Prognostic value of absolute lymphocyte count in patients with advanced esophageal cancer treated with immunotherapy: a retrospective analysis

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Background: Immunotherapy has become the standard of treatment for recurrent metastatic esophageal cancer (EC), and the value of efficacy predictive markers represented by programmed death-ligand 1 (PD-L1) is limited. The purpose of this study is to analyze the prognostic value of peripheral blood absolute lymphocyte count (ALC) at baseline in patients with recurrent metastatic EC treated with immunotherapy, and to further investigate the relationship between the minimal ALC value (Min ALC) and radiotherapy (RT) parameters.

Methods: The main inclusion criteria were: histologically or imaging confirmed recurrent or metastatic EC; complete routine blood test data. A total of 105 patients were included in a single-center institution, 65 of whom had previously received RT. The optimal cut-off value for baseline lymphopenia was determined by the receiver operating characteristic (ROC) curve. The prognostic value of baseline phase lymphopenia for immunotherapy were determined by cox regression analysis and the associated factors affecting lymphopenia were explored by logistic regression analysis.

Results: The cut-off value for baseline ALC predicting 1-year overall survival (OS) was 625 cells/μL. The OS was significantly lower in the lymphopenia group (ALC ≤625 cells/μL) than in the non-lymphopenia group (ALC >625 cells/μL) (median OS: 6 *vs.* 12 months, P=0.002). Multivariate analysis showed that pre-immunotherapy lymphopenia was an important factor influencing patient prognosis [hazard ratio (HR): 1.771, 95% confidence interval (CI): 1.051–2.985; P=0.032)] (adjusted for clinical factors including sex, age, tumor location, histology, degree of differentiation, distant metastasis, use of RT). Patients with a previous grade 4 (G4) Min ALC during RT were more likely to develop pre-immunotherapy lymphopenia following diagnosis of recurrent metastasis [odds ratio (OR): 10.809, 95% CI: 2.185–53.471; P=0.004]. Planning target volume (PTV) volume greater than 521.2 cm^3 (OR: 19.981, 95% CI: 1.372–290.985; P=0.028) was an independent risk factor affecting the G4 Min ALC during RT.

Conclusions: Lymphopenia is associated with a poorer immunotherapy prognosis in patients with recurrent metastatic EC and those with previous G4 Min ALC after RT. RT-related parameters, especially irradiation volume, can significantly affect lymphocyte counts.

Keywords: Esophageal cancer (EC); lymphopenia; radiotherapy (RT); immunotherapy

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Introduction

Esophageal cancer (EC) is one of the common gastrointestinal malignancies. Even with standard treatment, recurrence and metastasis occur in 27% to 50% of patients (1). The overall prognosis of patients with recurrence and metastasis is poor, with a median overall survival (OS) of only 6.0 to 8.2 months (2). It has been reported that time to recurrence, location of recurrence, number of recurrent metastatic organs and treatment after recurrent metastasis are independent factors affecting prognosis (3). In recent years, a series of clinical studies of immunotherapy combined with chemotherapy have significantly improved OS in patients with advanced EC with a controlled safety profile (4-7). Immunotherapy has greatly benefited the survival of patients, greatly improved their quality of life, and provided a new treatment option.

The immune system plays a central role in the fight against tumors. Lymphocytes are the primary carriers of organism-mediated cellular immunity, which specifically recognizes tumor cells through cytotoxic responses, antagonizes tumor cell proliferation, and promotes tumor cell apoptosis. Studies have shown that $CD4^+$ T cells and $CD8^+$ T lymphocytes can significantly improve the prognosis of patients with EC by directly destroying tumor cells or by secreting cytokines that activate effector cells (8,9).

Researchers have long used PD-L1 as a biomarker for tumor immunotherapy. However, PD-L1 expression assays not only lack uniform standards, but also require complex and expensive laboratory techniques. Patients on post-line therapy cannot have their expression measured by secondary biopsy (4-7). Therefore, there is an urgent need for simple and easy-to-use assays in the clinic to predict the prognosis of patients on immunotherapy. In clinical work, peripheral blood specimens are easier to obtain, have high patient acceptance, and facilitate long-term evaluation and monitoring (10). Previous reports have shown that cancer-related inflammatory indicators, such as the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), are associated with prognosis in EC (11). Lymphocytes are central to these inflammatory indicators. The peripheral blood absolute lymphocyte count (ALC) is associated with the autoimmune status of cancer patients, and lymphopenia indicates that the body is immunosuppressed (12). Lymphopenia is associated with poor prognosis in tumors such as cervical, nasopharyngeal, and lung cancers (13-15). Lymphocytes are the more radiation-sensitive cells in the blood system. Radiation exposure to bone marrow (BM), lymphoid tissue, or blood circulation can result in a significant decrease in lymphocytes and reduce the body's immune response against tumors (12,16). Lymphopenia after radiotherapy (RT) can result in a poorer prognosis for patients with solid tumors (17,18).

