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ORIGINAL RESEARCH

The Correlation Between Low-Dose Radiotherapy Area of the Mediastinum and CD8+T Cells and the Efficacy of Radiotherapy for Non-Small Cell Lung Cancer

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Background: Radiation therapy (RT) can cause changes in peripheral blood immune cells. The relationship between the efficacy of radiation therapy for non-small cell lung cancer (NSCLC) and immune cell changes and the study of how mediastinal radiation dose parameters affect immune cell changes is still unclear. This study aims to analyze the relationship between immune cell changes induced by radiotherapy and the efficacy of NSCLC radiotherapy, as well as the relationship between radiotherapy dose parameters and immune cell changes.

Materials and Methods: We retrospectively analyzed the data of NSCLC patients receiving mediastinal radiation therapy from 2020 to 2022. Collect lymphocytes and circulating immune cells within one week before and after radiotherapy and collect the dose-volume parameters of the whole mediastinum in the patient's RT planning system. Analyze the changes in lymphocytes and radiotherapy effects after radiotherapy, and explore the relationship between radiotherapy dose parameters and immune cell changes.

Results: A total of 72 patients were enrolled. Compared with before radiotherapy, the proportion of CD3+T cells, CD8+T cells, and CD8/Treg in peripheral blood significantly increased after radiotherapy ($P<0.05$). The increase in CD8+T cells and CD8/Treg after radiotherapy was correlated with Objective response rate (ORR) ($P<0.05$). Based on binary logistic univariate and multivariate regression analysis, an increase in CD8+T cells after radiotherapy is an independent predictor of objective tumor response after radiotherapy (OR=12.71, 95% CI=3.64–44.64, $P=0.01$), and Volume of 200 cGy irradiation (V2) is an independent positive predictor of an increase in CD8+T lymphocyte ratio after radiotherapy (high group, OR=3.40, 95% CI=1.13–10.36, $P=0.03$).

Conclusion: The increase in CD8+T cells after radiotherapy can positively predict the short-term efficacy of radiotherapy. Mediastinal low-dose radiation therapy can increase CD8+T cells, thereby improving the short-term efficacy of radiotherapy. These potentially related mechanisms are worth further verification and exploration.

Keywords: radiation therapy, non-small cell lung cancer, immune cells, radiotherapy dose parameters

Introduction

Lung cancer is the most common tumor in the world. According to the latest global cancer burden data released by the International Agency for Research on Cancer (IARC) of the World Health Organization in 2020, lung cancer is the most common tumor and one of the tumors with the highest mortality.[1]

Radiation therapy (RT) was an important treatment for solid tumors.[2] The mediastinal region is a common RT site for lung cancer. RT damages endothelial cells and causes radiation-induced inflammation. Damaged vessels inhibit the infiltration of lymphocytes into tumors, and immunosuppressive pathways are activated.[3]

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More and more studies have shown that the immune cells in breast cancer, prostate cancer, liver cancer, nasopharyngeal cancer and other tumors change before and after RT, which affects the efficacy of RT, for example, the CD8+T cells in nasopharyngeal cancer after radiotherapy are reduced, the CD8+T cells in esophageal cancer and colorectal cancer after radiotherapy are increased, and the CD8+T cells in anterior gland and breast cancer after radiotherapy have no significant change.[4–7] This shows that the patients have obvious immunological changes in a short time after RT, which can cause apoptosis and decrease T lymphocytes and affect the balance of peripheral blood immune cells. The degree of immune response induced by RT varies with the type of tumor.[8]

The immunomodulatory effect of different doses of radiotherapy on the tumor microenvironment can improve the efficacy of T cell immunotherapy. Low-dose RT can increase the homing ability of activated T cells and adjust the RT effect.[9] At present, it is not clear how the changes in immune cells and the effects of radiotherapy, and the dose parameters of mediastinal radiotherapy affect the changes in immune cells.

In this study, cancer patients receiving radiation therapy were analyzed. (1) Analyze the correlation between immune cell changes and radiotherapy efficacy caused by different radiation doses (2) Explore the relationship between radiation dose parameters and immune cell changes.

Methods and Materials

Patient Characteristics

Patients with lung cancer, including those cytologically or pathologically diagnosed, who underwent intensity-modulated radiotherapy (IMRT) in the Department of Radiation Oncology at The First Affiliated Hospital of Shandong First Medical University between October 2020 to December 2022 were enrolled. The study was conducted by the Declaration of Helsinki (as revised in 2013). The study was approved by the First Affiliated Hospital of Shandong First Medical University review board (No. 2022-S607). Individual consent for this retrospective analysis was waived. The participating hospitals informed and agreed on the study.

Eligible patients: ≥ 18 years old, male or female; NSCLC patients confirmed by cytology or pathology; Patients receiving chest RT; No acute infection or uncontrolled infection during radiotherapy. Exclusion criteria: Patients with a history of tumor or radiation therapy in the past; Patients with immune deficiency; Patients in Immunosuppressive drug therapy; Patients who have received Colony-stimulating factor within one week before immunocyte test; Suffering from serious complications such as heart and lung, liver and kidney, and immune deficiency. Patients with pneumoconiosis, drug-induced pneumonia, severe pulmonary insufficiency, and RT intolerance.

