

The Clinical Significance of Peripheral Blood Helper T Cell Subsets in the Efficacy of Radiotherapy for Patients with Esophageal Cancer

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Abstract

Background: Peripheral blood helper T cell subsets are pivotal in assessing immune function and predicting treatment outcomes in cancer patients. This study sought to determine the prognostic significance of these subsets in gauging the efficacy of radiotherapy for esophageal cancer patients. **Methods:** 80 patients diagnosed with esophageal cancer underwent a comprehensive analysis of their helper T cell subsets before and after radiotherapy. Treatment efficacy was meticulously evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines. **Results:** Radiotherapy induced significant immunological alterations, evidenced by a notable increase in the proportions of Th17 and Tfh cells, while the percentages of Th2, Treg, and Tfr cells decreased significantly (all $P < 0.05$). Post-treatment, the responsive group exhibited higher Tfh cell proportions ($P < 0.05$) and lower Treg cell percentages ($P < 0.05$) compared to the non-responsive group. Logistic regression analysis identified Tfh cells as a protective factor and Treg cells as a risk factor for treatment response ($P < 0.05$). Receiver operating characteristic (ROC) curve analysis further underscored the predictive utility of Tfh (AUC = 0.930) and Treg (AUC = 0.830) cells. **Conclusion:** The proportions of Tfh and Treg cells represent independent determinants of radiotherapy response and hold promise as robust biomarkers for predicting treatment outcomes in esophageal cancer patients, offering potential implications for personalized immunotherapeutic strategies.

Keywords

Esophageal cancer; Radiotherapy; Helper T cell subsets; Efficacy prediction

1. Introduction

Esophageal cancer, a prevalent and menacing form of malignancy, ranks among the leading causes of cancer-related deaths globally. According to the global cancer burden data released by the International Agency for Research on Cancer in 2020, there were approximately 604,100 new cases of esophageal cancer worldwide, with around 544,000 deaths [1]. Helper T cell subsets in peripheral blood play a crucial role in immune regulation. The changes in the numbers and functions of different helper T cell subsets, such as Th1, Th2, Th17, etc., can affect the immune response to tumor cells [2-4]. When patients receive radiotherapy, the immune system will be activated, and the helper T cell subsets in the peripheral blood may change, which may be closely related to the efficacy of radiotherapy [5, 6]. However, there are relatively few reports on the changes in the proportions of helper T cell subsets in the peripheral blood in esophageal cancer. This research endeavors to comprehensively assess the composition and functional characteristics of helper T cell subsets in the peripheral blood of esophageal cancer patients.

2. Materials and Methods

2.1 General Information

This study enrolled 80 esophageal cancer patients who underwent three-dimensional conformal radiotherapy or intensity-modulated radiotherapy at the Department of Radiotherapy of The First Affiliated Hospital of Bengbu Medical College between September 2022 and March 2024. The inclusion criteria were as follows: Patients aged 18 years and above; Those without a history of radiotherapy, kidney diseases, immunodeficiency, or other concurrent complications; Histopathologically confirmed esophageal cancer; A Karnofsky performance status score of ≥ 70 ; No prior history of malignant tumors or severe medical conditions. Patients were excluded according to the following exclusion criteria: Those who did not complete the treatment course or were removed due to treatment interruption, Pregnant or breastfeeding patients, and individuals with mental disorders that hindered their cooperation with the treatment. The study protocol was approved by the Ethics Committee of Bengbu Medical University (Approval Code: LKPZ [2024]051). Clinical data of patients meeting the above-mentioned inclusion and exclusion criteria were collected and organized, encompassing radiation dose, basic tumor characteristics, age, gender, smoking history, and drinking history, among others.

2.2 Detection Method of Helper T Cell Subsets in Peripheral Blood

Within one week before and after radiotherapy, 5 ml of peripheral venous blood was collected from the patients. An equal volume of lymphocyte separation medium was added. After centrifugation at 2000 rpm for 30 minutes, peripheral blood mononuclear cells were aspirated. The cells were washed twice with PBS, and the concentration of the cell suspension was adjusted to 2×10^6 cells per 100 μL .