In patients with thoracic tumors, the heart, lungs, large blood vessels, and lymph nodes are often exposed to the radiation field and are susceptible to lymphopenia after RT. A previous study have shown that the minimal ALC value (Min ALC) during RT is associated with the planning target volume (PTV) in EC, V10, and V20 of the heart (19). Larger PTVs and higher cardiopulmonary doses may expose a large number of circulating cells to radiation, thereby producing greater lymphocytic destruction. Therefore, RT-related parameters (irradiation volume and dose) may have an impact on the Min ALC. However, there are relatively few studies on the prognostic value of lymphopenia in patients with recurrent metastatic EC treated with immunotherapy and the effect of RT-related parameters on the Min ALC.

The purpose of this study was to investigate the prognostic value of pre-immunotherapy lymphopenia in patients with recurrent metastatic EC treated with immunotherapy and to assess the relationship between RT-related parameters and the Min ALC. We hypothesize that choosing the appropriate irradiation range to control the irradiation volume during RT can reduce the risk of Min ALC reduction, maintain the normal function of the patient's immune system, and help improve the patient's immunotherapy outcome. We present the following article in accordance with the STARD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2669/rc).

Methods

Patient selection and data collection

This single-center retrospective cohort study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethical Committee of the First Affiliated Hospital of Soochow University [(2021) No. 329]. Individual consent for this retrospective analysis was waived. The clinical data of patients with recurrent metastatic EC who received immunotherapy in our hospital from June 2018 to June 2020 were retrospectively analyzed.

The aim of the study was to assess the prognostic value of baseline ALC in patients with recurrent metastatic EC treated with immunotherapy and further analyzed the relationship between ALC and RT-related parameters. The inclusion criteria were as follows: (I) age ≥18 years; (II) histologically or imaging confirmed recurrent or metastatic EC; (III) complete routine blood test data before and during the follow-up period of immunotherapy in our hospital; (IV) systemic treatment of immunotherapy with or without RT; and (V) follow-up time ≥4 weeks after the start of immunotherapy. The exclusion criteria were as follows: (I) cases in which insufficient routine blood data were obtained from patients; and (II) patients with severe systemic or hematologic diseases. Finally, 105 patients with EC were included. Considering that immunotherapy for EC has started to be used clinically in the last few years and the number of cases is relatively small, all eligible samples were included in this study. The HR =1.771 for predicting OS according to ALC in the multivariate Cox regression model, with a *post-hoc* calculated statistical power of 79.11%, close to 80%.

General information about the patient was collected and recorded, such as age, gender, type of pathology, degree of differentiation, tumor location, recurrence or distant metastasis, type of immunotherapy drugs, number of courses of RT and interval between RT and immunotherapy. ALC within 1 week before immunotherapy was collected as the baseline or pre-immunotherapy ALC. For patients who had previously received RT, the ALCs were also collected at baseline and 1, 2, 3, 6, and 12 months after the start of RT. According to the Common Terminology Criteria for Adverse Events (CTCAE) 5.0, we defined a Min ALC <200 cells/μL within 3 months after the start of RT as G4 Min ALC.

Among the patients included in the study, 65 patients had previously received and completed the prescribed dose of RT (including postoperative adjuvant RT and radical RT). The organs at risk were outlined to include both lungs, the heart, and the spinal cord. Considering that a considerable the mediastinum (including structures such as the esophagus, heart, large vessels, and lymph nodes) has most of its volume exposed to the irradiation field, we defined the mediastinum for the first time as an organ in jeopardy for outlining (upper to the entrance of the thorax, lower to the diaphragm, with the posterior border of the sternum at the anterior boundary, the anterior border of the spine at the posterior boundary, and the borders of the lungs at the left and right sides), which helps to assess the volume of the large vessels, heart, and lymph nodes in the thorax of RT patients from a holistic perspective. The following dosimetric parameters were collected: mean PTV dose, PTV volume, mean heart dose, mean bilateral lung dose, mean mediastinal dose, as well as the V5, V10, V20, V30, and V40 of the heart, both lungs, and the mediastinum.

Patient follow-up

OS was defined as the period from the start of the patients' immunotherapy to the follow-up deadline or the date of death. All patients were followed up for survival every 3 months until their death via electronic medical records or by phone from June 2018 to July 2021.

