Impact of radiotherapy on immunological parameters, levels of inflammatory factors, and clinical prognosis in patients with esophageal cancer

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ABSTRACT

The aim of this study was to observe dynamic changes in immunological parameters and levels of inflammatory factors from pre-radiotherapy to post-radiotherapy in patients with esophageal cancer, and to evaluate the related clinical prognosis. In all, 110 patients with esophageal cancer who underwent radiotherapy were enrolled. Before radiotherapy, post-radiotherapy, and 3 months after radiotherapy, the percentages of T lymphocyte subsets and natural killer (NK) cells in peripheral blood were detected using flow cytometry. The levels of serum inflammatory factors were measured with the enzyme-linked immunosorbent assay (ELISA). Thirty peripheral blood samples from healthy people were similarly analysed as the control. Before radiotherapy, the percentages of CD4+ and CD8+ T cells and NK cells, and the CD4+/CD8+ rate in esophageal cancer patients were significantly different from those in the healthy control group (P < 0.001); the levels of inflammatory factors were increased significantly (P < 0.001). The percentages of the above cells and the levels of inflammatory factors also differed statistically significantly between pre- and post-radiotherapy (P < 0.001) in the esophageal cancer patients. Three months after radiotherapy, the percentages of CD3+ (P = 0.453), CD4+ (P = 0.108), and CD8+ T cells (P = 0.163) and NK cells (P = 0.103) had recovered to the level before radiotherapy; and the levels of TNF-α (P = 0.101), IL-6 (P = 0.302) and IL-8 (P = 0.250) were also restored. After radiotherapy, alterations in immunological parameters were associated with the irradiation volume and the myelosuppression condition. Patients with recovered immunological parameters showed a longer median survival time than those with poor recovery of immunological parameters. For esophageal cancer patients who were immunosuppressed and had an activated inflammatory response before radiotherapy, radiotherapy aggravated these symptoms, and this aggravation was positively associated with myelosuppression and irradiation volume. In addition, recovery of the immunological parameters indicated better prognosis.

Keywords: esophageal cancer; radiotherapy; T-lymphocyte subsets; natural killer cells; inflammatory factor

INTRODUCTION

Esophageal cancer (EC) represents the ninth most common malignancy and ranks as the sixth most frequent cause of cancer death in the world [1]. The incidence of esophageal cancer is very high in China, where most patients are diagnosed at middle or advanced stage and the surgical operation is not applicable [2]. The non-surgical approach for esophageal cancer includes radiotherapy and combination treatment. Despite the substantial advances made in radiotherapy and surgical diagnostic techniques for esophageal cancer over the past few decades, patients with late-stage esophageal cancer still have a poor prognosis and frequently a lethal outcome [3].

Tumor progression is usually accompanied by poor immune function, and the host immune system has an important role in affecting the therapeutic efficacy of radiotherapy [4]. The long-term weakened T cell immune function following radiotherapy can alter the host immune responses, and may be a key factor in the prognosis and outcome of esophageal cancer [5]. The lymphocyte subsets (including CD4+, CD8+ and CD3+ T cells) and the natural killer (NK) cells are the immune cells most essential for maintaining the immune function of patients [6]. Therefore, analysis of the immune parameters may have clinical significance in evaluating the prognosis of esophageal cancer patients who have undergone radiotherapy.
The assessment of immune function in tumor patients is mainly based on parameters concerning the T lymphocyte subsets and NK cells, including the percentages of CD3\(^+\), CD4\(^+\), and CD8\(^+\) T cells and NK cells and the ratio of CD4\(^+\)/CD8\(^+\) [7].

Moreover, inflammation has been proposed as a major physiological hallmark of malignancy [8]. Increasing evidence has demonstrated that the cancer-associated inflammatory response may be closely associated with poor outcome in patients with advanced cancers [9], including esophageal cancer [10]. Therefore, exploration of the possible mechanisms of cancer-induced inflammation may be beneficial for improving diagnosis, treatment and prognosis for esophageal cancer. Mounting evidence has indicated that the critical mediators of inflammation involved in esophageal cancer include the inflammatory factors TNF-\(\alpha\), IL-6 and IL-8 [11, 12].

Till now, most studies have focused on the influence of radiotherapy on the immune response in patients with esophageal cancer [13, 14], but the levels of the inflammatory factors and the clinical prognosis have rarely been looked at in conjunction with this. This study aimed to observe the dynamic changes in the immunological parameters and in the levels of inflammatory factors in patients with esophageal cancer, from pre-radiotherapy to post-radiotherapy, and to analyze the associated clinical prognostic factors.