Collecting and organizing clinical data of patients who meet the inclusion and exclusion criteria mentioned above for analysis. Collecting peripheral blood lymphocytes and immune cell subsets of patients within one week before and after radiotherapy, including lymphocytes, CD3+T cells, CD4+T cells, CD8+T cells, CD4/CD8, NK cells, B cells, Treg cells and CD8/Treg cells. Collect radiation therapy dose volume parameters (V2, V5, V10, V20, V30, V40, and V50) for the entire mediastinum in the radiation therapy planning system for analysis. After radiotherapy: before radiotherapy lymphocytes, CD3+T cells, CD4+T cells, CD8+T cells, CD4/CD8, NK cells, B cells, Treg cells, and CD8/Treg are defined as LymR, CD3R, CD4R, CD8R, CD4/CD8R, NKR, BR, TregR, and CD8/TregR. In addition, radiation doses, basic tumor characteristics, including tumor lymph node metastasis (TNM) staging, and other baseline information were collected, such as the patient's age, gender, smoking history, and alcohol consumption history.

Target Delineation

The mediastinum is delineated in the Eclipse planning system according to the IASLC criteria.[10,11] The upper boundary of the delineated volume is located at the lower edge of Cricoid cartilage, and the lower boundary is to the lower edge of the diaphragm, including various sized arteries and veins, hearts, lymph nodes and nerves, and it is defined as the mediastinum (M). The target planning evaluation volume is the mediastinal volume (M) minus the planned target volume (PTV), defined as M-PTV (Figure 1).

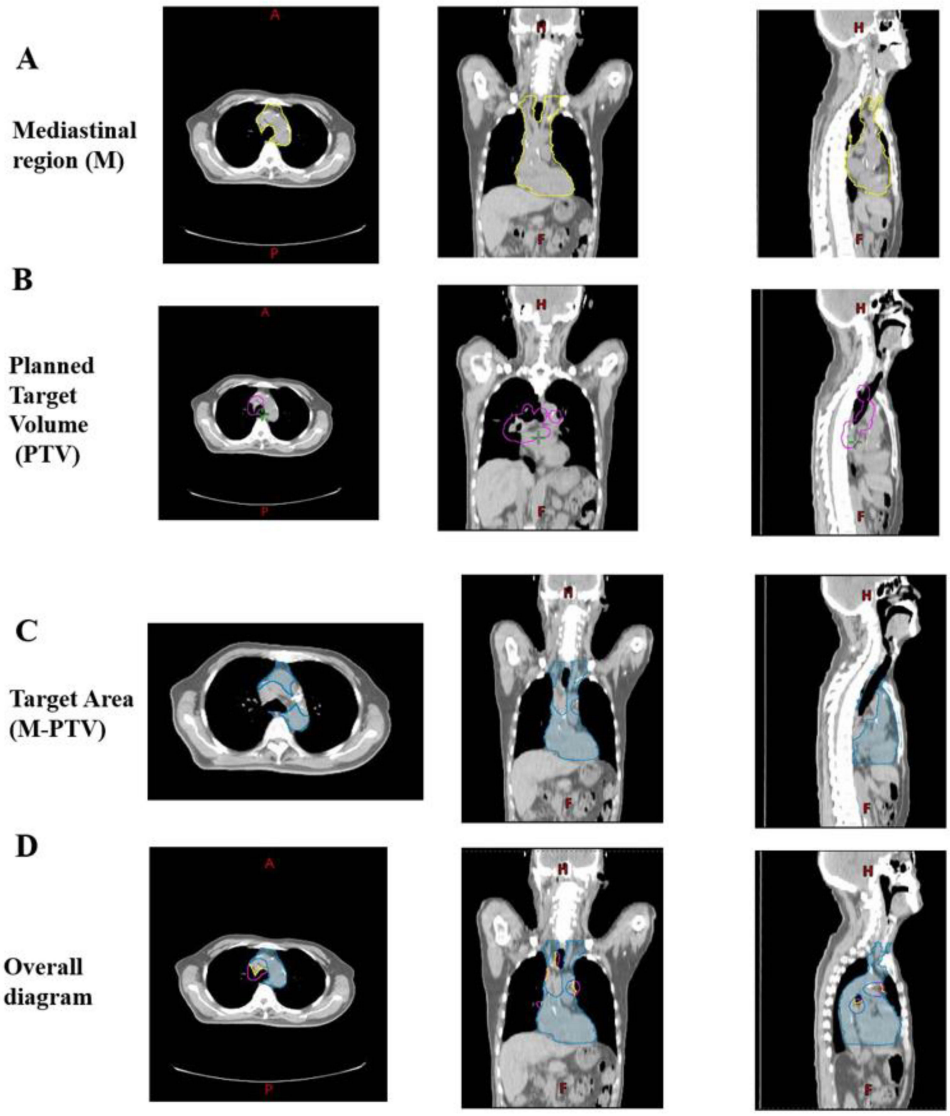

Figure 1 Example of target delineation of the target volume (M-PTV) for NSCLC radiotherapy. (**A**), the yellow area represents the mediastinal area (M) outlined in the general drawing. (**B**). The purple area represents the PTV area (PTV). (**C**), The blue area represents the target area of the study, which is the mediastinal area minus P (M-PTV). (**D**) represents the general layout.

