ *via* enzyme-linked immunosorbent assay (ELISA). To compare the changes in quality of life between the two groups before and after treatment, the European Organisation for Research and Treatment for Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) questionnaire was used, including 5 dimensions (physical function, role function, emotional function, cognitive function, social function and overall quality of life), and the score of each function was compared between the two groups. The incidence of adverse reactions during treatment was recorded.

The clinical data of patients were collected through outpatient follow-up or telephone follow-up, the blood routine, liver function and tumor markers examinations, enhanced CT or MRI were performed, and the patient's survival status was recorded. Overall survival (OS) refers to the duration from diagnosis with primary HCC to death or the end of follow-up. The follow-up was ended in January 2020, and the data of those lost to follow-up were processed as the censored value at the last valid follow-up in the survival analysis.

Statistics

SPSS 22.0 software was used for statistical analysis. Measurement data were expressed as mean ± standard deviation, and two-sample t-test was performed for intergroup comparison. Enumeration data were expressed as rate (%), and compared between groups using x^2 test. The survival curves were plotted using the Kaplan-Meier method, and log-rank test was performed to determine whether there was a statistically significant difference in the survival rate between two groups. P<0.05 suggested statistically significant difference.

Results

Levels of peripheral blood immune cells before and after treatment

Before treatment, there were no statistically significant differences in the levels of $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8+$ T cells, $CD4^+/CD8^+$ ratio, Tregs and NK cells between the two groups (p>0.05). After treatment, the levels of peripheral blood $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD4^+/CD8^+$ ratio, Tregs and NK cells significantly rose (p<0.05), while the level of $CD3^+CD8^+$ T cells significantly declined in both groups compared with those before treatment (p<0.05). After treatment, the levels of $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD4^+/CD8^+$ ratio, Tregs and NK cells were significantly higher, while the level of $CD3^+CD8^+$ T cells was significantly lower in RFA+TACE+CIK group than those in RFA+TACE group (p>0.05) (Table 2).

Table 2. Comparison of immunological indicators of patients in the two studied groups

	RFA+TACE+CIK group (n=58)	*RFA+TACE group (n=58)*	*p value*
$CD3^+$ T cell (%)			
Pretreatment	65.78±12.39	64.80±11.82	0.664
Posttreatment	69.93±10.13	67.12±9.46	0.045
$CD3^+CD4^+$ T cell (%)			
Pretreatment	30.03±6.20	30.45±6.26	0.717
Posttreatment	38.61±7.98	32.97±7.64	0.001
$CD3^+CD8^+$ T cell (%)			
Pretreatment	32.13±5.86	31.85±5.76	0.796
Posttreatment	27.41±6.59	30.08±6.79	0.034
$CD4^+/CD8^+$ ratio			
Pretreatment	0.92±0.44	0.97±0.37	0.509
Posttreatment	1.38±0.46	1.16±0.49	0.014
Treg cell (%)			
Pretreatment	7.74±3.84	7.42±3.91	0.657
Posttreatment	9.53±3.90	7.95±3.78	0.029
NK cell (%)			
Pretreatment	2.34±1.33	2.69±1.15	0.132
Posttreatment	8.32±2.16	5.27±2.50	0.001

TACE: Transcatheter arterial chemoembolization; RFA: Radiofrequency ablation; CIK: Cytokine-induced killer; NK: Natural Killer.

Table 3. Comparison of liver function indexes and serum inflammatory factors of patients in the two studied groups

	RFA+TACE+CIK group (n=58)	*RFA+TACE group (n=58)*	*p value*
ALT (U/L)			
Pretreatment	44.78±20.42	46.15±21.74	0.727
Posttreatment	53.41±11.58	56.29±10.69	0.167
AST (U/L)			
Pretreatment	47.17±20.77	49.26±20.62	0.588
Posttreatment	52.34±12.02	54.75±12.08	0.284
TBIL (μmol/L)			
Pretreatment	15.41±4.23	14.83±4.66	0.484
Posttreatment	17.67±5.13	16.68±5.10	0.299
AFP (ng/ml)			
Pretreatment	408.20±123.16	417.41±117.83	0.682
Posttreatment	154.61±22.72	178.69±31.23	0.001
IFN-γ (ng/L)			
Pretreatment	20.23±2.06	19.74±2.21	0.219
Posttreatment	23.25±3.49	24.33±2.90	0.073
IL-2 (ng/L)			
Pretreatment	8.36±1.87	8.63±1.55	0.399
Posttreatment	12.46±2.74	13.01±2.36	0.117
IL-6 (ng/L)			
Pretreatment	6.06±0.89	6.35±1.04	0.109
Posttreatment	5.42±1.27	5.78±1.45	0.072