Statistical analysis

Taking the patients' 1-year OS as the endpoint, the receiver operating characteristic (ROC) curve of ALC predicting OS before immunotherapy was drawn and the area under the curve (AUC) was calculated. The optimal cutoff value of lymphopenia was determined according to the Youden index. The patients were divided into two groups according to the cut-off value, and the clinical baseline data of the two groups were compared. The univariate and multivariate Cox regression model was used to identify risk factors affecting survival. Variables considered to be clinically relevant or with a P value <0.20 in univariate analysis were included in the multivariate Cox regression model. The Kaplan-Meier method was used to calculate the cumulative survival rate and the log-rank test was used to compare the survival differences between the two groups. Pearson analysis was used to determine the relationship between the Min ALC and PTV volume, mean PTV dose, mean heart dose, mean bilateral lung dose, and mean mediastinal dose. Spearman analysis was used to determine the relationship between the Min ALC and the V5, V10, V20, V30, and V40 of the heart, both lungs, and the mediastinum. ROC curves were plotted to analyze the cut-off values of RT-related parameters (V20, V30, and V40 of the heart; V5, V10, and V20 of both lungs; and V10, V20, V30, and V40 of the mediastinum) for predicting G4 lymphopenia during RT. Binary logistic regression analysis was used to determine the factors affecting the baseline ALC reduction correlation and the relationship between grade 4 (G4) Min ALC reduction after RT and RT-related parameters. Variables considered to be clinically relevant or with

Table 1 Patient and treatment characteristics

Characteristics	N (%)
Sex	
Male	84 (80.0)
Female	21 (20.0)
Age (years)	
<65	50 (47.6)
≥65	55 (52.4)
Tumor location	
Upper-middle	47 (44.8)
Lower	58 (55.2)
Histology	
Squamous cell carcinoma	97 (92.4)
Adenocarcinoma	8 (7.6)
Degree of differentiation	
Poor	37 (35.2)
Moderate/well	32 (30.5)
Unknown	36 (34.3)
Distant metastasis	
None	45 (42.9)
Single organ	44 (41.9)
Multiple organs	16 (15.2)
Number of previous chemotherapy lines	
0	40 (38.1)
1	45 (42.9)
≥2	20 (19.0)
Interval between last chemotherapy and immunotherapy	
<3 months	25 (38.5)
≥3 months	40 (61.5)
Number of previous RT sessions	
0	40 (38.1)
1	49 (46.7)
≥2	16 (15.2)
Interval between last RT and immunotherapy	
<3 months	28 (43.1)
≥3 months	37 (56.9)
Use of anti-angiogenic therapy	
Yes	33 (31.4)
No	72 (68.6)
Types of ICIs	
Pabolizumab	6 (5.7)
Camrelizumab	35 (33.3)
Sintilimab	56 (53.4)
Toripalimab	8 (7.6)
Status	
Alive	39 (37.1)
Dead	66 (62.9)

RT, radiotherapy; ICIs, immune checkpoint inhibitors.

a P value <0.20 in univariate analysis were included in the multivariate logistic regression model. All statistical calculations were two-sided tests, and P values less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics of the included patients

A total of 105 patients with EC were included in our study, and their clinical and follow-up data were collected (*Table 1*). Among the included patients, there were 84 men (80.0%) and 21 women (20.0%), aged 43–77 years old, with a median age of 65 years, of which 55 cases (52.4%) were at least 65 years old. There were 47 cases (44.8%) of upper and middle segment EC and 58 cases (55.2%) of lower segment EC. In terms of the pathological types, there were 97 cases (92.4%) of squamous carcinoma and eight cases (7.6%) of adenocarcinoma. The pathological grading was low differentiation in 37 patients (35.2%), intermediate/high differentiation in 32 patients (30.5%), and unknown differentiation in 36 patients (34.3%). Forty-five (42.9%) patients had no distant metastasis, 60 (57.1%) patients had distant metastasis, and there were 44 (41.9%) and 16 (15.2%) cases of single- and multiple-organ metastasis, respectively.