Detecting Immune Cells

Send the collected blood samples before and after radiotherapy to the First Affiliated Hospital of Shandong First Medical University for examination. Perform flow cytometry analysis in the laboratory. The specific steps are: 1. Use BD FACS Canto Clinical software to perform lymphocyte subpopulation detection. 2. Detection instrument BD FACS Canto II. 3. Method principle: Two/three color direct immunofluorescence method. Various monoclonal antibodies labeled with fluorescein are mixed into whole blood and bind to corresponding antigens on the leukocyte membrane. After hemolysis, washing (and fixation), analysis is performed on a flow cytometer to obtain the percentage of lymphocyte subpopulations. Add 4u1 monoclonal antibodies to the numbered test tubes as required. Add a mixed 50u1 anticoagulant to the test tube. Mix well, avoid light, and incubate at room temperature for 15 minutes. Bathing Blood: Take 10 × Dilute hemolysin to 1 with distilled water ×, Add 450u1 hemolysin, dissolve red blood cells, mix well, and avoid light for 10 minutes at room temperature. On-machine testing (samples can be washed or not washed according to the sample situation).

Evaluation Strategy

The main endpoint for evaluating the efficacy of radiotherapy is within 2–3 months after the end of the patient's radiotherapy, based on the changes in imaging solid tumor volume after radiotherapy, and evaluated using the Recipe 1.1 standard.[12] PR refers to a reduction of at least 30% in the sum of the maximum diameters of the tumor; SD means that the sum of the maximum diameters of the tumor will not shrink to PR or increase to PD; PD refers to an increase of at least 20% in the sum of the maximum diameters of the target lesion, or the emergence of new lesions. Objective response rate (ORR) refers to the proportion of patients whose tumor volume has decreased to a predetermined value and can maintain the shortest possible time frame. Usually, ORR is defined as CR+PR.

Data Analysis

All statistical analyses were conducted using SPSS 25.0 (SPSS Co., Ltd., Chicago, Illinois, USA). Log rank test was used to evaluate the difference in efficacy. Evaluation of changes in immune cells using nonparametric tests. The subject-receiver operating characteristic (ROC) curve selects the best cut-off value for each measurement and stratified the measurement. Univariate and multivariate analysis using a binary logistic regression model to evaluate the factors affecting the efficacy, $P< 0.05$ was considered statistically significant.

Result

Patient Characteristics

Table 1 summarizes the characteristics of the 72 patients who met the above inclusion criteria. The cohort's median age was 66.6 years (range: 49–87 years). The median dose of radiotherapy is 60 Gy/30f (range: 45–66 Gy/20-30f).

Table 1 Baseline Characteristics of Patients

Characteristic	No of the People (%)
Sex	
Female	12 (16.7%)
Male	60 (83.3%)
Age	
Median	66.6
Range	49–87
≥65	42 (58.3%)
<65	30 (41.7%)
History of smoking	
Yes	50 (69.4%)
No	22 (30.6%)
History of drinking	
Yes	41 (56.9%)
NO	31 (43.1%)
TNM stage*	
III	34 (47.2%)
IV	38 (52.8%)
Radiotherapy dose	
Median	60 Gy/30f
Range	45–66 Gy/20-30f

Note: *According to the 7th AJCC/International Union against Cancer staging system.
Abbreviation: AJCC, American Joint Committee for Cancer.

The Correlation Between Short-Term Efficacy of Radiation Therapy and Lymphocyte and Immune Cell Subsets

To elucidate the correlation between changes in lymphocyte and immune cell subsets and short-term efficacy of radiotherapy, analyze the trend of changes in lymphocyte and immune cell subsets before and after radiotherapy. After radiotherapy, CD3+T cells, CD8+T cells, and CD8/Treg increased compared to before radiotherapy, while lymphocytes, CD4+T cells, CD4/CD8, and B cells decreased compared to before radiotherapy, with significant statistical differences ($P<0.05$). There was no statistical difference between NK cells and Treg cells ($P>0.05$) (Figure 2).

Determining the Optimal Value of Lymphocyte and Circulating Immune Cell Subsets for Predicting Short-Term Radiotherapy Efficacy

The relationship between changes in lymphocyte and immune cell subsets before and after radiotherapy and the efficacy of short-term radiotherapy was analyzed. The threshold values of lymphocyte and circulating immune cell subsets were obtained based on ROC curve analysis and grouped. The area under the curve (AUC) of LymR, CD3R, CD4R, CD8R, CD4/CD8R, NKR, BR, TregR, and CD8/TregR were 0.63, 0.51, 0.73, 0.75, 0.74, 0.54, 0.54, 0.50, and 0.58, respectively. Set the point corresponding to the maximum Youden index=Sensitivity+Specificity − 1 as the optimal threshold value for predicting short-term radiotherapy efficacy, which is 1.38, 1.05, 0.99, 1.02, 1.08, 1.26, 0.45, 1.35, and 0.99, respectively (Figure 3).

Changes in Circulating Immune Cells Induced by Radiotherapy and Their Relationship with Radiotherapy Efficacy

According to the chi square test analysis between lymphocyte and immune cell subpopulations and short-term radiotherapy efficacy, CD8+T cells and CD8/Treg in the high group were correlated with higher ORR, while lymphocytes, CD4+T cells, CD4/CD8, and NK cells in the low group were correlated with ORR, $P<0.05$. Please refer to Table 2 for details.

Relationship Between Short-Term Efficacy of Radiotherapy and Immune Cells and Their Subsets

To explore the correlation between short-term radiotherapy efficacy and lymphocyte and immune cell subsets, the factors in each group were included in a univariate logistic regression analysis. The results showed that LymR (High group, OR=0.27, 95% CI=0.09–0.85, $P=0.03$), CD4R (High group, OR=0.19, 95% CI=0.06–0.58, $P=0.01$), CD8R (High group, OR=13.00, 95% CI=3.71–45.62, $P=0.01$), CD4/CD8R (High group, OR=0.20, 95% CI=0.07–0.58, $P=0.01$), NKR (High group, OR=3.54, 95% CI=1.05–11.96, $P=0.04$) and CD8/TregR (High group, OR=4.85, 95% CI=1.65–14.21, $P=0.01$) are predictors of short-term efficacy of radiotherapy. To further explore the independent predictive factors between lymphocyte and immune cell subsets and short-term efficacy of radiotherapy, a multivariate logistic regression analysis was conducted. The results showed that the increase in CD8+T cells after radiotherapy was a predictive factor for short-term efficacy after radiotherapy CD8R (High group, OR=12.71, 95% CI=3.62–44.64, $P=0.01$) (Table 3).