**MATERIALS AND METHODS**

**General information**

A total of 110 esophageal cancer patients who were unsuitable or unwilling to undergo surgical operation and who were admitted to the Department of Radiotherapy at the First People’s Hospital of Nantong between January 2013 and December 2016 were enrolled. Of the 110 patients, 62 were male and 48 were female, and they were aged 35–77 years, with a median age of 60 years; 106 of the patients were diagnosed with squamous cell carcinoma, and 4 with adenocarcinoma. There were 28 cases of upper esophageal cancer, 47 of middle esophageal cancer, and 35 of lower esophageal cancer, and there were 64 cases with a lesion longer than 5 cm. According to the 7th Union for International Cancer Control (UICC) esophageal cancer staging system [15], there were 33 cases in Stage II, 41 cases in Stage III, and 36 cases in Stage IV. All the enrolled patients were treated with radiotherapy alone.

The research protocol has been reviewed and approved by the Ethics Committee of the First People’s Hospital of Nantong (Institutional Review Board No. 201001-012). All patients signed an informed consent for radiotherapy, and healthy volunteers in the control group signed blood specimen test consent. Peripheral blood immune parameters and inflammatory markers were measured in all patients before radiotherapy, at the end of radiotherapy and 3 months after radiotherapy. All of the 110 esophageal cancer patients had complete follow-up data, with a follow-up range of 4–40 months and a median follow-up of 20 months. Thirty healthy volunteers were randomly selected from our hospital staff, including 20 males and 10 females, aged 26–70 years, with a median age of 46 years, and 30 healthy blood samples from these were taken as the control.

**Radiotherapy procedure**

Radiotherapy was performed using a Varian linear accelerator. In radiotherapy planning, 90 patients received intensity-modulated radiotherapy and 20 patients received 3D conformal radiotherapy. The radiotherapy dose was 50.4–70.0 Gy/5–7 weeks, performed with a conventional method: once per day, 1.8–2.0 Gy each time, 5 times per week; the median radiotherapy dose was 60.0 Gy.

The target volume of the radiotherapy was the planning target volume (PTV) generated in the radiotherapy planning. In brief, a CT scan was performed for radiotherapy planning, and then the irradiation target volume and the organs at risk (such as the lungs, spinal cord, and heart) were delineated on CT images. For the gross tumor volume (GTV), the primary lesion of the esophageal cancer and the metastatic lymph nodes (only metastatic lymph nodes with the shortest diameter >1 cm) were included. Subclinical lesions (determined by enlarging the lesion area for 3 cm along the longitudinal axis of esophagus) and the involved lymphatic drainage regions were included in the clinical target volume (CTV). The PTV was determined by enlarging the CTV for 0.3 cm in each direction, and this was automatically calculated using our hospital Eclipse TPS treatment planning system. The results indicated that the radiotherapy target volume ranged from 80 cm\(^3\) to 480 cm\(^3\), and had an average target volume of 298 cm\(^3\) and a median target volume of 300 cm\(^3\).

**Detection methods and parameters**

The proportion of T lymphocyte subsets and NK cells in peripheral blood were measured by flow cytometry in esophageal cancer patients at different stages of radiotherapy. At the end of radiotherapy, the short-term clinical effect in patients was assessed in terms of their level of complying with the Response Evaluation Criteria in Solid Tumors (RECIST): complete remission (CR), partial response (PR), stable disease (SD) and progressive disease (PD). CR or PR is judged as effective radiotherapy, while SD or PD is defined as Ineffective radiotherapy. The patients were followed up for 4–40 months by outpatient clinical appointment, hospitalization, or home visit. Three months after radiotherapy, if the percentages of CD3\(^+\), CD4\(^+\), and CD8\(^+\) T cells and NK cells were measured as within the normal range, a good recovery of immune function was deemed to have occurred; otherwise, the recovery was considered to have been poor.

A sample of 2 ml of peripheral venous blood was obtained from each patient, and the serum was separated by centrifugation. The levels of inflammatory factors TNF-\(\alpha\), IL-6 and IL-8 in the serum were determined by using the enzyme-linked immunosorbent assay (ELISA). The protocol for analyzing each of the inflammatory factors considered relevant was followed according to the ELISA instructions.

A complete blood count was undertaken weekly during radiotherapy, in order to evaluate the patient’s myelosuppression, mainly manifested as leukopenia. Myelosuppression was categorized into Grades 0, 1, 2, 3 and 4 by assessing the levels of hemoglobin, leukocytes, granulocytes and platelets, according to the World Health Organization (WHO) toxicity grading scale [16].