As for treatment, 40 patients (38.1%) were treated with immunotherapy as a first-line treatment, 45 (42.9%) as second line, and 20 (19.0%) as third line and above. Twenty-five (38.5%) patients had less than 3 months between immunotherapy and the previous cycle of chemotherapy,

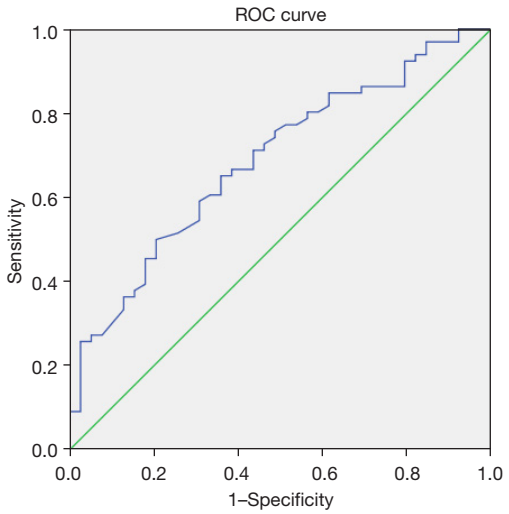

Figure 1 ROC curve of ALC predicting 1-year overall survival. ROC, receiver operating characteristic; ALC, absolute lymphocyte count.

and 40 (61.5%) had at least 3 months between immunotherapy and the previous cycle of chemotherapy. Also, 65 (61.9%) patients had received previous radiation therapy, of which 49 (46.7%) had received one session of RT and 16 (15.2%) had received two or more sessions of RT. The interval between RT and immunotherapy was less than 3 months in 28 cases (43.1%) and at least 3 months in 37 cases (56.9%). In 33 cases (31.4%), anti-tumor angiogenesis therapy was administered at the same time. The following types of immune checkpoint inhibitors (ICIs) classes were used: pabolizumab in six cases (5.7%), camrelizumab in 35 cases (33.3%), sintilimab in 56 cases (53.4%), and toripalimab in eight cases (7.6%). As of the last follow-up date, 66 (62.9%) patients had died of tumor recurrence and metastasis.

Cut-off value of the baseline ALC predicting survival

The ALC was collected within 1 week before immunotherapy and chemotherapy. Taking the patients' 1-year OS as the endpoint, the ROC curve of ALC predicting OS was drawn (*Figure 1*). When the ALC cut-off value was 625 cells/μL, the Youden index was the largest (0.295), the sensitivity was 0.5, the specificity was 0.795, and the AUC was 0.688 [95% confidence interval (CI): 0.586–0.791, P=0.001].

Taking 625 cells/μL as the cutoff value, the patients were divided into a low ALC or lymphopenia group (ALC ≤625 cells/μL, n=41) and a high ALC or non-lymphopenia group (ALC >625 cells/μL, n=64). A comparison of the baseline characteristics of the two groups of patients is shown in *Table 2*. There were significant differences between the number of courses of RT before immunotherapy (P=0.026), but there were no significant differences in the other clinicopathological characteristics between the two groups.

Analysis of the risk factors affecting OS of patients with EC

The median OS for all patients (n=105) was 8 months. As shown in *Figure 2*, 41 patients in the lymphopenia group had a median OS of 6 months and a 1-year survival rate of 14.6%, while 64 patients in the non-lymphopenia group had a median OS of 12 months and a 1-year survival rate of 51.6%, and these differences were statistically significant (χ^2=9.833, P=0.002).

The prognostic factors for OS were analyzed in *Table 3*. The univariate Cox regression analysis showed that lower segment EC [hazard ratio (HR): 1.887, 95% CI: 1.140–3.124; P=0.014], distant metastasis to multiple organs (HR: 2.065, 95% CI: 1.043–4.088; 0.037), and lymphopenia (ALC ≤625 cells/μL) before immunotherapy (HR: 2.068, 95% CI: 1.268–3.373; P=0.004) were significantly associated with poorer OS. In the multivariate Cox regression analysis, lower segment EC (HR: 1.833, 95% CI: 1.076–3.124; P=0.026), multiple-organ metastasis (HR: 2.156, 95% CI: 1.071–4.339; P=0.031), and baseline lymphopenia (HR: 1.771, 95% CI: 1.051–2.985; P=0.032) were significantly associated with poorer OS.

Analysis of the related factors affecting baseline ALC

Binary logistic regression was performed to identify the determinants affecting the baseline ALC (*Table 4*). The univariate binary logistic regression showed that the number of previous RT courses ≥2 [odds ratio (OR): 5.000, 95% CI: 1.448–17.271; P=0.011], the number of previous chemotherapy lines ≥2 (OR: 3.667, 95% CI: 1.179–11.408; P=0.025), and the presence of G4 Min ALC (Min ALC <200 cells/μL) during RT (OR: 8.510, 95% CI: 2.141–33.830; P=0.002) were factors influencing the reduction of ALC at baseline. In the multivariate binary logistic regression, patients presenting with prior G4 Min ALC during previous RT (OR: 10.809, 95% CI: 1.061–14.207; P=0.004) were more likely to develop pre-immunotherapy lymphopenia after recurrent metastasis.