Determining the Optimal Threshold Value of Mediastinal Radiation Dose Parameters and CD8+T Cells

Multivariate analysis based on binary metalogic regression showed that CD8+T cells were independent predictors of lymphocyte and circulating immune cell subsets for radiotherapy efficacy. In order to further investigate the correlation between changes in lymphocyte and circulating immune cell subpopulations and radiation dose parameters, the optimal threshold for radiation dose parameters was selected based on ROC curve analysis. The AUC values of V2, V5, V10, V20, V30, V40, and V50 are 0.68, 0.62, 0.56, 0.56, 0.6, 0.58, and 0.58, respectively, with critical values of 65.05, 49.3, 44.9, 39.5, 32.1, 9.6, and 2.95, respectively (Figure 4).

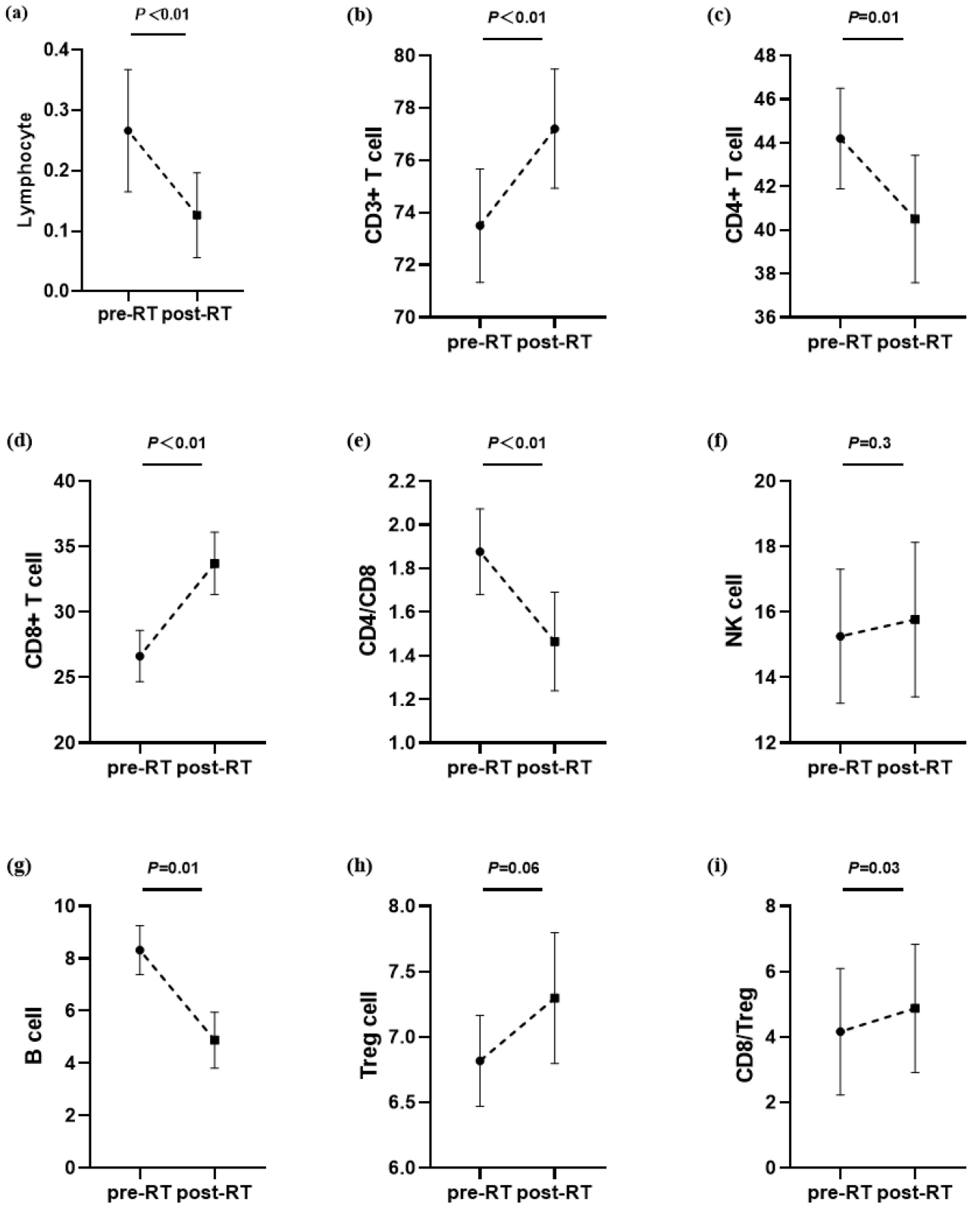

Figure 2 Changes in circulating immune cell subsets before and after radiotherapy. (**A**)–(**I**) represent the changes in lymphocyte and immune cell subsets before and after radiotherapy. After radiotherapy, CD3+T cells, CD8+T cells, and CD8/Treg increased, while CD4+T cells, CD4/CD8, and B cells decreased significantly ($P<0.05$). There was no statistically significant difference in lymphocytes, NK cells, and Treg cells ($P>0.05$). Pre RT, before radiotherapy; Post RT: After radiotherapy. (**A**) The Y-axis of the graph has no units. (**B**) The Y-axis of the graph has units of "% Lymphs", (**C**) The Y-axis of the graph has units of "% Lymphs", (**D**) The Y-axis of the graph has units of "% Lymphs", (**E**) The Y-axis of the graph has no units, (**F**) The Y-axis of the graph has units of "% Lymphs", (**G**) The Y-axis of the graph has units of "% Lymphs", (**H**) The Y-axis of the graph has units of "% CD4+T Lymphs", (**I**) The Y-axis of the graph has no units.