Detection of CD4⁺CD25⁺Foxp3⁺ Treg cells, CD4⁺CXCR5⁺ICOS⁺ Tfh cells, and CD4⁺CXCR5⁺ICOS⁺Foxp3⁺ Tfr cells: Take 100 μL of the cell suspension and add it to a microplate. Add 0.05 μL of Fixable Viability Stain 700 (BD Biosciences, San Jose, CA, USA), and then add 1 μL of anti-human CD4-Percp-cy5.5 (BioLegend, San Diego, CA, USA), 1 μL of anti-human ICOS-BV605 (BioLegend), 1 μL of anti-human CD25-BV510 (BioLegend), and 1 μL of anti-human CXCR5-BV786 (BioLegend) monoclonal antibodies, respectively. Incubate in the dark for 30 minutes, and then add 200 μL of PBS to wash the cells twice. Add 200 μL of cell membrane permeabilization solution, incubate in the dark for 30 minutes, and then wash the cells twice with PBS. Finally, add 1 μL of anti-human CD4-Percp-cy5.5 (BioLegend) and 1 μL of anti-human Foxp3-PE (BioLegend) monoclonal antibodies, respectively, incubate in the dark for 30 minutes, wash the cells twice with PBS, resuspend them, and detect them with a flow cytometer.

Detection of CD4⁺IFN- γ ⁺ Th1 cells, CD4⁺IL4⁺ Th2 cells, and CD4⁺IL-17⁺ Th17 cells: Take 100 μL of the cell suspension and add an equal volume of cell stimulant. After incubating for 6 hours, wash the cells twice with PBS. Add 0.05 μL of Fixable Viability Stain 700 and 1 μL of anti-human CD4-Percp-cy5.5 (BioLegend) monoclonal antibody, incubate for 30 minutes, and then wash the cells twice with PBS. Add 200 μL of cell membrane permeabilization solution (Thermo Fisher Scientific), incubate in the dark for 30 minutes, and then wash the cells twice with the membrane permeabilization washing solution. Add 1 μL of anti-human CD4-Percp-cy5.5 (BioLegend), 1 μL of anti-human IFN- γ -APC (BioLegend), 1 μL of anti-human IL-4-BV421 (BioLegend), and 1 μL of anti-human IL-17-PE (Thermo Fisher Scientific), respectively. Incubate in the dark for 30 minutes, wash the cells twice with PBS, resuspend them, and detect them with a flow cytometer.

2.3 Efficacy Evaluation

Four weeks post-treatment, an associate chief radiologist and an associate chief radiotherapy physician, both blinded to the research data results, evaluated the tumor size, location, and lymph node metastasis. They performed this assessment using contrast-enhanced CT scans of the chest, abdomen, and neck. Based on the Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1) [7], the treatment response was categorized into four grades. A complete response (CR) was defined as the complete disappearance of all lesions. A partial response (PR) was characterized by a decrease of more than 30% in the longest diameter of the target lesions compared to the baseline measurement. Disease progression (PD) was identified when new target lesions emerged or when there was an increase of less than 20% in the target lesions. Stable disease (SD) was determined when the lesion size decreased by less than 30% or increased by less than 20%.

2.4 Statistical Analysis

Statistical analyses were performed using GraphPad Prism 10.0 software. Enumeration data were expressed as rates (%), and comparisons between two groups were conducted using the Mann-Whitney U test (nonparametric). When there were more than two groups, the Kruskal-Wallis test (nonparametric) was performed. The paired t-test was used to examine the proportions of cell subsets before and after radiotherapy. The chi-square (χ^2) test was used to analyze the enumeration data, which were expressed as n. The SPSS software was used for logistic regression analysis to explore the factors affecting the efficacy of radiotherapy. The receiver operating characteristic (ROC) curve was employed to analyze the sensitivity and specificity. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1 Patient Characteristics

Table 1 summarizes the characteristics of 80 patients who met the above inclusion criteria. There were 56 male patients and 24 female patients, and there was no statistically significant difference between male and female patients ($\chi^2 = 0.785$, $P = 0.654$). The median age of this cohort was 65 years (range: 35-80 years). The median radiation dose was 60 Gy/30F (range: 50-66 Gy/25-33F).