**Statistical analysis**

SPSS 17.0 statistical software was applied for data processing, and measurement data were expressed as mean ± standard deviation (SD). An independent sample t-test was used to compare data between two groups, and one-way analysis of variance (ANOVA) was used to compare data between multiple groups. The Kaplan-
Table 1. The correlation between the T-lymphocyte subsets and NK cells proportions and clinical pathological characteristics before radiotherapy (x ± SD)

| Clinical pathological characteristics | Cases | CD3⁺% | Statistical value | P value | CD4⁺% | Statistical value | P value | CD8⁺% | Statistical value | P value | CD4⁺/CD8⁺ | Statistical value | P value | NK cells (%) | Statistical value | P value |
|--------------------------------------|-------|-------|------------------|---------|-------|------------------|---------|-------|------------------|---------|-------------|------------------|---------|---------------|------------------|---------|
| Age (years)                          |       |       |                  |         |       |                  |         |       |                  |         |             |                  |         |               |                  |         |
| <60                                  | 52    | 57.17 ± 9.28 | t = 1.156 | 0.250 | 27.60 ± 7.69 | t = 0.847 | 0.399 | 31.20 ± 7.19 | t = 0.835 | 0.406 | 0.90 ± 0.36 | t = 1.506 | 0.135 | 11.25 ± 4.68 | t = 0.738 | 0.462 |
| ≥60                                  | 58    | 59.11 ± 8.30 |       |       | 28.85 ± 7.74 |       |       | 32.34 ± 7.16 |       |       | 0.99 ± 0.32 |       |       | 11.87 ± 4.19 |       |     |
| Sex                                  |       |       |                  |         |       |                  |         |       |                  |         |             |                  |         |               |                  |         |
| male                                 | 62    | 58.55 ± 8.96 | t = 0.492 | 0.624 | 28.47 ± 7.60 | t = 0.315 | 0.754 | 32.10 ± 7.04 | t = 0.491 | 0.625 | 0.96 ± 0.34 | t = 0.441 | 0.660 | 11.86 ± 4.41 | t = 0.773 | 0.441 |
| female                               | 48    | 57.72 ± 8.64 |       |       | 27.99 ± 7.91 |       |       | 31.42 ± 7.37 |       |       | 0.93 ± 0.35 |       |       | 11.21 ± 4.45 |       |     |
| Lesion part                          |       |       |                  |         |       |                  |         |       |                  |         |             |                  |         |               |                  |         |
| upper                                | 28    | 55.28 ± 10.41 | F = 2.120 | 0.125 | 26.20 ± 8.80 | F = 1.358 | 0.262 | 29.91 ± 8.25 | F = 1.311 | 0.274 | 0.91 ± 0.39 | F = 0.217 | 0.806 | 10.84 ± 4.88 | F = 0.714 | 0.492 |
| middle                               | 47    | 59.05 ± 8.03 |       |       | 29.06 ± 7.44 |       |       | 32.43 ± 6.77 |       |       | 0.95 ± 0.33 |       |       | 12.10 ± 4.35 |       |     |
| lower                                | 35    | 59.37 ± 8.06 |       |       | 28.83 ± 7.01 |       |       | 32.47 ± 6.67 |       |       | 0.97 ± 0.34 |       |       | 11.47 ± 4.16 |       |     |
| Lesion length (cm)                   |       |       |                  |         |       |                  |         |       |                  |         |             |                  |         |               |                  |         |
| <5                                   | 46    | 57.89 ± 9.55 | t = 0.302 | 0.763 | 27.95 ± 7.92 | t = 0.353 | 0.724 | 31.71 ± 7.49 | t = 0.106 | 0.916 | 0.96 ± 0.36 | t = 0.210 | 0.834 | 11.94 ± 4.55 | t = 0.735 | 0.464 |
| ≥5                                   | 64    | 58.40 ± 8.27 |       |       | 28.48 ± 7.60 |       |       | 31.86 ± 6.98 |       |       | 0.94 ± 0.33 |       |       | 11.31 ± 4.34 |       |     |
| Pathological type                    |       |       |                  |         |       |                  |         |       |                  |         |             |                  |         |               |                  |         |
| squamous cell carcinoma              | 106   | 58.04 ± 8.76 | t = 0.906 | 0.367 | 28.17 ± 7.69 | t = 0.617 | 0.539 | 31.79 ± 7.19 | t = 0.085 | 0.933 | 0.95 ± 0.35 | t = 0.134 | 0.893 | 11.52 ± 4.39 | t = 0.700 | 0.485 |
| adenocarcinoma                       | 4     | 62.10 ± 10.12 |       |       | 30.60 ± 8.98 |       |       | 32.10 ± 7.41 |       |       | 0.93 ± 0.27 |       |       | 13.10 ± 5.56 |       |     |
| Clinical Stage                       |       |       |                  |         |       |                  |         |       |                  |         |             |                  |         |               |                  |         |
| II                                   | 33    | 58.02 ± 9.40 | F = 0.143 | 0.867 | 28.40 ± 8.26 | F = 0.274 | 0.761 | 31.61 ± 7.62 | F = 0.187 | 0.830 | 0.95 ± 0.35 | F = 0.009 | 0.991 | 11.32 ± 4.66 | F = 0.876 | 0.419 |
| III                                  | 41    | 58.75 ± 8.59 |       |       | 28.80 ± 8.05 |       |       | 32.33 ± 7.36 |       |       | 0.94 ± 0.35 |       |       | 12.28 ± 4.53 |       |     |
| IV                                   | 36    | 57.70 ± 8.66 |       |       | 27.51 ± 6.88 |       |       | 31.37 ± 6.66 |       |       | 0.95 ± 0.34 |       |       | 11.00 ± 4.07 |       |     |
Meier method was used for survival analysis, and a Log-rank test was used for comparison between groups. The significance level was assumed to be \( \alpha = 0.05 \).