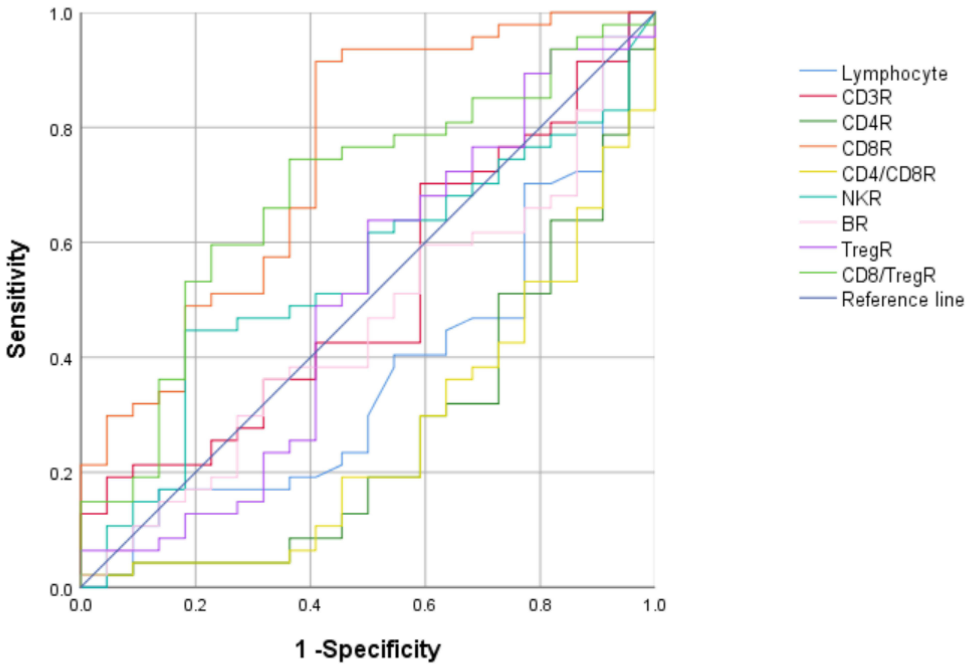

Figure 3 Receiver operating characteristic (ROC) curves for short-term response and circulating immune cell subsets in all patients. Area under lymphocyte ratio curve AUC=0.63 (light blue line), area under CD3+T cell ratio curve AUC=0.51 (red line), area under CD4+T cell ratio curve AUC=0.73 (dark green line), area under CD8+T cell ratio curve AUC=0.75 (Orange line), area under CD4/CD8 ratio curve AUC=0.74 (yellow line), area under NK cell ratio curve AUC=0.54 (blue green line), The area under the B cell ratio curve AUC=0.46 (pink line), the area under the Treg cell ratio curve AUC=0.50 (purple line), and the area under the CD8/Treg ratio curve AUC=0.68 (green line). LymR, post radiotherapy: lymphocyte ratio before radiotherapy; CD3R, post radiotherapy: CD3+T cell ratio before radiotherapy; CD4R, post radiotherapy: CD4+T cell ratio before radiotherapy; CD8R, post radiotherapy: CD8+T cell ratio before radiotherapy; CD4/CD8R, after radiotherapy: CD4/CD8 ratio before radiotherapy; NKR, post radiotherapy: NK cell ratio before radiotherapy; BR, after radiotherapy: B cell ratio before radiotherapy; TregR, post radiotherapy: Treg cell ratio before radiotherapy; CD8/TregR, post radiotherapy: CD8/Treg ratio before radiotherapy.

Correlation Between Mediastinal Radiation Dose Parameters and CD8+T Cells

Univariate analysis based on CD8+T cells and mediastinal radiation dose parameters showed V2 (high group, OR=3.4, 95% CI=1.13–10.36, *P*=0.03), V5 (high group, OR=3.14, 95% CI=1.04–9.48, *P*=0.04), and V20 (high group, OR=4.71,

Table 2 Circulating Immune Subgroup ORR

	Sensitive Group	Non-Sensitive Group	*P*
	No (%)	No (%)	
Sex			0.82
Men	42 (58.3%)	18 (25%)	
Women	8(11.1%)	4(5.6%)	
History of smoking			0.49
Yes	36 (50%)	14 (19.4%)	
No	14 (19.4%)	8 (11.1%)	
History of drinking			0.49
Yes	29 (40.3%)	12 (16.7%)	
No	21 (29.2%)	10 (13.9%)	
Synchronous radiochemotherapy			0.80
Yes	15 (20.8%)	7 (9.7%)	
No	35 (48.7%)	15 (20.8%)	
TNM*			0.76
III	23 (31.9%)	11 (15.3%)	
IV	27 (37.5%)	11 (15.3%)	

(*Continued*)

Table 2 (Continued).