TACE: Transcatheter arterial chemoembolization; RFA: Radiofrequency ablation; CIK: Cytokine-induced killer; ALT: Alanine transaminase; AST: Aspartate aminotransferase; TBIL: Total bilirubin; AFP: Alpha fetoprotein; IFN: Interferon; IL: Interleukin.

Comparison of levels of liver function indexes before and after treatment

Before treatment, no statistically significant differences were found in the levels of liver function indexes ALT, AST, TBIL and AFP between the two groups (p>0.05). After treatment, the levels of serum ALT, AST and TBIL rose in both groups compared with those before treatment, and they had no statistically significant differences between the two groups (p>0.05). After treatment, the level of AFP obviously declined in both groups compared with that before treatment, and it was significantly lower in RFA+TACE+CIK group than that in RFA+TACE group (p<0.001).

Comparison of levels of serum inflammatory factors before and after treatment

The levels of serum IFN-γ, IL-2 and IL-6 had no statistically significant differences between the two groups before treatment (p>0.05). After treatment, the levels of serum IFN-γ and IL-2 were increased, but the level of IL-6 was decreased in both groups, while they had no statistically significant differences between the two groups (p>0.05) (Table 3).

Comparison of quality-of-life score between the two groups after treatment

Before treatment and at 1 month after treatment, the patient's life status was recorded. According to the EORTC QLQ-C30 questionnaire, the physical functioning, role functioning, emotional functioning, cognitive functioning and social functioning in the function module were all greatly improved in both groups after treatment, and the scores were all significantly higher in RFA+TACE+CIK group than those in RFA+TACE group (p<0.05). After treatment, the general functioning score rose from (58.55±11.82) and (59.39±10.97) points to (74.74±15.58) and (68.42±14.85) points, respectively, in RFA+TACE+CIK group and RFA+TACE group, and it was significantly higher in RFA+TACE+CIK group than that in RFA+TACE group (p=0.027) (Table 4).

Comparison of incidence of adverse reactions between the two groups

The main treatment-related adverse reactions included fever, nausea and vomiting, hepatalgia, lumbago and backache, fatigue and poor appetite, transaminase elevation and jaundice. There were 9 (15.5%) cases and 15 (25.9%) cases of hepatalgia, 6 (10.3%) cases and 8 (13.8%) cases of lumbago and backache, 12 (20.7%) cases and 17 (29.3%) cases of transaminase elevation, and 7 (12.1%) cases and 5 (8.6%) cases of jaundice, respectively, in RFA+TACE+CIK group and RFA+TACE group. The adverse reactions were tolerable for all patients and improved after symptomatic treatment. The incidence of adverse reactions had no statistically significant difference between the two groups (p>0.05) (Table 5).

Table 4. Comparison of posttreatment EORTC-QLQ-C30 scale scores of the studied patients in two different groups

Complications	*RFA+TACE+CIK group (n=58)*	*RFA+TACE group (n=58)*	*p value*
Physical Functioning			
Pretreatment	56.72±10.35	57.41±11.03	0.729
Posttreatment	75.22±15.45	68.58±16.18	0.026
Role Functioning			
Pretreatment	55.93±14.64	56.86±15.59	0.741
Posttreatment	74.75±17.63	67.14±16.90	0.019
Emotional Functioning			
Pretreatment	57.90±13.35	57.10±14.66	0.759
Posttreatment	74.53±12.15	68.07±17.54	0.023
Cognitive Functioning			
Pretreatment	65.16±12.98	65.93±13.09	0.751
Posttreatment	77.39±14.28	70.44±15.52	0.014
Social Functioning			
Pretreatment	56.38±12.78	57.63±13.73	0.613
Posttreatment	69.36±13.65	63.83±16.41	0.041
General Functioning			
Pretreatment	58.55±11.82	59.39±10.97	0.692
Posttreatment	74.74±15.58	68.42±14.85	0.027

TACE: Transcatheter arterial chemoembolization; RFA: Radiofrequency ablation; CIK: Cytokine-induced killer.