Table 2 Comparison of the baseline information between patients in the lymphopenia and non-lymphopenia groups

Variables	Lymphopenia (n=41, %)	Non-lymphopenia (n=64, %)	χ^2	P value
Sex			0.360	0.548
Male	34 (82.9)	50 (78.1)		
Female	7 (17.1)	14 (21.9)		
Age (years)			0.984	0.321
<65	16 (39.0)	31 (48.4)		
≥65	25 (61.0)	33 (51.6)		
Tumor location			0.896	0.344
Upper middle	22 (53.7)	28 (43.8)		
Lower	19 (46.3)	36 (56.2)		
Histology			0.718	0.397
SqCCa	39 (95.1)	58 (90.6)		
Adenocarcinoma	2 (4.9)	6 (9.4)		
Degree of differentiation			1.340	0.512
Poorly	14 (34.1)	23 (35.9)		
Moderate/well	15 (36.6)	17 (26.6)		
Unknown	12 (29.3)	24 (37.5)		
Distant metastasis			0.755	0.686
None	17 (41.5)	28 (43.8)		
Single organ	19 (46.3)	25 (39.1)		
Multiple organs	5 (12.2)	11 (17.1)		
Number of previous chemotherapy lines			6.006	0.050
0	10 (24.4)	30 (46.9)		
1	20 (48.8)	25 (39.1)		
≥2	11 (26.8)	9 (14.0)		
Interval between last chemotherapy and immunotherapy			2.467	0.116
<3 months	15 (48.4)	10 (29.4)		
≥3 months	16 (51.6)	24 (70.6)		
Number of previous RT sessions			7.313	0.026
0	10 (24.4)	30 (46.9)		
1	21 (51.2)	28 (43.8)		
≥2	10 (24.4)	6 (9.3)		
Interval between last RT and immunotherapy			0.031	0.859
<3 months	13 (41.9)	15 (44.1)		
≥3 months	18 (58.1)	19 (55.9)		

Table 2 (*continued*)

Table 2 (*continued*)

Variables	Lymphopenia (n=41, %)	Non-lymphopenia (n=64, %)	χ^2	P value
Use of anti-angiogenic therapy			1.316	0.251
Yes	18 (43.9)	21 (32.8)		
No	23 (56.1)	43 (67.2)		
Types of PD-1 ICIs			0.980	0.806
Pabolizumab	3 (7.3)	3 (4.7)		
Camrelizumab	14 (34.1)	21 (32.8)		
Sintilimab	20 (48.8)	36 (56.3)		
Toripalimab	4 (9.8)	4 (6.2)		

SqCCa, squamous cell carcinoma; RT, radiotherapy; PD-1, programmed cell death 1; ICIs, immune checkpoint inhibitors.

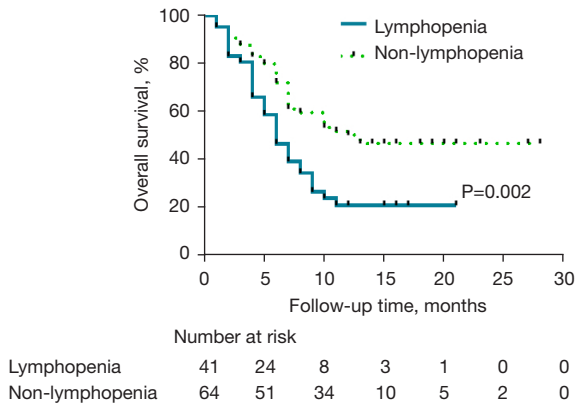

Figure 2 The relationship between ALC before immunotherapy and patient prognosis. ALC, absolute lymphocyte count.

Relationship between the Min ALC and RT-related parameters

The Min ALC during RT was reviewed in 65 patients who had previously received RT, with a median Min ALC of 250 cells/μL (70–1,360 cells/μL). Among them, 17 patients had post-RT G4 Min ALC. The median PTV volume was 390.8 cm^3 (79.1–885.4 cm^3) and the median mean PTV dose was 5,641.6 cGy (3,133.9–6,910.6 cGy) in all RT patients.

Pearson analysis (Figure S1) showed that Min ALC after RT was significantly negatively correlated with the PTV volume (r=–0.370, P=0.002) but was not correlated with the mean PTV dose (r=–0.035, P=0.782). Figures S2-S4 demonstrate the relationship between the Min ALC and the mean heart dose, mean bilateral lung dose, mean mediastinal dose, as well as V5, V10, V20, V30, and V40 of the heart, both lungs, and the mediastinum. We observed that higher V5 (r=–0.343, P=0.005) and V10 (r=–0.322, P=0.009) of both lungs were significantly associated with lower Min ALC (P<0.01). Higher V20 (r=–0.255, P=0.041), V30 (r=–0.280, P=0.024), and V40 (r=–0.246, P=0.048) of the heart, V20 of both lungs (r=–0.275, P=0.027), and V10 (r=–0.254, P=0.041), V20 (r=–0.284, P=0.022), V30 (r=–0.278, P=0.025), and V40 (r=–0.267, P=0.032) of the mediastinum correlated with lower Min ALC (P<0.05).