Table 1. Baseline Characteristics of Patients

Characteristic	No. of People (%)
Sex	
Female	24 (30.00%)
Male	56 (70.00%)
Age	
Median	65
Range	35-80
History of smoking	
Yes	60 (75.00%)
No	20 (25.00%)
History of drinking	
Yes	22 (27.50%)
No	58 (72.50%)
Radiotherapy dose	
Median	60 Gy/30F
Range	50-66 Gy/25-33F

3.2 Correlation Between the Short-term Efficacy of Radiotherapy and the Peripheral Blood Helper T Cell Subsets

In order to elucidate the relationship between the alterations in peripheral blood helper T cell subsets and the short-term therapeutic effect of radiotherapy, an analysis was conducted on the variation patterns of these subsets in peripheral blood before and after radiotherapy. Post-radiotherapy, when compared to the pre-treatment levels, the percentages of Th17 cells and Tfh cells showed an increase, whereas the percentages of Th2, Treg, and Tfr cells decreased. These changes were statistically significant ($P < 0.05$) (Figure 1). Conversely, for Th1 cells, no statistically significant difference was observed ($P > 0.05$) (Figure 1).

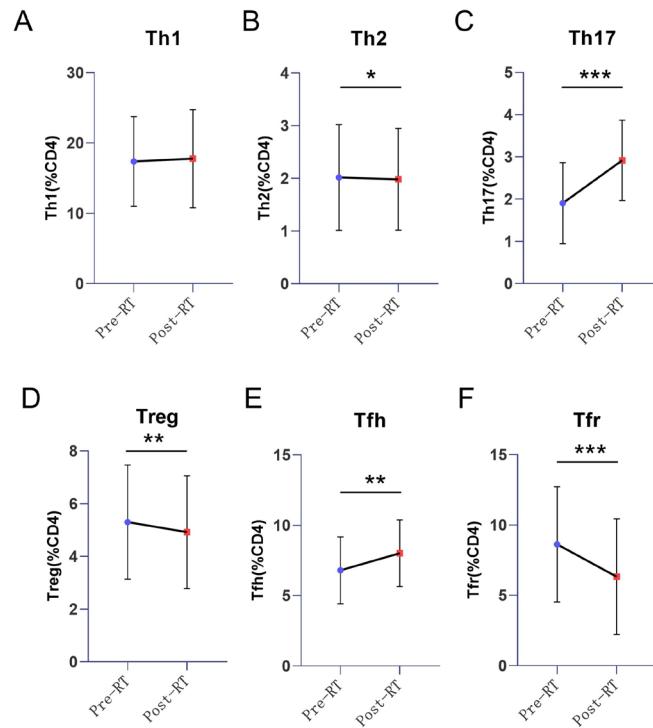


Figure 1. Changes in proportions of peripheral blood helper T-cell subsets before and after radiotherapy. (A)-(F) represent the cell proportions of Th1, Th2, Th17, Treg, Tfh, and Tfr in the patients before and after radiotherapy.

3.3 Relationship Between the Proportions of Peripheral Blood Helper T Cell Subsets Before Radiotherapy and the Efficacy of Radiotherapy