	Sensitive Group	Non-Sensitive Group	*P*
	No (%)	No (%)	
LymR			0.02
High group	24 (33.4%)	17 (23.6%)	
Low group	26 (36.1%)	5 (6.9%)	
CD3R			0.24
High group	22 (30.6%)	13 (18.1%)	
Low group	28 (38.8%)	9 (12.5%)	
CD4R			0.01
High group	17 (23.6%)	16 (22.2%)	
Low group	33 (45.8%)	6 (8.3%)	
CD8R			0.01
High group	45 (62.5%)	9 (12.5%)	
Low group	5 (6.9%)	13 (18.1%)	
CD4/CD8R			0.01
High group	11 (15.3%)	13 (18.1%)	
Low group	39 (54.1%)	9 (12.5%)	
NKR			0.04
High group	22 (30.5%)	4 (5.6%)	
Low group	28 (38.9%)	18 (25%)	
BR			0.11
High group	34 (47.2%)	19 (26.4%)	
Low group	16 (22.2%)	3 (4.2%)	
TregR			0.08
High group	8 (11.1%)	7 (9.8%)	
Low group	42 (58.3%)	15 (20.8%)	
CD8/TregR			0.01
High group	37 (51.4%)	8 (11.1%)	
Low group	13 (18.1%)	14 (19.4%)	

Note: *According to the staging system of the 7th AJCC/International Alliance Against Cancer. The sensitive group is CR+PR, while the non-sensitive group is SD+PD. Lymphocytes, post radiotherapy: lymphocyte ratio before radiotherapy; CD3R, post radiotherapy: CD3+T cell ratio before radiotherapy; CD4R, post radiotherapy: CD4+T cell ratio before radiotherapy; CD8R, post radiotherapy: CD8+T cell ratio before radiotherapy; CD4/CD8R, after radiotherapy: CD4/CD8 ratio before radiotherapy; B cell ratio before radiotherapy; TregR, post radiotherapy: Treg cell ratio before radiotherapy; CD8/TregR, post radiotherapy: CD8/Treg ratio before radiotherapy.
Abbreviations: AJCC, United States Joint Commission on Cancer; NKR, post radiotherapy; NK cell ratio before radiotherapy; BR, after radiotherapy.

95% CI=0.98–22.63, P=0.05). Incorporating P<0.05 into binary multivariate logistic regression analysis showed that V2 independently positively predicted an increase in CD8+T cells (high group, OR=3.4, 95% CI=1.13–10.36, P=0.03) (Table 4).

Discussion

In this study of 72 patients with lung cancer receiving radiotherapy, CD8+T cells are related to the radiotherapy effect, and CD8+T cells increase after radiotherapy, thus benefiting patients. It is further found that the radiation dose parameters play a key role in the changes in circulating immune cells, increasing the radiation dose parameters can improve the circulating immune cells after radiotherapy.

At present, anti-tumor immunity is an important factor affecting the efficacy of radiotherapy and tumor prognosis. It is closely related to the type of primary tumor, radiotherapy site, radiotherapy parameters (V2, V5, V10, etc.), dose planning (low or conventional segmentation), and blood collection time point (before or after radiotherapy). Chao Liu et al research has shown that CD8+T cells have anti-tumor immune response effects in NSCLC patients. Pre-treatment CD8+CD28+T cells can

Table 3 Univariate and Logistic Regression Analysis of Factors Affecting Short-Term Efficacy of Radiotherapy

Factors	Univariate Regression Analysis			Multivariate Regression Analysis		
	OR	(95%) CIs	*P*	OR	(95%) CIs	*P*
Sex	0.86	0.23–3.21	0.82			–
Combined chemotherapy	1.09	0.37–3.21	0.88			–
Smoking history	0.68	0.23–1.98	0.48			–
Drinking history	0.87	0.32–2.39	0.79			–
TNM*	0.85	0.31–2.32	0.75			–
LymR	0.27	0.09–0.85	0.03			–
CD3R	0.54	0.20–1.50	0.24			–
CD4R	0.19	0.06–0.58	0.01			–
CD8R	13.00	3.71–45.62	0.01	12.71	3.62–44.64	0.01
CD4/CD8R	0.20	0.07–0.58	0.01			–
BR	0.34	0.09–1.30	0.11			–
NKR	3.54	1.05–11.96	0.04			–
TregR	0.36	0.11–1.19	0.09			–
CD8/TregR	4.85	1.65–14.21	0.01			–

Notes: *According to the 7th AJCC/International Anti Cancer Alliance staging system; LymR, lymphocyte ratio; CD3R, CD3+T cell ratio; CD4R, CD4+T cell ratio; CD8R, CD8+T cell ratio; CD4/CD8R, CD4/CD8 ratio; NKR, NK cell ratio; BR, B cell ratio; TregR, Treg cell ratio; CD8/TregR, CD8/Treg ratio; OR, ratio; (95%) CIs, (95%) confidence interval.
Abbreviation: AJCC, United States Joint Commission on Cancer.

predict the early tumor response of NSCLC lung metastasis patients to stereotactic ablation radiotherapy (SABR).[13] In our study, we found that peripheral blood lymphocytes showed a downward trend after radiotherapy, but the proportion of CD8+T cells increased. Relevant studies also show that radiation therapy can reduce the number of peripheral blood lymphocytes.[14–18]

