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Prognostic value of lymphocyte subset levels in hepatocellular carcinoma following conventionally fractionated *vs.* stereotactic body radiotherapy

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Abstract

Aim: The aim of this study was to compare the effects of conventionally fractionated radiotherapy (CFRT) and stereotactic body radiotherapy (SBRT) on peripheral lymphocyte subset levels in patients with hepatocellular carcinoma (HCC), and to assess the association between radiotherapy (RT)-induced lymphopenia and patient prognosis.

Methods: A retrospective analysis was conducted on 137 HCC patients who underwent either CFRT or SBRT between July 2011 and January 2018. Variables were obtained within 1 week before RT, and 1 day and 2 months post-RT, respectively. Peripheral lymphocyte subsets, including CD4+, CD8+, CD19+, and NK cells, were measured using flow cytometry. Univariate and multivariate Cox regression analyses were conducted to investigate independent prognostic factors for overall survival (OS).

Results: The one-year and two-year OS rates were 80.0% and 55.0%, respectively. Multivariate analysis identified tumor size > 4.5 cm, multiple tumors, and post-RT CD4+ T cell count < 231/μL and CD8+ T cell count < 179/μL as independent factors associated with inferior OS in HCC patients. Severe lymphopenia (< 0.5×10^9/L) occurred in 70.0% of patients following CFRT compared to 23.0% in SBRT patients. Patients who received SBRT exhibited higher total lymphocyte counts and subset levels 1 day and 2 months post-treatment compared to those receiving CFRT ($P < 0.05$). Logistic regression analysis identified the number of RT fractions as an independent factor for

severe lymphopenia. Further analysis revealed that CD19+ B cells were predominantly depleted and recovered more slowly than other populations, whereas CD8+ T cells demonstrated rapid recovery. Among SBRT patients, higher levels of CD4+ and CD8+ T cells post-treatment were associated with longer OS ($P < 0.05$).

Conclusion: SBRT may induce less severe lymphopenia than CFRT. Decreases in CD4+ and CD8+ T cell levels post-SBRT may independently predict worse OS in HCC patients.

Keywords: Radiotherapy, hepatocellular carcinoma, lymphopenia, overall survival

INTRODUCTION

Hepatocellular carcinoma (HCC) ranks as a prevalent malignant tumor and the third leading cause of cancer-related mortality worldwide[1]. China contributes to half of the total HCC cases and deaths globally. Currently, surgical interventions remain the primary treatment approach for HCC patients[2]. However, certain patients are deemed ineligible for surgery due to donor shortages, limited liver function, extensive tumor burden, or comorbidities.

Advances in conformal photon-based techniques, such as intensity-modulated radiotherapy (RT) and volumetric modulated arc therapy, have enhanced the role of RT in the management of HCC[3]. Irradiation exerts dual effects on HCC treatment. It directly kills tumor cells by inducing DNA damage and breakage while simultaneously eliciting systemic immune-modulating effects[4]. However, RT can decrease the total peripheral lymphocyte count (TPLC), thereby weakening antitumor immune responses, which is correlated with poor prognosis in HCC patients[5-7]. Furthermore, studies have shown that RT-induced lymphopenia (RIL) is strongly associated with poor outcomes in patients with pancreatic, cervical, esophageal, lung, and central nervous system malignancies[8-12]. Therefore, reducing the incidence of RIL is of paramount importance.

Conventionally fractionated radiotherapy (CFRT), typically delivered in 5 to 6 weeks, has been widely used in the treatment of advanced HCC. More recently, stereotactic body radiotherapy (SBRT), capable of delivering a more ablative dose in a short period, has emerged as an effective treatment approach for HCC. Compared to CFRT, SBRT offers substantial local control, improved overall survival (OS), and reduced toxicity for patients with HCC[13]. Notably, SBRT may present a lower risk of inducing lymphopenia[14].

Our previous research demonstrated that peripheral lymphocyte levels, including CD8+ T and CD56+ NK cells, following SBRT are closely associated with survival in HCC patients[7]. However, the impacts of different RT fractionation schemes on circulating lymphocyte populations (CLPs) remain unclear. This study aimed to elucidate the differential effects of SBRT and CFRT on lymphocyte subgroups and to characterize the influence of RIL on survival outcomes in HCC patients.