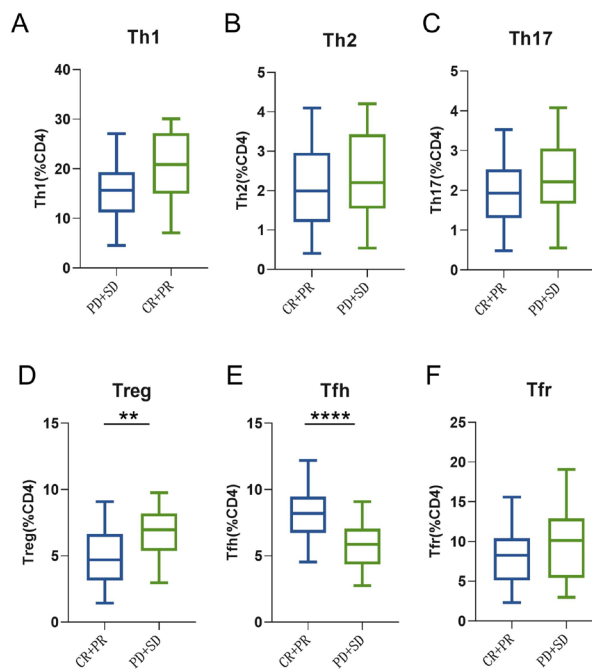


Figure 2. Differences in the Proportions of Peripheral Blood Helper T-cell Subsets before Radiotherapy between Responding and Non-responding Groups. (A)-(F) represent the cell proportions of Th1, Th2, Th17, Treg, Tfh, and Tfr between responding and non-responding groups.

Patients were divided into a responsive group (CR + PR; n = 47) and a non-responsive group (PD + SD; n = 33) according to their treatment response at 4 weeks. A comparative analysis demonstrated that the proportions of Tfh ($P < 0.001$) cells in the responsive group were significantly higher than those in the non-responsive group (Figure 2). In contrast, the proportions of Treg ($P < 0.01$) cells in the responsive group were significantly lower than those in the non-responsive group (Figure 2).

3.4 Logistic Regression Analysis of the Factors Influencing the Efficacy of Radiotherapy

To investigate the relationship between the short-term efficacy of radiotherapy and the peripheral blood helper T cell subsets prior to radiotherapy, logistic regression analysis was performed on the proportions of these subsets. The treatment efficacy was set as the dependent variable (with 0 representing responsive and 1 representing non-responsive), while the proportions of peripheral blood helper T cell subsets served as the independent variables. Through this, a Logistic regression equation was developed. The univariate logistic regression results indicated that Tfh (OR = 3.725, 95% CI = 1.597-8.689, $P < 0.05$) acted as an independent protective factor for a favorable treatment response. Conversely, the proportions of Treg (OR = 0.016, 95%CI = 0.290-0.729, $P < 0.05$) cells were identified as risk factors for a positive treatment outcome (Table 2).

Table 2. Univariate logistic regression analysis of factors affecting treatment efficacy

Project	β	SE	Wald	P	OR	95% CI
Th1	0.016	0.016	0.178	0.701	1.016	(0.985, 1.048)
Th2	-0.048	0.182	0.352	0.672	0.953	(0.667, 1.361)
Th17	0.135	0.125	0.975	0.216	1.145	(0.896, 1.462)
Treg	-0.777	0.235	8.588	0.016	0.460	(0.290, 0.729)
Tfh	1.315	0.432	23.238	<0.0001	3.725	(1.597, 8.689)
Tfr	-0.15	0.20	0.563	0.453	0.861	(0.581, 1.275)

3.5 Predictive Value of the Proportions of Peripheral Blood Helper T Cell Subsets Before Radiotherapy for Treatment Efficacy

Taking the response after 4 weeks of treatment as the standard, the ROC curve was applied to evaluate the predictive value of Treg and Tfh for the effectiveness of treatment. As shown in Figure 3, the proportions of Tfh (AUC:0.930) and Treg (AUC:0.830) cells had predictive value for the response to treatment.

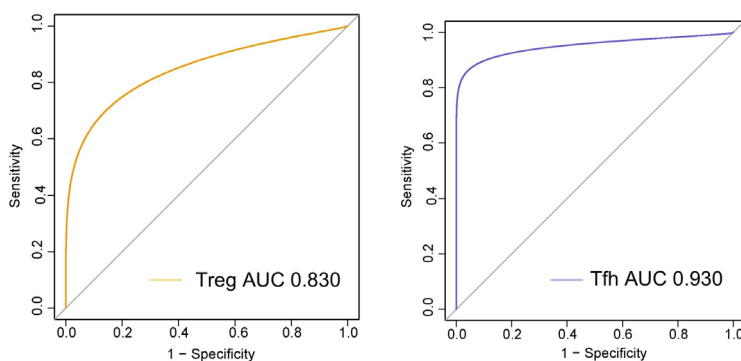


Figure 3. Predictive Value of the Proportions of Treg and Tfh before Radiotherapy for the Efficacy of Radiotherapy.