**RESULTS**

The correlation between the proportions of the T-lymphocyte subsets and the NK cells with the clinical pathological characteristics before radiotherapy

As shown in Table 1, the pre-radiotherapy immunological parameters in patients with esophageal cancer \((n = 110)\) were independent of clinical pathological features such as age, gender, lesion location, lesion length, pathological type, clinical stage, etc.

Effects of radiotherapy on T lymphocyte subsets and NK cells in patients with esophageal cancer

As shown in Fig. 1 and Table 2, the percentages of CD4\(^+\) T cells and NK cells and the CD4\(^+\)/CD8\(^+\) ratio in the peripheral blood of patients with esophageal cancer before radiotherapy were significantly decreased when compared with the control group \((n = 30)\), and the percentage of CD8\(^+\) was significantly increased. However, the percentage of CD3\(^+\) T cells showed no significant differences between the esophageal cancer patients and the control group. At various stages after radiotherapy, the percentages of CD3\(^+\), CD4\(^+\), and CD8\(^+\) T cells and NK cells and the CD4\(^+\)/CD8\(^+\) ratio showed a dynamic variation. The percentages of CD3\(^+\), CD4\(^+\) T cells and NK cells and the CD4\(^+\)/CD8\(^+\) ratio in peripheral blood of esophageal cancer patients after radiotherapy were significantly increased, and the percentage of CD8\(^+\) T cells was significantly reduced, when compared with the peripheral blood specimens before radiotherapy. Three months after radiotherapy, the immune parameters like the percentages of the CD3\(^+\), CD4\(^+\) and CD8\(^+\) T cells and NK cells and the CD4\(^+\)/CD8\(^+\) ratio in peripheral blood of esophageal cancer patients after radiotherapy were significantly increased, and the percentage of CD8\(^+\) T cells was significantly reduced, when compared with the peripheral blood specimens before radiotherapy.

Effects of radiotherapy on inflammatory factors TNF-\(\alpha\), IL-6 and IL-8 in patients with esophageal cancer

As shown in Fig. 2 and Table 3, the levels of TNF-\(\alpha\), IL-6 and IL-8 in esophageal cancer patients before radiotherapy were significantly
higher than those in the control group, and the difference was statistically significant. The levels of TNF-α, IL-6 and IL-8 in the peripheral blood of the patients changed dynamically in the period after radiotherapy: the expression first increased and then decreased. At the end of radiotherapy, the levels of TNF-α, IL-6 and IL-8 had increased significantly compared with those before radiotherapy. Three months after radiotherapy, the levels of the inflammatory factors had decreased to slightly lower than the levels before radiotherapy, but no significant difference was found between the pre-and 3-months post-radiotherapy levels.

**Changes after radiotherapy in immune parameters and related clinical factors in patients with esophageal cancer**

**Effects of radiotherapy target volume and radiotherapy dose on immune parameters in patients with esophageal cancer**

Statistical analysis of the patients’ PTVs revealed that the minimum volume was 120 cm³, the maximum volume was 598 cm³, the median volume was 300 cm³, and the average volume was 308 cm³. The target volume was categorized into ≥300 cm³ (n = 61) or <300 cm³ (n = 49) and had a median target volume of 300 cm³ as the boundary between the two categories. Our results showed that the immune parameters (including percentages of CD3⁺ and CD4⁺ T cells and NK cells, and the CD4⁺/CD8⁺ ratio) were dramatically decreased at the end of radiotherapy in the >300 cm³ group, whereas the percentage of CD8⁺ T cells statistically increased, as shown in Fig. 3 and Table 4. The irradiated patients were divided into groups who had received >60 Gy (n = 89) and those who had received <60 Gy (n = 21), and the median dose of 60 Gy formed the boundary between the groups. Our results indicated that the radiotherapy dose did not have a significant effect on the immune parameters after radiotherapy, as seen in Fig. 4 and Table 5.