RT-related parameters predict the cut-off value of G4 Min ALC

The accuracy of RT-related parameters (V20, V30, and V40 of the heart; V5, V10, and V20 of both lungs; and V10, V20, V30, and V40 of the mediastinum) in predicting the G4 Min ALC after RT was analyzed by ROC curves. As shown in *Figure 3*, the parameters corresponding to P<0.05 were included in the ROC curve, and the cut-off values for PTV volume, V20, V30, and V40 of the heart; V5, V10 of both lungs; and V10, V20, and V30 of the mediastinum were 521.2 cm^3, 16.55%, 8.7%, 4.85%, 45.65%, 32.65%, 70.2%, 47.3%, and 45.3%, respectively (P=0.014, P=0.033, P=0.023, P=0.048, P=0.01, P=0.037, P=0.015, P=0.013, and P=0.021). V20 of both lungs and V40 of the mediastinum were not included in the ROC curves (P=0.074 and P=0.050). Using these cut-off values, the risk of developing G4 Min ALC during RT could be better predicted (*Table 5*).

Table 3 Prognostic factor analysis for overall survival

Variables	UVA			MVA		
	HR	95% CI	P value	HR	95% CI	P value
Male *vs.* female	1.352	0.769–2.377	0.294	1.408	0.780–2.541	0.257
Age (≥65 years)	0.809	0.499–1.312	0.390	0.915	0.547–1.530	0.735
Upper-middle *vs.* lower	1.887	1.140–3.124	0.014	1.833	1.076–3.124	0.026
SqCCa *vs.* adenocarcinoma	1.480	0.675–3.244	0.328	1.282	0.556–2.960	0.560
Degree of differentiation						
Poor *vs.* moderate/well	0.880	0.485–1.599	0.675	0.949	0.496–1.819	0.875
Poor *vs.* unknown	0.853	0.481–1.514	0.587	0.996	0.548–1.813	0.991
Distant metastasis						
None *vs.* single organ	1.488	0.863–2.566	0.153	1.582	0.888–2.817	0.120
None *vs.* multiple organs	2.065	1.043–4.088	0.037	2.156	1.071–4.339	0.031
Use of radiotherapy (no *vs.* yes)	1.674	0.988–2.837	0.056	1.683	0.947–2.993	0.076
ALC (≤625 cells/μL)	2.068	1.268–3.373	0.004	1.771	1.051–2.985	0.032

SqCCa, squamous cell carcinoma; ALC, absolute lymphocyte count; UVA, univariate analysis; MVA, multivariate analysis; HR, hazard ratio; CI, confidence interval.

Table 4 Binary logistic regression affecting ALC

Variables	Univariate logistic regression			Multivariate logistic regression		
	OR	95% CI	P value	OR	95% CI	P value
Male *vs.* female	0.735	0.269–2.012	0.549	0.808	0.254–2.565	0.717
Age (≥65 years)	0.672	0.306–1.477	0.322	0.661	0.245–1.783	0.413
Upper-middle *vs.* lower	1.468	0.662–3.255	0.345	1.165	0.429–3.165	0.765
SqCCa *vs.* adenocarcinoma	0.496	0.095–2.584	0.405	0.385	0.048–3.061	0.367
Degree of differentiation						
Poor *vs.* moderate/well	1.450	0.554–3.790	0.449	1.659	0.487–5.646	0.418
Poor *vs.* unknown	0.821	0.315–2.145	0.688	0.935	0.285–3.069	0.912
Distant metastasis						
None *vs.* single organ	1.252	0.536–2.923	0.604	1.103	0.394–3.090	0.851
None *vs.* multiple organs	0.749	0.222–2.528	0.641	0.574	0.138–2.390	0.445
Number of previous RT sessions						
None *vs.* 1 session	2.250	0.904–5.603	0.081	0.944	0.298–2.989	0.922
None *vs.* ≥2 sessions	5.000	1.448–17.271	0.011	1.915	0.423–8.665	0.399
Number of previous chemotherapy lines						
None *vs.* 1 line	2.400	0.950–6.060	0.064	1.716	0.600–4.903	0.314
None *vs.* ≥2 lines	3.667	1.179–11.408	0.025	2.332	0.591–9.210	0.227
Min ALC during RT (≥200 *vs.* <200 cells/μL)	8.510	2.141–33.830	0.002	10.809	2.185–53.471	0.004

ALC, absolute lymphocyte count; Min ALC, minimal ALC value; OR, odds ratio; CI, confidence interval; RT, radiotherapy; SqCCa, squamous cell carcinoma.