3.6 Toxicity Evaluation

The incidence of side effects in the overall patients was 27.5% (22/80). Among them, there were 3 cases of acute pneumonia, 7 cases of bone marrow suppression, 2 cases of cardiac toxicity, 4 cases of hypothyroidism, 5 cases of increased amylase, and 1 case of increased muscle enzymes. No esophagitis or pneumonia of grade ≥ 3 was found, and there was no radiotherapy-related death.

4. Discussion

Currently, anti-tumor immunity is an important factor affecting the efficacy of radiotherapy and the prognosis of tumors [8]. It is closely related to the type of primary tumor, the radiotherapy site, radiotherapy parameters, dose planning, and the time points of blood collection (before and after radiotherapy). Relevant studies have shown that radiotherapy affects the number of peripheral blood lymphocytes. For example, the number of CD8⁺ T cells decreases in nasopharyngeal carcinoma after radiotherapy, the number of CD8⁺ T cells increases in esophageal cancer and colorectal cancer after radiotherapy, and there is no significant change in the number of CD8⁺ T cells in prostate cancer and breast cancer after radiotherapy [9-13]. This indicates that patients experience obvious immunological changes in a short period after radiotherapy, which can lead to apoptosis and a decrease in T lymphocytes, affecting the balance of peripheral blood immune cells. The degree of the immune response induced by radiotherapy varies depending on the type of tumor [14]. Studies have also reported that the number of peripheral blood lymphocytes is closely related to the prognosis of tumor patients [15]. An increase in the number of CD4⁺CD25⁺Foxp3⁺ Treg is associated with a poor prognosis in patients with advanced breast cancer, and patients with a decreased Treg ratio after chemotherapy have a better prognosis [16]. This study aimed to evaluate the value of peripheral blood helper T cell subsets in patients with esophageal cancer for the short-term efficacy of radiotherapy.

Helper T cells can secrete various cytokines, activate macrophages and natural killer cells, and enhance their ability to attack tumor cells. Among them, Treg cells exert immunosuppressive effects by secreting IL-10 and transforming growth factor- β [17]. Treg cells are T cells that can recognize self-antigenic peptides presented by MHC molecules of target cells through the T cell antigen receptor and have certain immunosuppressive functions. They maintain self-tolerance by down-regulating the immune response level of the body against foreign or self-antigens, which is mainly manifested in two aspects: immunological anergy and immunosuppression. Tfh cells are a key subset that helps B cells differentiate into plasma cells. An overly strong Tfh helper signal will lead to excessive differentiation of B cells, interfere with the recognition of tumor cells, and assist in their immune escape [18, 19]. The proportions of Tfh cells before radiotherapy are related to the short-term efficacy of radiotherapy. After radiotherapy, the proportion of Tfh cells increases, while the proportion of Treg cells decreases, which is beneficial to patients. The significant increase in the proportion of Tfh cells after radiotherapy may reflect the remodeling of the immune microenvironment induced by radiotherapy, enabling Tfh cells to play a greater role in anti-tumor immunity. At the same time, the decrease in the proportion of Treg cells may reduce immunosuppression, thereby enhancing the ability of the immune system to attack tumors. However, there are still certain limitations. This study is a single-center retrospective study with a relatively small sample size, which may increase statistical bias. Further studies with large samples and multiple centers are needed for verification.

5. Conclusion

The proportions of Tfh and Treg cells among the peripheral blood helper T cell subsets have a certain predictive value for the short-term efficacy of radiotherapy, and it is of positive significance for guiding the individualized treatment of patients with esophageal cancer.

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Ethics Statement

This study was approved by the Ethics Committee of Bengbu Medical University (Approval Code: LKPZ [2024]051). This study followed the Declaration of Helsinki (2013 revision).

Authors' Contributions

Study administration and design: Shilong Song, Hongbo Xu. Methodology: Qimeng Tao. Acquisition and interpretation of data: Zhiwei Tian, Jingjing Sun. Writing original manuscript: Shilong Song. Study supervision: Hongbo Xu. All authors read and approved the final manuscript.