**Effects of hemogram during the radiotherapy on post-radiotherapy immune parameters in patients with esophageal cancer**

Myelosuppression in patients during radiotherapy was analyzed, and our results showed that a normal hemogram was observed in 50 cases. Sixty cases were found with myelosuppression, including 35 cases with Grade 1 myelosuppression, 20 cases with Grade 2 myelosuppression, and 5 cases with Grade 3 myelosuppression. Compared with those without myelosuppression, the percentages of CD3⁺ and CD4⁺ T cells and NK cells and the CD4⁺/CD8⁺ ratio decreased significantly after radiotherapy in patients with myelosuppression, and

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**Table 3. Changes in inflammatory factors at different stages of radiotherapy for esophageal cancer patients (X ± SD)**

| Parameters | Pre-radiotherapy (n = 110) | Immediately post-radiotherapy (n = 110) | 3 months post-radiotherapy (n = 110) | Control (n = 30) | Immediately post-radiotherapy: Pre-radiotherapy | 3 months post-radiotherapy: Pre-radiotherapy |
|------------|---------------------------|----------------------------------------|------------------------------------|-----------------|-----------------------------------------------|-----------------------------------------------|
| TNF-α(ng/l) | 5.64 ± 0.71               | 7.26 ± 0.94                            | 5.50 ± 0.60                        | 4.42 ± 0.45     | 8.950 <0.001                                 | 14.255 <0.001                                 |
| IL-6(μg/l)  | 17.49 ± 2.84              | 30.10 ± 4.63                           | 17.14 ± 2.15                       | 4.52 ± 0.35     | 24.845 <0.001                               | 24.332 <0.001                                |
| IL-8(μg/l)  | 328.55 ± 37.96            | 595.35 ± 66.02                         | 322.23 ± 43.21                    | 186.90 ± 22.88  | 19.462 <0.001                               | 36.737 <0.001                                |

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**Fig. 2.** Changes in inflammatory factors through the various stages of radiotherapy for esophageal cancer patients. The columns with different colors represent different immune targets. Values are presented as mean ± SD.
The percentage of CD8$^+$ T cells increased significantly, as presented in Fig. 5 and Table 6.

**The relationship between change in immune parameters and short-term clinical effect**

The short-term clinical effect of radiotherapy in patients was assessed in terms of their level of complying with the RECIST criteria. In the current study, 71 cases (CR in 11 cases and PR in 60 cases) were judged as having received effective radiotherapy, and 39 cases (SD in 30 cases and PD in 9 cases) were considered as having received ineffective radiotherapy. Compared with the effective group, the percentages of CD3$^+$ and CD4$^+$ T cells and NK cells, and the CD4$^+$/CD8$^+$ ratio were markedly decreased in the ineffective radiotherapy group, with significant differences, whereas no significant difference was found between the CD8$^+$ T cells proportions in the two groups, as shown in Fig. 6 and Table 7.

The correlation of recovery of immune parameters with prognosis and clinical characteristics 3 months after radiotherapy

**Correlation between recovery of immune parameters and prognosis**

The patients were followed up for 4–40 months, and the median survival time was 19 months. Three months after radiotherapy, the percentage of CD3$^+$, CD4$^+$ and CD8$^+$ T cells and NK cells was determined to assess the level of recovery of immune function. It was found that good recovery of immune function was present in 63 patients 3 months after radiotherapy, and the median survival time of those patients was 21 months; the immune function of 47 cases recovered poorly, and the median survival time in that group of patients was 17 months. The difference in the median survival time was statistically significant, as shown in Fig. 7 ($\chi^2 = 19.821$, $P < 0.001$).

**Correlation between immune parameters recovery and clinical characteristics**

Three months after radiotherapy, 63 patients showed good recovery of immune function, whereas 47 patients showed poor recovery of immune function. To analyze the factors that related to recovery of the immune parameters 3 months after radiotherapy, patients were grouped according to their ages (<60 or $\geq$60 years), clinical stages (Stage II, III and IV) and PTV (<300 cm$^3$ or $\geq$300 cm$^3$). As shown in Table 8, significant correlation was found between the immune parameters recovery and clinical stage ($P < 0.005$); the recovery of immune parameters differed significantly in patients with different PTVs ($P < 0.005$). There was no significant difference was noted between the level of immune parameters recovery in patients in the different age groups ($P > 0.005$).

**DISCUSSION**
The curative efficacy of the radiotherapy and the prognosis of patients with esophageal cancer are affected by various complex...
T lymphocytes have cytotoxic activity against cancer, playing a critical role in antitumor immunity [23]. The CD4+/CD8+ ratio reflects the immune balance state, acting as a significant indicator of immune function in esophageal cancer patients [24]. NK cells are cytotoxic effectors of the innate immune system that are able to recognize and eradicate tumor cells without prior antigenic exposure, and they may be able to be used as an independent prognostic predictor and indicator of immune function [25]. In this study, the percentages of CD4+ T cells and NK cells, and the CD4+/CD8+ ratio in the peripheral blood of esophageal cancer patients before radiotherapy were significantly decreased compared with the control group, and the percentage of CD8+ T cells was significantly increased. An immunosuppressive condition was noted. This may be attributed to the production of inhibitory cytokines (induced by tumor cells), which inactivated the immunologic effector cells and suppressed the body immune function.