Table 5. Comparison of adverse reactions of patients in the two studied groups

	RFA+TACE+CIK group (n=58) *n (%)*	*RFA+TACE group (n=58)* *n (%)*	*p value*
Fever	10 (17.2)	8 (13.8)	0.798
Nausea and vomiting	19 (32.8)	16 (27.6)	0.686
Hepatalgia	9 (15.5)	15 (25.9)	0.252
Lumbago and backache	6 (10.3)	8 (13.8)	0.777
Fatigue and poor appetite	40 (69.0)	35 (60.3)	0.437
Transaminase elevation	12 (20.7)	17 (29.3)	0.391
Jaundice	7 (12.1)	5 (8.6)	0.762

TACE: Transcatheter arterial chemoembolization; RFA: Radiofrequency ablation; CIK: Cytokine-induced killer

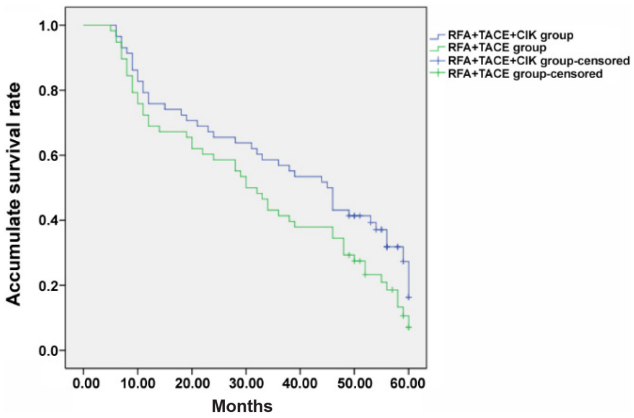

Figure 1. Kaplan-Meier survival curves of patients in RFA+TACE+CIK group and RFA+TACE group. The overall survival rate of patients in RFA+TACE+CIK group was significantly higher than that of RFA+TACE group (p=0.045).

Follow-up results of patient's survival

All patients were followed up for 5-60 months. The mean OS of patients was (42.1±5.6) months and (37.8±4.8) months, respectively, in RFA+TACE+CIK group and RFA+TACE group. The 1-, 3- and 5-year OS rates were 75.9% (44/58) *vs.* 69.0% (40/58), 56.9% (33/58) *vs.* 42.4% (24/58), and 29.3% (17/58) *vs.* 13.8% (8/58), respectively, in the two groups. The Kaplan-Meier survival curves of the two groups by the end of the follow-up period were shown in Figure 1. The results of log-rank test showed that the OS in RFA+TACE+CIK group was significantly superior to that in RFA+TACE group (p=0.045).

Discussion

HCC is a common malignant tumor, and its treatment method should be selected based on not only the tumor progression, but also the possible severe liver disease complicated. TACE has definite efficacy against HCC, and it can preserve the liver function to the greatest extent and cause fewer severe complications, so it has become the preferred treatment for inoperable patients [11]. However, TACE does not completely result in necrosis of tumor lesions, and it will damage the immune function of patients, with high rates of local recurrence and distant metastasis of HCC after treatment [12]. At present, RFA combined with TACE has been gradually recognized in the local control of HCC not treated with surgery. In recent years, a series of different studies have confirmed that anti-tumor immunity is closely related to the prognosis of patients [13]. Enhancing the local and systemic anti-tumor immune responses in HCC can reduce the recurrence and metastasis rates of HCC after treatment, and improve the prognosis of patients [14]. CIK cells can raise the proportion of immune cells in the body to inhibit tumor recurrence. Therefore, it is of great significance to explore the combination of RFA and TACE with CIK cell therapy for the prognosis of HCC [15, 16].