Competing Interest

The authors declare that they have no competing interests.

References

- [1] Zhu H, Ma X, Ye T, et al. Esophageal cancer in China: practice and research in the new era. *Int J Cancer*. 2023;152(9):1741-51.
- [2] Togashi Y, Shitara K, Nishikawa H. Regulatory T cells in cancer immunosuppression - implications for anticancer therapy. *Nat Rev Clin Oncol*. 2019;16(6):356-71.
- [3] Zheng Z, Wieder T, Mauerer B, et al. T cells in colorectal cancer: unravelling the function of different T cell subsets in the tumor microenvironment. *Int J Mol Sci*. 2023;24(14):11673.
- [4] Thommen DS, Schumacher TN. T cell dysfunction in cancer. *Cancer Cell*. 2018;33(4):547-62.
- [5] Jarosz-Biej M, Smolarczyk R, Cichoń T, et al. Tumor microenvironment as a “game changer” in cancer radiotherapy. *Int J Mol Sci*. 2019;20(13):3212.
- [6] Joerger M, Finn SP, Cuffe S, et al. The IL-17-Th1/Th17 pathway: an attractive target for lung cancer therapy? *Expert Opin Ther Targets*. 2016;20(11):1339-56.
- [7] Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-47.
- [8] Ozga AJ, Chow MT, Luster AD. Chemokines and the immune response to cancer. *Immunity*. 2021;54(5):859-74.
- [9] Sage EK, Schmid TE, Sedelmayr M, et al. Comparative analysis of the effects of radiotherapy versus radiotherapy after adjuvant chemotherapy on the composition of lymphocyte subpopulations in breast cancer patients. *Radiother Oncol*. 2016;118(1):176-80.
- [10] Sage EK, Schmid TE, Geinitz H, et al. Effects of definitive and salvage radiotherapy on the distribution of lymphocyte subpopulations in prostate cancer patients. *Strahlenther Onkol*. 2017;193(8):648-55.
- [11] Tao C, Chen Y, Jiang F, et al. A prognostic model combining CD4/CD8 ratio and N stage predicts the risk of distant metastasis for patients with nasopharyngeal carcinoma treated by intensity modulated radiotherapy. *Oncotarget*. 2016;7(29):46653-61.
- [12] Shiraishi Y, Fang P, Xu C, et al. Severe lymphopenia during neoadjuvant chemoradiation for esophageal cancer: a propensity matched analysis of the relative risk of proton versus photon-based radiation therapy. *Radiother Oncol*. 2018;128(1):154-60.
- [13] Lissoni P, Meregalli S, Bonetto E, et al. Radiotherapy-induced lymphocytopenia: changes in total lymphocyte count and in lymphocyte subpopulations under pelvic irradiation in gynecologic neoplasms. *J Biol Regul Homeost Agents*. 2005;19(3-4):153-8.
- [14] Hao J, Li M, Zhang T, et al. Prognostic value of tumor-infiltrating lymphocytes differs depending on lymphocyte subsets in esophageal squamous cell carcinoma: an updated meta-analysis. *Front Oncol*. 2020;10:614.
- [15] Fang P, Jiang W, Davuluri R, et al. High lymphocyte count during neoadjuvant chemoradiotherapy is associated with improved pathologic complete response in esophageal cancer. *Radiother Oncol*. 2018;128(3):584-90.
- [16] Seif F, Torki Z, Zalpoor H, et al. Breast cancer tumor microenvironment affects Treg/IL-17-producing Treg/Th17 cell axis: molecular and therapeutic perspectives. *Mol Ther Oncolytics*. 2023;28:132-57.
- [17] Knochelmann HM, Dwyer CJ, Bailey SR, et al. When worlds collide: Th17 and Treg cells in cancer and autoimmunity. *Cell Mol Immunol*. 2018;15(5):458-69.
- [18] Crotty S. T follicular helper cell biology: a decade of discovery and diseases. *Immunity*. 2019;50(5):1132-48.
- [19] Gutiérrez-Melo N, Baumjohann D. T follicular helper cells in cancer. *Trends Cancer*. 2023;9(4):309-25.