Our study also showed dynamic changes in the percentages of CD3+, CD4+ and CD8+ T cells and NK cells and in the ratio of CD4+ and CD8+ in peripheral blood of esophageal cancer patients at different stages after radiotherapy. When compared with healthy controls, the percentages of CD3+ and CD4+T cells and NK cells, and the ratio of CD4+/CD8+ in the peripheral blood of patients with esophageal cancer decreased significantly after radiotherapy, and the percentage of CD8+ T cells increased significantly, which means the patient’s immune function was further reduced. This can be explained if the radiation not only killed tumor cells but also damaged T lymphocytes [26]. Moreover, NK cells are highly sensitive to radiotherapy, and decreased NK activity and numbers were observed after radiotherapy [27, 28]. Furthermore, it has been shown that radiotherapy seems to weaken cell membrane function and cause a large loss of surface antigens, which in turn leads to aggravated immune suppression [29, 30].

The presence of numerous inflammatory factors in the tumor microenvironment causes vascular dilatation, amplifies the inflammatory response, promotes the growth and metastasis of tumor
cells, and stimulates the production of blood vessels and lymphatic vessels [31, 32]. The representative indicators of inflammatory response include TNF-α, IL-6 and IL-8 [33], and they have been widely evaluated in esophageal cancer [12, 34]. In this study, levels of inflammatory factors such as TNF-α, IL-6 and IL-8 in esophageal cancer patients before radiotherapy were significantly higher than those in the control group, suggesting that the patients were in an inflammatory response activation state. Inflammatory factors fluctuated at different stages after radiotherapy, first rising and then falling, which suggests that the inflammatory response was further enhanced in the short term after radiotherapy, but that it was gradually restored to the level of pre-radiotherapy within 3 months after radiotherapy. We speculate this may be the result of rebalancing of the immunity and inflammation responses.

After radiotherapy, the decrease in immune function was related to the volume of the irradiation and to the level of myelosuppression. This may be because a larger radiotherapy target volume will correspondingly increase the damage effect of radiation on the body’s immune cells, thus leading to aggravated immunosuppression. Furthermore, some patients with a large PTV, whose myelosuppression was unresolved at 3 months after radiotherapy, possibly had residual tumors or tumor recurrences. Therefore, recovery from myelosuppression may be interrupted by residual tumors and cancer-related side effects, such as anorexia, undernutrition or pain, and this deserves further exploration. In addition, esophageal cancer patients with myelosuppression in radiotherapy have a more pronounced decrease in immune parameters, which may be related to the inhibition of immune function caused by leucopenia [35]. In addition, our study indicated that post-radiotherapy immune parameters were associated with different curative effects in patients: the immune

Table 6. Effect of myelosuppression on T lymphocyte subsets and NK cells in patients with esophageal cancer after radiotherapy (x ± SD)

| Parameters | No myelosuppression (n = 50) | Myelosuppression (n = 60) | t   | P   |
|------------|-------------------------------|--------------------------|-----|-----|
| CD3⁺%      | 54.11 ± 8.33                  | 50.47 ± 9.51             | 2.111 | 0.037|
| CD4⁺%      | 20.58 ± 6.24                  | 17.38 ± 7.59             | 2.382 | 0.019|
| CD8⁺%      | 37.99 ± 8.97                  | 41.65 ± 9.40             | 2.077 | 0.040|
| CD4⁺/CD8⁺  | 0.69 ± 0.26                   | 0.52 ± 0.34              | 2.812 | 0.006|
| NK cells%  | 8.63 ± 3.68                   | 6.72 ± 3.68              | 2.696 | 0.008|

Figure 5. Effects of hemogram on immune function post-radiotherapy in patients with esophageal cancer. The columns with different colors represent different immune targets. Values are presented as mean ± SD.

Fig. 6. The relationship between the changes in immune parameters and short-term clinical effect. Values are presented as mean ± SD.
parameters were notably improved in the patient group for whom the radiotherapy was deemed effective, suggesting that the body immune function recovered when the esophageal cancer was controlled. This may be due to the attenuation of immune suppressor released by tumor cells [36]. In addition, the clinical stage, PTV and age of patients showed varying degrees of influence on the recovery of immune parameters, which indicated the patients’ immune state and degree of myelosupression after radiotherapy. Most importantly, the survival analysis in our study illuminated the fact that recovery of the immune parameters indicated better prognosis for patients, suggesting that recovered immune function exerted a crucial role in repressing the remaining tumor and delaying its recurrence.

In summary, esophageal cancer patients are immunosuppressed and have activated inflammatory reactions pre-radiotherapy. The implementation of radiotherapy further aggravated these conditions.