METHODS

Patient selection

This research received approval from the Institutional Review Board of the Ethics Committee of Zhongshan Hospital Affiliated to Fudan University (B2018-272) and adhered to the principles outlined in the Declaration of Helsinki. We conducted a single-center retrospective examination of medical records from patients diagnosed with unresectable HCC between July 2011 and January 2018. Inclusion criteria for patients undergoing RT were as follows: (1) HCC diagnosis confirmed by histologic or imaging criteria based on the National Comprehensive Cancer Network guidelines for hepatobiliary cancers; (2) age over 18 years; (3) Child-Pugh class A or B; (4) Eastern Co-operative Oncology Group performance status 0 or 1; (5)

laboratory tests conducted within one week before, 1 month, and 2 months after RT; and (6) one or more radiological assessments performed prior to and following RT. Patients meeting any of the following criteria were excluded from the study: (1) presence of distant metastasis; (2) double primary malignancy; or (3) follow-up duration less than 6 months post-completion of RT; and (4) receipt of other local treatments within 1 month before RT.

Radiation treatment

All enrolled patients underwent respiratory training prior to the initiation of RT. Patients underwent computed tomography (CT) simulation and four-dimensional CT (4D-CT) scans in the supine position. The gross tumor volume (GTV) encompassed all tumors identified via CT imaging. For tumors not well visualized on CT scans, pre-treatment magnetic resonance imaging (MRI) studies were co-registered with the planning CT. 4D-CT simulations were employed to generate an internal target volume (ITV), and the planning target volume (PTV) was defined as the ITV plus a radial margin of 3 mm. Patients receiving SBRT were administered 5-10 fractions five times per week, with a median dose of 48 Gray (Gy). CFRT was characterized by a radiation dose of 1.8 or 2 Gy per fraction, prescribed to the isodose surface covering 95% of the PTV. The total dose ranges from 46 to 70 Gy, with a median dose of 58 Gy.

Follow-up and patient outcomes

The follow-up duration was defined as the period from the initiation of RT to determine the median follow-up and time-to-event estimates. Baseline peripheral blood cell counts, including lymphocyte subsets, were obtained before RT, while post-treatment values were assessed 1 day and 2 months after the completion of RT. In cases where peripheral cell records were unavailable for a specific month, the closest available values were utilized. Follow-up CT or MRI studies were conducted 6-8 weeks following RT and subsequently every 3 months. Follow-up imaging studies were conducted every 6-8 weeks following RT and every 3 months thereafter, with the most recent follow-up completed in December 2023. OS was calculated at the patient level as the duration from the first RT session until death from any cause or the last follow-up.

Statistics and analysis

Descriptive statistics were presented as means ± standard deviation or as medians and interquartile range, depending on the normality of data distribution assessed using Kolmogorov-Smirnov tests. Quantitative variable comparisons were assessed using two-sided *t*-tests or Mann-Whitney tests, as appropriate. The effects of RT on lymphocyte subsets at different times were compared using repeated-measures analysis of variance. Logistic regression analyses were conducted to identify factors associated with severe post-RT lymphopenia. The primary endpoint was OS. Cumulative survival was calculated using the Kaplan-Meier method, and cutoff values for continuous variables affecting patient prognosis were determined using the receiver operating characteristic curve. Univariate and multivariate analyses were conducted using Cox regression models, presenting hazard ratios (HRs) and 95% confidence intervals (95%CIs). IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA), was used for data analysis.

RESULTS

Patient characteristics and clinical outcomes

A cohort of 137 patients with HCC treated with either SBRT (n = 84) or CFRT (n = 53) were enrolled in the study. The treatment and tumor characteristics of patients are summarized in Table 1. Among the total cohort, 101 patients (73.7%) had received previous treatments, including surgery, transarterial chemoembolization, radiofrequency ablation, and percutaneous ethanol injection. The median tumor size was 2.5 cm for patients treated with SBRT and 5.1 cm (range, 1.2-14.0) for patients receiving CFRT. The median number of fractions was 6 (range 5-10) for patients receiving SBRT and 25 (range 20-30) for those receiving CFRT. The median biologically equivalent dose (BED) was 77 Gy (range, 65-140 Gy) and 72 Gy