Impact of RT-related parameters on G4 Min ALC during RT

Binary logistic regression was used to determine the impact of RT-related parameters on the G4 Min ALC during RT (*Table 6*). Among them, RT-related parameters such as PTV volume, V20, V30, and V40 of the heart, V5, and V10 of both lungs, and V10, V20, and V30 of the mediastinum were included as dichotomous variables, and V20 of both lungs and V40 of the mediastinum were included as numerical variables. The univariate binary logistic regression showed that PTV volume >521.2 cm^3 (OR: 9.778, 95% CI: 2.416–39.576; P=0.001), V20 of the heart >16.55% (OR: 6.900, 95% CI: 1.421–33.511; P=0.017), V30 of the heart >8.7% (OR: 14.720, 95% CI: 1.806–119.984; P=0.012), V40 of the heart >4.85% (OR: 16.000, 95% CI: 1.963–130.400; P=0.010), V5 of both lungs >45.65% (OR: 11.447, 95% CI: 2.347–55.842; P=0.003), V10 of both lungs >32.65% (OR: 5.417, 95% CI: 1.531–19.170; P=0.009), V10 of the mediastinum >70.2% (OR: 6.462, 95% CI: 1.904–21.934; P=0.003), V20 of the mediastinum >47.3% (OR. 16.000, 95% CI: 1.963–130.400; P=0.010), and V30 of the mediastinum >45.3% (OR: 8.510, 95% CI: 2.141–33.830; P=0.002) were factors influencing the Min ALC during RT. In the multivariate binary logistic regression, PTV volume >521.2 cm^3 (OR: 19.981, 95% CI: 1.372–290.985; P=0.028) was identified as an independent risk factor influencing the G4 Min ALC during RT.

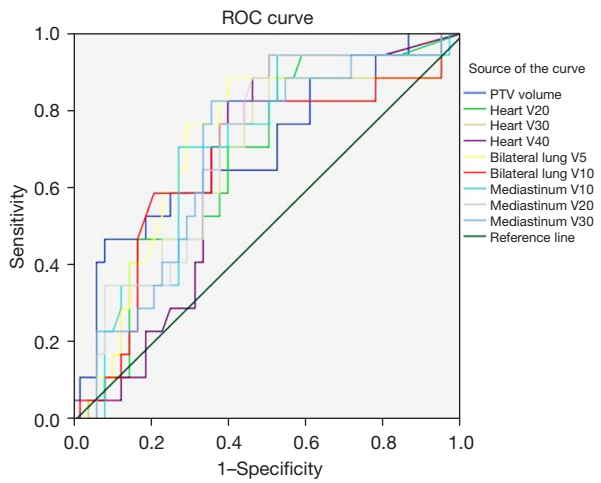

Figure 3 ROC curve analysis for determining the cut-off value of radiotherapy parameters for predicting the grade 4 Min ALC. Heart Vn: the percentage of heart receiving n Gy; bilateral lung Vn: the percentage of both lungs receiving n Gy; mediastinum Vn: the percentage of the mediastinum receiving n Gy. ROC, receiver operating characteristic; PTV, planning target volume; ALC, absolute lymphocyte count; Min ALC, minimal ALC value.

Discussion

Generally, cellular immunity plays a major role in the anti-tumor process, and lymphocytes play a key role in mediating the body's cellular immune response against tumors. $CD8^+$ T cells kill tumor cells by releasing cytolytic factors and promoting cell apoptosis. Han *et al.* concluded

Table 5 Predictive cut-off value of radiotherapy parameters and its determining ability to G4 Min ALC