### Table 7. Comparison of T lymphocyte subsets and NK cells in esophageal cancer patients with different curative effects (\( \bar{x} \pm SD \))

| Parameters       | Effective radiotherapy group \((n = 71)\) | Ineffective radiotherapy group \((n = 39)\) | \(t\) value | \(P\) value |
|------------------|------------------------------------------|-------------------------------------------|-------------|-------------|
| CD3⁺%            | 53.91 ± 7.96                             | 48.86 ± 10.29                            | 2.862       | 0.005       |
| CD4⁺%            | 20.23 ± 6.53                             | 16.30 ± 7.62                             | 2.837       | 0.005       |
| CD8⁺%            | 40.29 ± 9.40                             | 39.43 ± 9.36                             | 0.459       | 0.647       |
| CD4⁺/CD8⁺        | 0.65 ± 0.29                              | 0.50 ± 0.34                              | 2.307       | 0.023       |
| NK cells%        | 8.30 ± 3.49                              | 6.30 ± 4.00                              | 2.720       | 0.008       |

### Survival curve

![Survival curve](image)

**Fig. 7.** Comparison of survival curves of esophageal cancer patients with different immune recovery status at 3 months after radiotherapy.

### Table 8. Correlation between immune parameters recovery and clinical characteristics 3 months following radiotherapy

| Clinical characteristics | Cases \((n = 110)\) | Immune parameters recovery | \(\chi^2\) | \(P\) value |
|--------------------------|---------------------|----------------------------|-------------|-------------|
| Age (years)              |                     |                            |             |             |
| <60                      | 52 (100%)           | 35 (67.3%)                 | 17 (32.7%)  | 4.058       | 0.054       |
| ≥60                      | 58 (100%)           | 28 (48.3%)                 | 30 (51.7%)  |             |             |
| Clinical stage           |                     |                            |             |             |
| II                       | 33 (100%)           | 25 (75.8%)                 | 8 (24.2%)   | 11.425      | 0.003       |
| III                      | 41 (100%)           | 25 (61%)                   | 16 (39%)    |             |             |
| IV                       | 36 (100%)           | 13 (36.1%)                 | 23 (63.9%)  |             |             |
| PTV (cm³)                |                     |                            |             |             |
| ≥300                     | 61 (100%)           | 29 (47.5%)                 | 32 (52.5%)  | 5.300       | 0.032       |
| <300                     | 49 (100%)           | 34 (69.4%)                 | 15 (30.6%)  |             |             |
Moreover, the degree of aggravation was related to both the level of myelosuppression and the irradiation volume. Three months after radiotherapy, the immune parameters of the patients in the effective radiotherapy group had recovered greatly, and this was associated with good prognosis. Together, these findings indicate that interventions that enhance immune function during radiotherapy may have a positive effect on improving a patient’s prognosis. As a comprehensive treatment of tumors, radiotherapy has dual effects on the immune response of tumor patients. On the one hand, radiation is used to kill tumor cells, but it non-selectively kills immune cells at the same time, which leads to low immune function and the inhibited immune response of patients. On the other hand, radiotherapy indirectly enhances the immune response of patients through activating an inflammatory reaction. In the organism as a whole, the immune system is not only modulated by radiation, but also responds to the change in the tumor microenvironment. Therefore, a balance between the immune system and inflammation needs be achieved in patients, and this presents a challenge for clinical and scientific researchers.

CONFLICT OF INTEREST

The authors have no actual or potential conflicts of interest to declare.