Table 1. Patient characteristics

Characteristic	All patients (*n* = 137)	SBRT-treated patients (*n* = 84)	CFRT-treated patients (*n* = 53)	*P*-value
Median age (range)	62 (22-87)	59 (22-86)	58 (30-87)	0.259
Sex				0.230
Male	113 (82.5%)	71 (84.5%)	42 (79.2%)	
Female	24 (17.5%)	13 (15.5%)	11 (20.8%)	
Child-Pugh				0.295
A	118 (86.1%)	71 (84.5%)	47 (88.7%)	
B	19 (13.9%)	13 (15.5%)	6 (11.3%)	
Median tumor size (cm)	3.0 (0.8-14.0)	2.5 (0.8-6.8)	5.1 (1.2-14.0)	< 0.0001
Mean AFP (ng/mL)	3741 ± 12057	977 ± 2344	7524 ± 17449	< 0.0001
TPLC before RT (1×10^9/L)	1.31 ± 0.59	1.39 ± 0.58	1.23 ± 0.60	0.060
Viral etiology hepatitis				0.152
Yes	95 (69.3%)	57 (67.9%)	38 (71.7%)	
No	42 (30.7%)	27 (32.1%)	15 (28.3%)	
Previous treatments				0.231
Yes	101 (73.7%)	64 (76.1%)	39 (73.6%)	
No	36 (26.3%)	20 (23.9%)	14 (26.4%)	
Median of fractions (range)	10 (5-30)	6 (5-10)	25 (20-30)	< 0.0001
Median GTV (cc)	38 (1.6-1530)	16 (1.6-465)	285 (17-1530)	< 0.0001
Median BED (Gy)	77 (55-140)	77 (65-140)	72 (55-91)	0.087

AFP: Alpha-fetoprotein; BED: biologically equivalent dose; CFRT: conventionally fractionated radiotherapy; GTV: gross tumor volume; RT: radiotherapy; SBRT: stereotactic body radiotherapy; TPLC: total peripheral lymphocyte count.

(range, 55-91 Gy) for patients treated with SBRT and CFRT, respectively.

The median follow-up time was 35 months (range, 7.5-80 months). Median survival time was 29 months (SBRT 69 months *vs.* CFRT 17 months). The one-year and two-year OS rates were 80.0% and 55.0%, respectively (93.0% and 77.6% for SBRT, 66.0% and 38.0% for CFRT, respectively).

Changes in absolute counts of lymphocyte subsets induced by SBRT vs. CFRT

No significant differences were observed in pre-treatment TPLC between SBRT and CFRT-treated patients [Figure 1]. However, severe lymphopenia (< 0.5×10^9/L) occurred in 23.0% of patients receiving SBRT and 70.0% in those receiving CFRT. Further analysis was performed according to different CLPs. As is shown in Figure 1, peripheral B cells were profoundly depleted after both SBRT and CFRT (decreased by 78.9% and 90.5%, respectively), followed by NK cells (decreased by 47.3% and 71.8%, respectively). The counts of CD4+ T cells and CD8+ T cells decreased by 46.5% and 50.4% for SBRT and 66.3% and 52.6% for CFRT, respectively [Supplementary Table 1]. The mean values of TPLC and CLPs at different time points post-RT were listed in Table 2.

Two months post-RT, TPLC and CLP counts showed partial recovery. B lymphocytes demonstrated the slowest recovery. Interestingly, CD8+ T cell counts returned to baseline in both the SBRT and CFRT groups, while CD4+ T cell recovery was slower, leading to a reduced CD4/CD8 ratio, particularly in the CFRT group. In contrast, SBRT had a less significant effect on CD4/CD8 ratio [Figure 1 and Table 2]. The mean values of TPLC and CLPs at different time points are summarized in Table 2.

Factors associated with severe post-RT lymphopenia

Logistic regression analyses were performed to identify factors influencing severe lymphopenia in patients.