The body's immune defense function is constructed and kept mainly by T cell subsets with different functions. $CD3^+$ is a common receptor for T lymphocytes, which only exists on the surface of T lymphocytes. T lymphocytes can be classified into two different functional subsets, namely $CD4^+$ helper T lymphocytes (Th) and $CD8^+$ suppressive T lymphocytes (Ts), and the two different subsets interact with each other to regulate and maintain the body's immune balance [17, 18]. Th can regulate the immune response, help B cells produce or secrete antibodies, and promote the secretion of specific functional cytokines. Ts possess the immunosuppressive function and cytotoxic killing effect [19, 20]. Most cancer patients suffer from T cell subset dysfunction and/or imbalance. Among them, the changes in $CD4^+/CD8^+$ ratio are closely related to the degree of pathological differentiation of patients. $CD4^+/CD8^+$ ratio declines in poorly-differentiated tumors, and $CD4^+/CD8^+$ ratio <1 usually indicates the immunosuppressive state in patients. Th1/Th2 balance is of great importance for normal immune surveillance of tumor patients. Th con-

tains two important cell subsets (Th1 and Th2). Th1 promotes the immune function mainly through secreting Th1-type cytokines, such as IFN-γ, TNF-α, IL-2, IL-12 and IL-15, while Th2 inhibits the immune function mainly through secreting Th2-type cytokines, such as IL-4, IL-5, IL-6 and IL-10. In this study, after treatment, the levels of peripheral blood $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD4^+/CD8^+$ ratio, Tregs and NK cells significantly rose ($p<0.05$), while the level of $CD3^+CD8^+$ T cells significantly declined in both groups compared with those before treatment ($p<0.05$). After treatment, the levels of $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD4^+/CD8^+$ ratio, Tregs and NK cells were significantly higher, while the level of $CD3^+CD8^+$ T cells was significantly lower in RFA+TACE+CIK group than those in RFA+TACE group, showing statistically significant differences between the two groups ($p>0.05$). Besides, $CD4^+/CD8^+$ ratio was <1 before treatment but >1 after treatment, indicating that the immunosuppressive state in patients is reversed after autologous CIK cell therapy. After treatment, the levels of serum IL-2 and IFN-γ were higher, while the level of IL-6 was lower in both groups than those before treatment, confirming that the immune function of patients is enhanced after treatment.

It is reported that autologous CIK cell therapy can effectively remove residual lesions, inhibit tumor metastasis, reduce postoperative tumor recurrence, prolong the survival time of patients, greatly improve the treatment effect, and raise the quality of life of patients [21, 22]. In this study, the results revealed that the scores of all dimensions of EORTC QLQ-C30 in RFA+TACE+CIK group after treatment were all higher than those in RFA+TACE group. According to follow-up results, the OS in RFA+TACE+CIK group was significantly superior to that in RFA+TACE group ($p=0.045$).

This study was a retrospective study, so the sample size was small, the follow-up period was short, the follow-up content was not comprehensive enough, and the tumor recurrence and progression were not further analyzed during follow-up. Therefore, the conclusion made in this study remains to be verified by more rigorous prospective large-sample multicenter randomized studies.

Conclusions

RFA and TACE combined with postoperative autologous CIK cell reinfusion have significant efficacy in the treatment of primary HCC, which can enhance the immune function, improve the postoperative quality of life and raise the survival rate of patients, with tolerable adverse reactions.

Conflict of interests

The authors declare no conflict of interests.