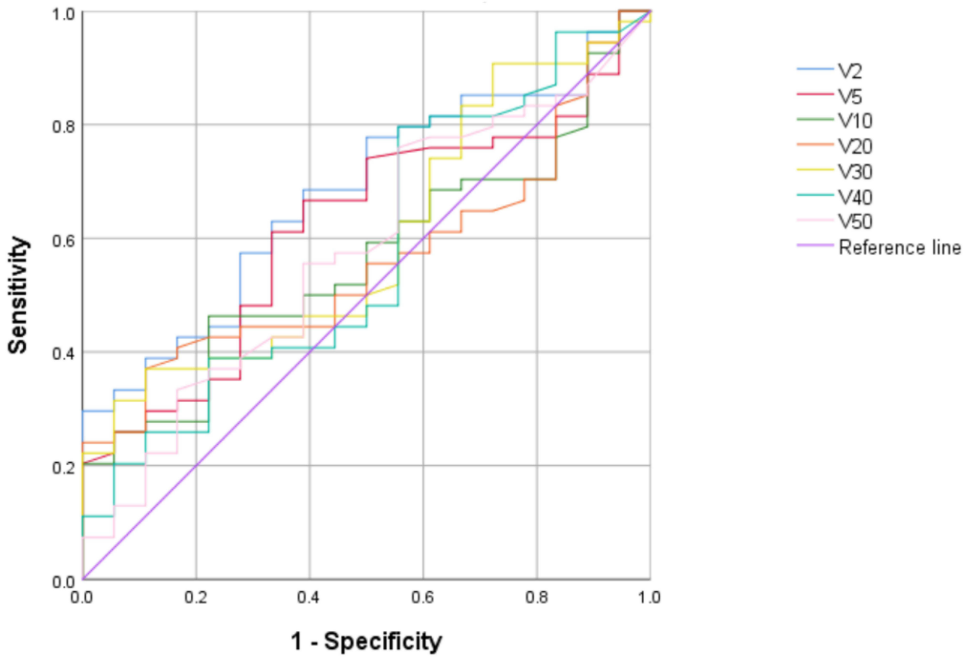

Figure 4 Receiver operating characteristic curves (ROC) for dose parameters. AUC under V2 curve=0.68 (blue line), AUC under V5 curve=0.62 (red line), and AUC under V10 curve=0.56 (green line); Area under V20 curve AUC=0.56 (Orange line); Area under V30 curve AUC=0.60 (yellow line); Area under V40 curve AUC=0.58 (blue green line); Area under V50 curve AUC=0.58 (pink line); V2, the volume subjected to 200cGy irradiation; V5, the volume subjected to 500cGy irradiation; V10; Volume of exposure to 1000cGy; V20,; The volume of exposure to 2000cGy; V30, volume irradiated with 3000cGy; V40, volume irradiated with 4000cGy; V50, the volume subjected to 5000cGy irradiation.

Table 4 Binary Logistic Regression Analysis of Radiotherapy Parameters and CD8+ T Cells

	Mean±SD	Binary Univariate Logistic Regression Analysis			Binary Multivariate Logistic Regression Analysis		
		OR	95% CI	*P*	OR	95% CI	*P*
V2	71.48±19.60	3.40	1.13–10.36	0.03	3.40	1.13–10.36	0.03
V5	55.25±20.50	3.14	1.04–9.48	0.04			
V10	45.13±18.66	3.02	0.88–10.36	0.08			
V20	33.66±15.57	4.71	0.98–22.63	0.05			
V30	23.68±11.82	7.81	0.96–63.59	0.06			
V40	15.71±9.71	2.49	0.78–7.90	0.12			
V50	7.08±6.89	2.52	0.62–7.73	0.11			

Notes: Mean, average; SD, standard deviation; V2, the volume subjected to 200cGy irradiation; V5, the volume subjected to 500cGy irradiation; V10, the volume of exposure to 1000cGy; V20, the volume subjected to 2000cGy irradiation; V30, volume irradiated with 3000cGy; V40, volume irradiated with 4000cGy; V50, volume exposed to 5000cGy irradiation; OR, ratio; (95%) CIs, (95%) confidence interval.

In tumor patients, lymphocytes are closely related to the prognosis of patients.[19] At present, the study shows that lymphopenia will reduce the survival period of NSCLC patients receiving radiotherapy.[20] In our study, we found that in patients with NSCLC, although the peripheral blood lymphocytes of patients after radiotherapy decreased significantly compared with those before radiotherapy, which also verified that the lymphocytes showed a downward trend after radiotherapy, and radiotherapy harmed the total lymphocyte changes, when the immune cell subgroups were taken as the observation object, the study found that the radiation sensitivity of different subgroups was very different. At present, studies have confirmed that different lymphocyte subsets in peripheral blood have different sensitivity to radiation, and radiotherapy causes a redistribution of lymphocyte subsets in peripheral blood.[16]

Our study suggested that the proportion of CD8+T cells increased in NSCLC patients after radiotherapy, and CD8+T cells correlated with radiotherapy's efficacy. Liu et al found that the proportion of CD8+T cells in NSCLC after radiotherapy increased compared to baseline in the total population. The RFS of patients with a higher proportion of CD8+T cells was significantly prolonged one month after radiotherapy, which is similar to the results of our study.[21] Previous studies have proved that the increase of CD8+T cells and the decrease of CD4+/CD8+T lymphocyte ratio in patients with lung or liver metastasis are beneficial to patients receiving stereotactic systemic radiotherapy (SBRT).[22] However, in previous studies, it was found that the CD4/CD8 ratio and CD19+cell count in patients with prostate cancer treated with carbon ion radiotherapy CR or PR were always higher than those in the SD group. However, the CD3+ T cell and CD8+ T cell counts in CR and PR groups were lower than those in the SD group.[23] There are several explanations for these seemingly contradictory observations. This may be due to the great difference in the radioimmunoassay of different types of tumors.[24] The distribution of lymphocyte subtypes in the human body is not uniform. For example, there are more B cells in the spleen, while lymphocytes containing immature antigens in the lymph nodes gather. Therefore, some lymphocyte subsets are preferentially consumed. It may also depend on the radiation sensitivity of specific subgroups and exposure sites.[25]