Table 2. The mean values of TPLC and CLP at different time points after RT

Counts of lymphocytes	All patients (*n* = 137)	SBRT-treated patients (*n* = 84)	CFRT-treated patients (*n* = 53)	*P*-value
TPLC before RT (1×10^9/L)	1.32 ± 0.60	1.42 ± 0.60	1.19 ± 0.60	0.056
CD4 (cells/μL)	489.13 ± 229.47	513.29 ± 208.78	463.52 ± 249.10	0.212
CD8 (cells/μL)	322.81 ± 184.89	352.35 ± 204.56	290.86 ± 156.82	0.152
NK (cells/μL)	294.76 ± 258.67	322.88 ± 286.19	264.96 ± 224.96	0.295
B (cells/μL)	141.77 ± 103.59	172.52 ± 84.48	109.18 ± 112.50	< 0.001
TPLC at 1 day post-RT (1×10^9/L)	0.58 ± 0.33	0.70 ± 0.30	0.40 ± 0.30	< 0.001
CD4 (cells/μL)	222.74 ± 146.17	274.60 ± 138.02	156.26 ± 129.76	< 0.001
CD8 (cells/μL)	158.60 ± 136.96	174.70 ± 101.97	137.97 ± 171.04	< 0.01
NK (cells/μL)	128.31 ± 123.15	170.13 ± 141.76	74.69 ± 62.92	< 0.001
B (cells/μL)	25.02 ± 27.29	36.41 ± 29.16	10.41 ± 15.43	< 0.001
TPLC at 2 months post-RT (1×10^9/L)	1.08 ± 0.56	1.18 ± 0.61	0.84 ± 0.35	0.050
CD4 (cells/μL)	312.25 ± 240.26	377.47 ± 273.17	199.20 ± 100.41	0.020
CD8 (cells/μL)	368.10 ± 249.01	345.42 ± 233.46	407.40 ± 277.89	0.716
NK (cells/μL)	296.35 ± 230.40	381.55 ± 242.04	148.67 ± 101.03	0.001
B (cells/μL)	82.29 ± 56.34	100.76 ± 57.61	50.27 ± 37.53	0.018

CFRT: Conventionally fractionated radiotherapy; CLP: circulating lymphocyte population; RT: radiotherapy; SBRT: stereotactic body radiotherapy; TPLC: total peripheral lymphocyte count.

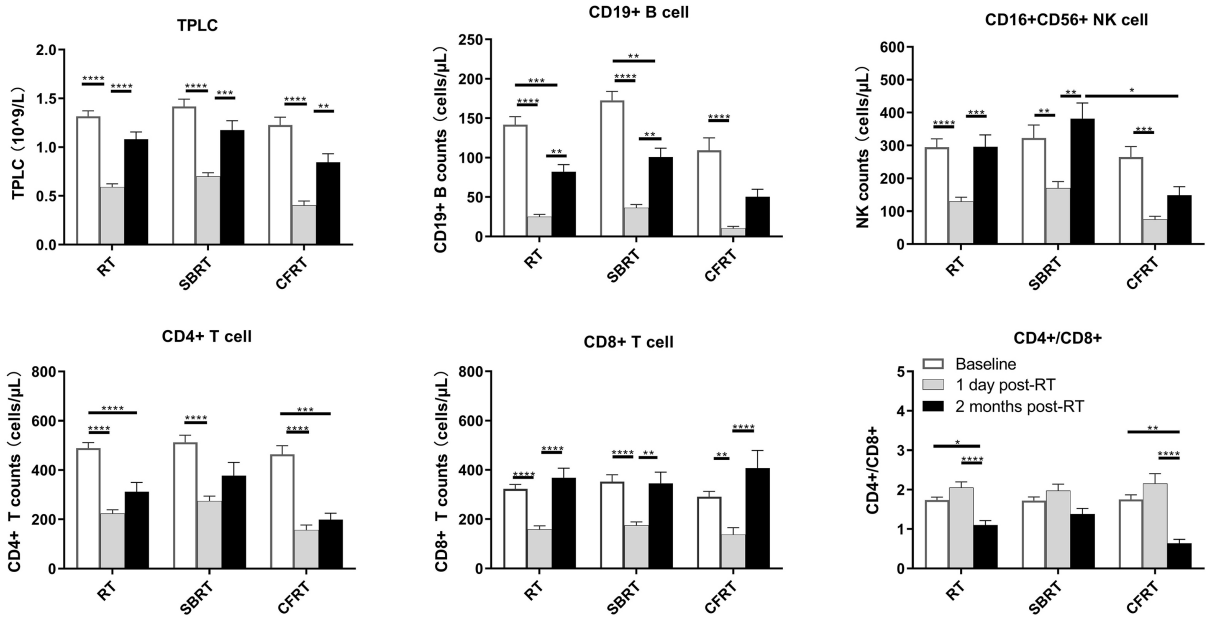

Figure 1. Impacts of different RT modalities on lymphocyte subset levels in patients with HCC. The absolute counts of TPLC, CD19+ B cells, NK cells, CD4+ T cells, and CD8+ T cells, as well as the CD4+/CD8+ ratio, were measured at baseline (within 1 week prior to RT) and at subsequent time points post-RT (1 day and 2 months). Statistically significant differences are indicated by $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. RT: Radiotherapy; HCC: hepatocellular carcinoma; TPLC: total peripheral lymphocytes; CFRT: conventionally fractionated radiotherapy; SBRT: stereotactic body radiotherapy.