References

1. Hartke J, Johnson M, Ghabril M. The diagnosis and treatment of hepatocellular carcinoma. Semin Diagn Pathol 2017;34:153-9.
2. Zhu Y, Feng B, Mei L, Sun R, Guo C, Zhu J. Clinical efficacy of TACE combined with Apatinib in the treatment of advanced hepatocellular carcinoma. J BUON 2019;24:608-14.
3. Lv Y, Xu A, Wang N et al. Retrospective study of TACE in the treatment of lobaplatin-induced thrombocytopenia in primary hepatocellular carcinoma. J BUON 2019;24:2385-93.
4. Liu Q, Dai X, Zhou X, Ye F, Zhou Y. Comparison of TACE combined with and without iodine-125 seeds implantation therapy for advanced stage hepatocellular carcinoma: a systematic review and meta-analysis. J BUON 2019;24:642-9.
5. Shimose S, Tanaka M, Iwamoto H et al. Prognostic impact of transcatheter arterial chemoembolization (TACE) combined with radiofrequency ablation in patients with unresectable hepatocellular carcinoma: Comparison with TACE alone using decision-tree analysis after propensity score matching. Hepatol Res 2019;49:919-28.
6. Wang L, Wang FS. Clinical immunology and immunotherapy for hepatocellular carcinoma: current progress and challenges. Hepatol Int 2019;13:521-33.
7. Sim HW, Knox J. Hepatocellular carcinoma in the era of immunotherapy. Curr Probl Cancer 2018;42:40-8.
8. Lee JH, Lee JH, Lim YS et al. Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. Gastroenterology 2015;148:1383-91.
9. Huang ZM, Li W, Li S et al. Cytokine-induced killer cells in combination with transcatheter arterial chemoembolization and radiofrequency ablation for hepatocellular carcinoma patients. J Immunother 2013;36:287-93.
10. Li X, Dai D, Song X, Liu J, Zhu L, Xu W. A meta-analysis of cytokine-induced killer cells therapy in combination with minimally invasive treatment for hepatocellular carcinoma. Clin Res Hepatol Gastroenterol 2014;38:583-91.

11. Yang Z, Chen G, Cui Y et al. The safety and efficacy of TACE combined with apatinib on patients with advanced hepatocellular carcinoma: a retrospective study. 2019;20:321-7.
12. Sieghart W, Hucke F, Peck-Radosavljevic M. Transarterial chemoembolization: modalities, indication, and patient selection. J Hepatol 2015;62:1187-95.
13. Lauvau G, Chorro L, Spaulding E, Soudja SM. Inflammatory monocyte effector mechanisms. Cell Immunol 2014;291:32-40.
14. Yoon JS, Song BG, Lee JH et al. Adjuvant cytokine-induced killer cell immunotherapy for hepatocellular carcinoma: a propensity score-matched analysis of real-world data. BMC Cancer 2019;19:523.
15. Cui J, Wang N, Zhao H et al. Combination of radiofrequency ablation and sequential cellular immunotherapy improves progression-free survival for patients with hepatocellular carcinoma. Int J Cancer 2014;134:342-51.
16. Yan S, Xu D, Sun B. Combination of radiofrequency ablation with transarterial chemoembolization for hepatocellular carcinoma: a meta-analysis. Dig Dis Sci 2013;58:2107-13.
17. Sheu BC, Hsu SM, Ho HN, Lin RH, Torng PL, Huang SC. Reversed CD4/CD8 ratios of tumor-infiltrating lymphocytes are correlated with the progression of human cervical carcinoma. Cancer 1999;86:1537-43.
18. Stanton SE, Disis ML. Clinical significance of tumor-infiltrating lymphocytes in breast cancer. J Immunother Cancer 2016;4:59.
19. Sasada T, Suekane S. Variation of tumor-infiltrating lymphocytes in human cancers: controversy on clinical significance. Immunotherapy-Uk 2011;3:1235-51.
20. Toia F, Buccheri S, Anfosso A et al. Skewed Differentiation of Circulating Vgamma9Vdelta2 T Lymphocytes in Melanoma and Impact on Clinical Outcome. PLos One 2016;11:e149570.
21. Cao J, Kong FH, Liu X, Wang XB. Immunotherapy with dendritic cells and cytokine-induced killer cells for hepatocellular carcinoma: A meta-analysis. World J Gastroenterol 2019;25:3649-63.
22. Yu R, Yang B, Chi X et al. Efficacy of cytokine-induced killer cell infusion as an adjuvant immunotherapy for hepatocellular carcinoma: a systematic review and meta-analysis. Drug Des Devel Ther 2017;11:851-64.