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REFERENCES

1. Zhang M, Zhou S, Zhang L et al. Role of cancer-related inflammation in esophageal cancer. Crit Rev Eukaryot Gene Expr 2013; 23:27–35.
2. Vaghjiani RG, Molena D. Surgical management of esophageal cancer. Chin Clin Oncol 2017; 6:47.
3. Huang FL, Yu SJ. Esophageal cancer: Risk factors, genetic association, and treatment. Asian J Surgery 2018;41:210–15.
4. Yoshimoto Y, Kono K, Suzuki Y. Anti-tumor immune responses induced by radiotherapy: A review. Fukushima J Med Sci 2015; 61:13–22.
5. Hong M, Jiang Z, Zhou YF. Effects of thermotherapy on Th1/Th2 cells in esophageal cancer patients treated with radiotherapy. Asian Pac J Cancer Prev 2014;15:2359–62.
6. Talvar J, Garraitt G, Goncalves-Mendes N et al. Immunonutrition stimulates immune functions and antioxidant defense capacities of leukocytes in radiochemotherapy-treated head & neck and esophageal cancer patients: a double-blind randomized clinical trial. Clin Nutr 2015;34:810–7.
7. Zhao G, Cao S, Zhang K et al. [Effect of early enteral nutrition on immune response and clinical outcomes after esophageal cancer surgery]. Zhonghua Wei Chang Wai Ke Za Zhi 2014;17: 356–60.
8. Kamangar F, Abnet CC, Hutchinson AA et al. Polymorphisms in inflammation-related genes and risk of gastric cancer (Finland). Cancer Causes Control 2006;17:117–25.
9. McMillan DC, Elahi MM, Sattar N et al. Measurement of the systemic inflammatory response predicts cancer-specific and non-cancer survival in patients with cancer. Nutr Cancer 2001; 41:64–9.
10. Guillem P, Triboulet JP. Elevated serum levels of C-reactive protein are indicative of a poor prognosis in patients with esophageal cancer. Dis Esophagus 2005;18:146–50.
11. Hardikar S, Onstad L, Song X et al. Inflammation and oxidative stress markers and esophageal adenocarcinoma incidence in a Barrett’s esophagus cohort. Cancer Epidemiol Biomarkers Prev 2014;23:2393–403.
12. Xing YL, Wang YC. Influence of autologous and homologous blood transfusion on interleukins and tumor necrosis factor-alpha in peri-operative patients with esophageal cancer. Asian Pac J Cancer Prev 2014;15:7831–4.
13. Suzuki Y, Mimura K, Yoshimoto Y et al. Immunogenic tumor cell death induced by chemoradiotherapy in patients with esophageal squamous cell carcinoma. Cancer Res 2012;72: 3967–76.
14. Ma JL, Jin L, Li YD et al. The intensity of radiotherapy-elicited immune response is associated with esophageal cancer clearance. J Immunol Res 2014;2014:794249.
15. Huang Y, Guo W, Shi S et al. Evaluation of the 7th edition of the UICC-AJCC tumor, node, metastasis classification for esophageal cancer in a Chinese cohort. J Thorac Dis 2016;8:1672–80.
16. Miller AB, Hoogstraten B, Staquet M et al. Reporting results of cancer treatment. Cancer 1981;47:207–14.
17. Lin EW, Karakashova TA, Hicks PD et al. The tumor microenvironment in esophageal cancer. Oncogene 2016;35:5337–49.
18. Xie J, Wang J, Cheng S et al. Expression of immune checkpoints in T cells of esophageal cancer patients. Oncotarget 2016;7: 63669–78.
19. Nakajima M, Kato H, Miyazaki T et al. Prognostic significance of heat shock protein 110 expression and T lymphocyte infiltration in esophageal cancer. Hepatogastroenterology 2011;58:1555–60.
20. Xu B, Chen L, Li J et al. Prognostic value of tumor infiltrating NK cells and macrophages in stage II–III esophageal cancer patients. Oncotarget 2016;7:74904–16.
21. Tian C, Lu S, Fan Q et al. Prognostic significance of tumor-infiltrating CD8+ or CD3+ T lymphocytes and interleukin-2 expression in radically resected non–small cell lung cancer. Chin Med J (Engl) 2015;128:105–10.
22. Sun B, Zhang Y. Overview of orchestration of CD4+ T cell subsets in immune responses. Adv Exp Med Biol 2014;841: 1–13.
23. Hu S, Rotschafer JH, Lokensgard JR et al. Activated CD8+ T lymphocytes inhibit neural stem/progenitor cell proliferation: role of interferon-gamma. PLoS One 2014;9:e105219.
24. Long H, Yang H, Lin Y et al. Fish oil-supplemented parenteral nutrition in patients following esophageal cancer surgery: effect on inflammation and immune function. Nutr Cancer 2013;65:71–5.
25. Bigley AB, Simpson RJ, NK cells and exercise: implications for cancer immunotherapy and survivorship. Discov Med 2015;19: 433–45.
26. Job G, PfleunSchuh M, Bauer M et al. The influence of radiation therapy on T-lymphocyte subpopulations defined by monoclonal antibodies. Int J Radiat Oncol Biol Phys 1984;10: 2077–81.
27. McGinnes K, Florence J, Penny R. The effect of radiotherapy on the natural killer (NK)-cell activity of cancer patients. *J Clin Immunol* 1987;7:210–7.

28. 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;19:153–8.

29. Reginato E, Lindenmann J, Langner C et al. Photodynamic therapy downregulates the function of regulatory T cells in patients with esophageal squamous cell carcinoma. *Photochem Photobiol Sci* 2014;13:1281–9.

30. Narita M, Kanda T, Abe T et al. Immune responses in patients with esophageal cancer treated with SART1 peptide-pulsed dendritic cell vaccine. *Int J Oncol* 2015;46:1699–1709.

31. Zitvogel L, Pietrocola F, Kroemer G. Nutrition, inflammation and cancer. *Nat Immunol* 2017;18:843.

32. Maghsudlu M, Yazd EF. Heat-induced inflammation and its role in esophageal cancer. *J Dig Dis* 2017;18:431–44.

33. Liu CH, Abrams ND, Carrick DM et al. Biomarkers of chronic inflammation in disease development and prevention: challenges and opportunities. *Nat Immunol* 2017;18:1175–80.

34. Groblewska M, Mroczko B, Sosnowska D et al. Interleukin 6 and C-reactive protein in esophageal cancer. *Clin Chim Acta* 2012;413:1583–90.

35. Zhang WC, Sun R, Zhang J et al. Recombinant human prolactin protects against irradiation-induced myelosuppression. *Cell Mol Immunol* 2005;2:379–85.

36. Sermeus A, Leonard W, Engels B et al. Advances in radiotherapy and targeted therapies for rectal cancer. *World J Gastroenterol* 2014;20:1–5.