Univariate analysis revealed significant associations between severe lymphopenia and the presence of portal vein tumor thrombus ($P < 0.01$), tumor size ($P = 0.01$), GTV ($P = 0.02$), BED ($P = 0.01$), and the number of RT fractions ($P < 0.01$). After addressing multicollinearity, multivariate analysis demonstrated that the

number of RT fractions ($P = 0.022$) was an independent predictor of severe lymphopenia [Table 3].

Univariate and multivariate Cox regression analyses for patients with HCC

Factors including age, presence of hepatitis and portal venous thrombus, previous treatments, tumor size, tumor number, Child-Pugh grade, baseline alpha-fetoprotein (AFP), baseline and post-treatment TPLC and CLP counts, GTV, BED, and fractions were analyzed at the univariate level. The results demonstrated that portal venous thrombus present, tumor size ≥ 4.5 cm, multiple tumors, a high level of AFP, GTV, and fractions, decreased BED, and low levels of post-treatment TPLC and CLP counts were significantly associated with poor OS (Table 3, $P < 0.05$ for each). After adjusting for covariates, the Cox regression model revealed that tumor size ≥ 4.5 cm (HR: 1.25; 95%CI: 1.10-1.43; $P = 0.001$), multiple tumors (HR: 3.39; 95%CI: 1.71-6.74; $P < 0.001$), post-treatment CD4 < 231 cells/μL (HR: 0.53; 95%CI: 0.32-0.90; $P = 0.018$), and post-treatment CD8 < 179 cells/μL (HR: 0.74; 95%CI: 0.55-0.99; $P = 0.043$) were identified as independent adverse factors for OS [Table 4].

Correlation between CLP and OS in patients after SBRT and CFRT

Cumulative survivals for CLPs following RT were calculated using the Kaplan-Meier method [shown in Figure 2]. Significant differences were observed in the OS curves between higher and lower levels of CD4+ and CD8+T cells ($P < 0.05$ for each). To further assess the impact of post-RT CLP on survival under different RT modalities, patients were stratified based on whether they received SBRT or CFRT. As illustrated in Figure 3, patients with lower CD4+ and CD8+ T cell counts after SBRT exhibited significantly worse OS ($P < 0.05$ for each). However, this trend was not seen in the CFRT group. In the CFRT group, the effect of post-treatment CD4+ T cells on OS became noticeable after the two-year survival mark, whereas post-treatment CD8+ T cell levels showed no significant correlation with OS.

DISCUSSION

The pivotal role of the immune system in cancer control is widely acknowledged, highlighting the importance of functional lymphocytes in identifying and eliminating malignant cells[15,16]. The impacts of RT on the immune system are intricate and diverse. While irradiation primarily targets tumor cells for destruction, it can also influence adjacent normal tissue and immune cells[17,18].

The phenomenon of RIL was initially delineated in 1916[19-21]. Studies have shown that radiation exposure can lead to a substantial decrease of 60% to 80% in peripheral lymphocytes[22]. In this study, we observed that SBRT caused less severe RIL compared to CFRT, consistent with findings by Wild *et al.*, suggesting that SBRT has advantages in minimizing normal tissue damage and reducing immunosuppression[14]. In line with our previous research[23], the study further demonstrated that the number of radiation fractions is an independent predictor of severe lymphopenia. These findings closely align with the hypothesized mechanisms underlying treatment-induced lymphopenia[24,25]. As the number of fractions increases, the percentage of circulating blood receiving ≥ 0.5 Gy rises dramatically. A single fraction exposes only 5% of circulating blood to 0.5 Gy, but 30 fractions result in 99% of circulating blood receiving ≥ 0.5 Gy. Similarly, a study comparing CFRT (50.4 Gy in 28 fractions) with hypofractionated neoadjuvant chemoradiotherapy (30 Gy in 10 fractions) in pancreatic cancer patients found a significant correlation between CFRT and RIL, with marked reductions in CD4+ and CD8+ T cell subsets. In contrast, hypofractionated chemoradiotherapy resulted in only a slight decrease in T cell levels, with more rapid recovery [26]. These findings highlight the advantage of SBRT, which delivers high radiation doses with fewer fractions, in effectively preserving circulating lymphocytes and potentially reducing treatment-induced immunosuppression.