In tumor patients, different doses of radiotherapy have different immunomodulatory effects on the tumor microenvironment, which may lead to changes in the effect of immunotherapy.[9] Our study found that low-dose radiotherapy (2Gy) can improve CD8+T cells after radiotherapy and thus affect the radiotherapy effect of NSCLC by comparing the volume of different doses in the target area. It may be that low-dose RT provides a more effective immunotherapy platform by promoting the reprogramming of the tumor microenvironment and promoting the infiltration of T cells into the tumor. For example, 2Gy × 1f is the strongest in inducing the infiltration of immune cells, the expression of immune-related genes, the infiltration of T cells, and the expression of T cell recruitment chemokine in low-dose RT. It is the best plan to control tumor growth.[26–28]

Sparry et al also showed that low-dose RT can enhance T cell function by increasing CD8+T cell diversity, T cell receptor signal, and cell proliferation.[29] In animal and mouse experiments, BALB/c mice received a single low dose of RT (0.1 or 0.2 Gy), and then received an intravenous injection of homologous L1 sarcoma cells 2 hours later. Compared with the sham radiation control group, lung metastasis was significantly reduced.[30] Similarly, in rats with locally implanted hepatoma cell lines, 0.2 Gy whole-body irradiation inhibited lung metastasis, although, at the same local irradiation dose, remote metastasis could not be effectively controlled.[31] It shows that a single low-dose RT correlates with the curative effect of lung cancer.

RT combined with immunotherapy can improve the anti-tumor effect. At the same time, low dose RT, the activation of DC (CD40 agonist, TLR agonist) and ICI can be further increased by drug intervention (such as anti-PD-1/PDL1, anti-CTLA4, anti-LAG-3), thus increasing the tumorigenicity of T cells.[31] For example, the combination of 2 * 2Gy radiation dose and TLR9 agonist has produced important clinical reactions in patients with advanced lymphoma.[32] Low-dose RT (for example, 2GY) is beneficial in combination with a variety of Immunomodulatory therapeutic (IMT) drugs for immune regulation to achieve IMT. It may be because radiation is used as an immune reprogramming agent rather than directly killing tumor cells. Low-dose radiotherapy activates innate and adaptive immunity by activating inflammatory mechanisms. To improve the immunogenicity of tumors.[9]

Our research aims to protect the lymphatic drainage area so that CD8+T cells can be increased after radiotherapy at low dose, to achieve a better radiotherapy effect. Low-dose lymphoid organs, circulating blood pool, and heart are closely related to the decline of immunosuppression.[33,34] Therefore, the RT protocol can be optimized to potentially reduce the immunosuppressive effect, for example, by protecting the lymphatic drainage area, thus affecting immunotherapy. The most sensitive cells to radiation are lymphocytes in the whole body and the hematopoietic system. Lymphocytes are located in the blood (circulating lymphocytes), spleen, and thymus (children and adolescents). CD8+T cells in parenchymal lymphoid organs (lymph nodes and spleen) are the most sensitive to radiation. Compared with circulating CD8+T cells in non-irradiated tumors, CD8+T cells in tumors are more resistant to radiation and promote exercise and interferon secretion. This is because the tumor microenvironment has changed, in which transforming growth factor β It is a key regulatory factor for the production of T cells in tumors that are more resistant to radiation. Therefore, CD8+T cells are the most prominent cell type in anti-tumor immune response, indicating the importance of prognosis.[35–37] A low dose can increase CD8+T cells after radiotherapy so that patients can get a better radiotherapy effect. Therefore, in the era of a combination of radiotherapy and immunotherapy, to protect immune cells, it is recommended to reduce exposure to the lymphatic drainage area of the cancer target area.

The current study has several limitations. Due to limited experimental equipment, it is not possible to obtain absolute values and activation markers of lymphocytes and immune cells. The nature of retrospective analyses and their experience at a single institution inevitably introduces selection bias and bias in radiation therapy planning. The cases are limited and have no classification by tumor type. Prognostic follow-up time is insufficient. A larger prospective study will be conducted in collaboration with other centers to confirm the general applicability of this conclusion.

The study found that the low dose area is related to the high CD8+T cell ratio, which may increase the effect of radiotherapy. Therefore, the effect of radiotherapy on the lymphatic drainage area is more complicated and requires more in-depth study.

Abbreviations

ROC, Receiver Operating Characteristic; RT, Radiation Therapy; IMRT, Intensity-Modulated Radiation Therapy; PTV, Planning Target Volume.

Patient Data Confidentiality Statement

The patient data involved in this study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Shandong First Medical University, with an ethics code of 2022-S607. Take all necessary measures to protect patient data during research and use, ensuring that unauthorized third parties do not access or use this data. Promise not to use patient data for commercial or other purposes without consent. Make every effort to protect the privacy and security of patients, and take appropriate technical and organizational measures to ensure the confidentiality and integrity of patient data.

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The present study was approved by the Ethic Committee of The First Affiliated Hospital of Shandong First Medical University, and informed consent was waived because this is a retrospective study.

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Disclosure

The authors declare that they have no competing interests.

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