Table 3. Multivariate analysis (logistic regression approach) of predictors of severe lymphopenia (TPLC < 0.5 × 10^9/L) 1 day after radiation

Characteristic	HR (95%CI)	*P*-value
Portal venous thrombus present	1.502 (0.424-5.325) < 0.001	0.529
GTV	1.001 (0.999-1.004) 0.017	0.279
BED	0.983 (0.947-1.020) 0.001	0.364
Fractions	1.087 (1.012-1.168) < 0.001	0.022

BED: Biological equivalent dose; CI: confidence interval; GTV: gross tumor volume; TPLC: total peripheral lymphocyte count; HR: hazard ratio.

Table 4. Univariate and multivariate analysis of prognostic factors for OS

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
Age (≥ 60 years)	0.98 (0.96-1.00)	0.09		
Presence of hepatitis	1.71 (0.98-2.99)	0.06		
Previous treatments present	1.07 (0.61-1.86)	0.82		
Portal venous thrombus present	2.59 (1.60-4.20)	< 0.001	1.24 (0.60-2.57)	0.562
Tumor size (≥ 4.5 cm)	1.20 (1.11-1.27)	< 0.001	1.25 (1.10-1.43)	0.001
Multiple tumors	2.47 (1.51-4.05)	0.04	3.39 (1.71-6.74)	< 0.001
Child B vs Child A	1.11 (0.52-2.37)	0.78		
AFP	1.11 (0.91-1.32)	0.01	1.01 (1.0-1.02)	0.24
Baseline TPLC	0.70 (0.45-1.08)	0.098		
TPLC at 1 day post-RT ≤ 0.5 (1 × 10^9/L)	2.55 (1.47-4.40)	< 0.001		
CD3 T cells	0.71 (0.60-0.83)	< 0.001		
CD4 T cells	0.49 (0.35-0.67)	< 0.001	0.53 (0.32-0.90)	0.018
CD8 T cells	0.36 (0.53-0.91)	0.007	0.74 (0.55-0.99)	0.043
NK cells	0.69 (0.51-0.95)	0.023	1.20 (0.84-1.69)	0.33
B cells	0.78 (0.61-1.10)	< 0.001	1.01 (0.99-1.03)	0.07
GTV	1.002 (1.001-1.003)	0.017		
BED	0.97 (0.95-0.99)	0.001	1.00 (0.98-1.03)	0.642
Fractions	1.07 (1.04-1.11)	< 0.001		

AFP: Alpha-fetoprotein; CI: confidence interval; BED: biological equivalent dose; GTV: gross tumor volume; OS: overall survival; RT: radiotherapy; TPLC: total peripheral lymphocyte count; HR: hazard ratio.

Among the various subsets of lymphocytes, CD19+ B cells exhibited pronounced depletion, with a notably slower recovery rate compared to other populations, particularly evident in the CFRT group. Clave *et al.* assessed the *in vivo* radiosensitivity of lymphocytes through the administration of whole-body irradiation to patients before undergoing bone marrow transplantation[27]. Similar to our conclusion, the research revealed that B cells exhibited the highest degree of sensitivity, followed by T cells (CD4+, CD8+) and NK cells. B cells play a crucial role in tumor immune surveillance by facilitating the recognition and elimination of malignant cells through antibody production and modulation of the immune response[28,29]. Additionally, the study found a significant decrease in the CD4/CD8 ratio in patients treated with CFRT, consistent with previous research on the immunosuppressive effects of CFRT[26]. Similarly, Yang *et al.* reported a decline in the CD4+/CD8+ T cell ratio following RT among individuals with head and neck cancer[30]. This alteration in the ratio appeared unrelated to minor variations in the radiosensitivity of CD4+ and CD8+ T cells.

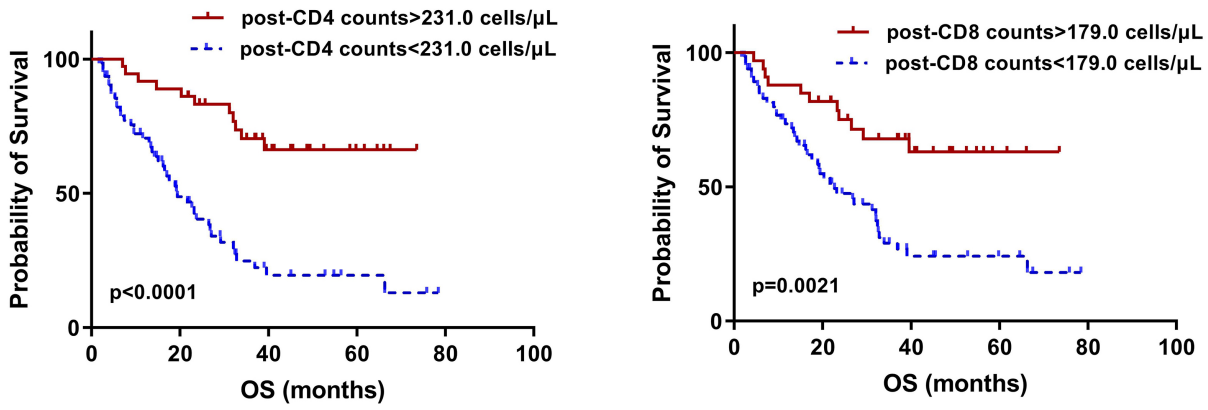

Figure 2. Kaplan-Meier OS curves for CD4+ and CD8+ T cells in patients with hepatocellular carcinoma after radiotherapy. One day post-radiotherapy, significant differences in OS were observed between patients with high (red) and low (blue) levels of CD4+ and CD8+ T cells ($P < 0.05$ for each). OS: Overall survival.

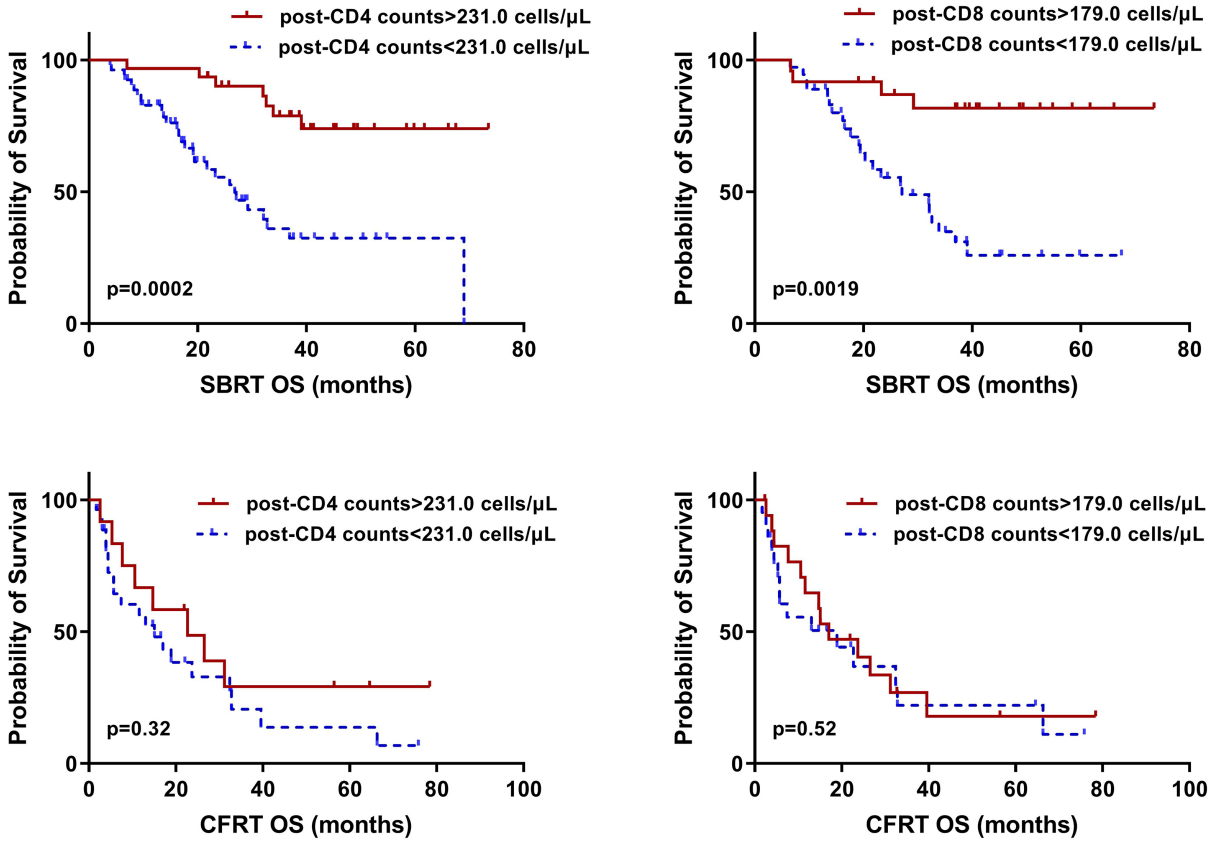

Figure 3. Kaplan-Meier OS curves for CD4+ and CD8+ T cells in patients treated with SBRT *vs.* CFRT. One day post-treatment, significant differences in OS were observed between high (red) and low (blue) levels of CD4+ and CD8+ T cells in both SBRT and CFRT groups ($P < 0.05$ for each). OS: Overall survival; CFRT: conventionally fractionated radiotherapy; SBRT: stereotactic body radiotherapy.

Instead, it likely stemmed from radiation-triggered activation and recruitment of CD8+ T cells, which counterbalanced the reduction in CD8+ T cells. In contrast, the SBRT group showed only minor changes in the CD4/CD8 ratio, suggesting that SBRT may have a potential advantage in maintaining immune balance. However, further research is needed to elucidate the role of CD4/CD8 ratio changes in the long-term

prognosis after RT.

Previous studies have indicated that RT influences patient prognosis by depleting T lymphocytes in the bloodstream[31]. Hypofractionated regimens in HCC treatment have been shown to reduce lymphocyte depletion and accelerate post-treatment recovery[32]. Furthermore, our study demonstrated a significant correlation between decreased post-RT CD4+ and CD8+ T lymphocyte counts and reduced OS in the SBRT cohort. These findings highlight the importance of developing interventions to mitigate RIL, which could improve treatment outcomes and survival in HCC patients.

Preventing RIL remains a critical area of focus. While complete avoidance of this condition may not always be feasible, implementing strategies to mitigate its impact is essential. Continuous monitoring of lymphocyte counts during and after RT is crucial for early detection of lymphocyte depletion. Additionally, RT regimens can be tailored to minimize lymphocyte damage, such as using SBRT to deliver higher doses per fraction while reducing the overall number of fractions. Emerging techniques, such as proton beam therapy, can further limit the impact of radiation on lymphocytes by reducing dosimetric scatter and minimizing the volume of tissue exposed to radiation[33]. Optimizing RT plans to spare major blood vessels, the spleen, and other hematopoietic organs may also help preserve lymphocyte levels. Furthermore, adjunctive therapies, such as cytokine interventions, have shown promise in alleviating RIL. Byun *et al.* demonstrated that exogenous interleukin-7 administration post-RT not only restored lymphocyte counts but also enhanced antitumor efficacy compared to RT alone[34,35].

One limitation of this study is the relatively small sample size and its retrospective design, which may introduce bias. Additionally, many patients received prior treatments that could have affected immune function and lymphocyte counts. Although multivariate Cox regression analysis accounted for their effects on patient OS, potential data biases may persist. Future prospective studies are planned to address these limitations.

In summary, this study demonstrates that SBRT has significant advantages in reducing RIL, with post-SBRT CD4+ and CD8+ T cell levels serving as independent prognostic factors in HCC patients. These findings support the use of SBRT as an effective treatment option for HCC and provide insights into developing future therapeutic strategies targeting RIL.

DECLARATIONS

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Authors' contributions

Drafted the manuscript: Wang ST
Participated in the collection, analysis, and interpretation of data: Chen YX, Gao YN, Yang P, Zhao QQ
Conceived the study, participated in its design and coordination, and critically revised the manuscript: Chen YX, Zhuang Y, Zeng ZC
All authors read and approved the final version of the manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of interest
All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
The study was ethically reviewed and approved by the Ethics Committee of Zhongshan Hospital Affiliated to Fudan University (B2018-272). Written informed consent was obtained from all subjects.

Consent for publication
Not applicable.

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