Parameters	Cut-off value	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)	P value
PTV volume	521.2 cm^3	0.917 (0.791–0.973)	0.471 (0.239–0.715)	0.702 (0.552–0.852)	0.014
Heart V20	16.55%	0.479 (0.335–0.626)	0.882 (0.623–0.979)	0.675 (0.535–0.815)	0.033
Heart V30	8.7%	0.479 (0.335–0.626)	0.941 (0.692–0.997)	0.686 (0.550–0.822)	0.023
Heart V40	4.85%	0.500 (0.354–0.646)	0.941 (0.692–0.997)	0.662 (0.527–0.798)	0.048
Bilateral lung V5	45.65%	0.604 (0.453–0.739)	0.882 (0.623–0.979)	0.712 (0.565–0.859)	0.010
Bilateral lung V10	32.65%	0.625 (0.473–0.757)	0.765 (0.498–0.922)	0.672 (0.516–0.827)	0.037
Mediastinum V10	70.2%	0.729 (0.579–0.843)	0.706 (0.440–0.886)	0.700 (0.562–0.839)	0.015
Mediastinum V20	47.3%	0.500 (0.354–0.646)	0.941 (0.692–0.997)	0.703 (0.566–0.840)	0.013
Mediastinum V30	45.3%	0.646 (0.494–0.774)	0.824 (0.558–0.953)	0.690 (0.549–0.831)	0.021

Heart Vn: the percentage of heart receiving n Gy; bilateral lung Vn: the percentage of both lungs receiving n Gy; mediastinum Vn: the percentage of the mediastinum receiving n Gy. AUC, area under the curve; CI, confidence interval; G4, grade 4; ALC, absolute lymphocyte count; Min ALC, minimal ALC value; PTV, planning target volume.

Table 6 Binary logistic regression affecting the minimum absolute lymphocyte count

Variables	Univariate logistic regression			Multivariate logistic regression		
	OR	95% CI	P value	OR	95% CI	P value
PTV volume (>521.2 cm^3)	9.778	2.416–39.576	0.001	19.981	1.372–290.985	0.028
Heart DVH						
V20 (>16.55%)	6.900	1.421–33.511	0.017		NS	
V30 (>8.7%)	14.720	1.806–119.984	0.012		NS	
V40 (>4.85%)	16.000	1.963–130.400	0.010	2.240	0.257–19.492	0.465
Bilateral lung DVH						
V5 (>45.65%)	11.447	2.347–55.842	0.003	24.380	0.602–987.563	0.091
V10 (>32.65%)	5.417	1.531–19.170	0.009	7.893	0.402–155.066	0.174
V20 (%)	1.041	0.967–1.119	0.285	0.767	0.546–1.075	0.124
Mediastinum DVH						
V10 (>70.2%)	6.462	1.904–21.934	0.003	2.240	0.257–19.492	0.465
V20 (>47.3%)	16.000	1.963–130.400	0.010		NS	
V30 (>45.3%)	8.510	2.141–33.830	0.002	7.413	0.330–166.632	0.207
V40 (%)	1.028	0.989–1.068	0.164	0.922	0.836–1.018	0.107

Heart Vn: the percentage of heart receiving n Gy; bilateral lung Vn: the percentage of both lungs receiving n Gy; mediastinum Vn: the percentage of the mediastinum receiving n Gy. DVH, dose-volume histogram; OR, odds ratio; CI, confidence interval; PTV, planning target volume; NS, non-significant.

that cancer patients with a high degree of $CD8^+$ T-cell infiltration have a better prognosis (8). Furthermore, activated $CD4^+$ T cells can induce an inflammatory response similar to delayed-type hypersensitivity, and promote immune cells such as macrophages and natural killer (NK) cells to exert anti-tumor effects. Oh *et al.* found that $CD4^+$ T cells can kill bladder cancer cells via major histocompatibility complex (MHC) II-dependent pathways, and the genetic characteristics of $CD4^+$ T cells predicted the clinical prognosis of 244 patients with metastatic bladder cancer treated with programmed death-ligand 1 (PD-L1) (9). A recent study has also confirmed the role of B cells in tumor immunity. Cabrita *et al.* reported that the presence of B cells is associated with a better response to neoadjuvant immunotherapy in melanoma (20). A study believes that the functional status of the immune system is an important biomarker for predicting the effect of treatment (21). Therefore, it is vital to maintain a complete immune system to improve the therapeutic outcomes of cancer patients during treatment.

ALC represents a patient's immune function status, and lymphopenia may be associated with poor prognosis of patients treated with immunotherapy. Previous studies have reported that extracranial RT or extended RT sessions increase the risk of severe lymphopenia in patients with non-small cell lung cancer, melanoma, and renal cell carcinoma treated with palliative RT, which in turn affects their prognosis with immunotherapy (22). Byun *et al.* included 134 patients with advanced or metastatic melanoma treated with ICI monotherapy and showed that treatment initiation lymphopenia (ALC <1,000 cells/μL) within 3 months was an independent risk factor for poor prognosis with immunotherapy [OS: HR =1.89, P=0.006; progression-free survival (PFS): HR =1.70, P=0.010] (23). Similarly, Chen *et al.* showed that lung V5 was associated with conventional RT-induced lymphopenia and that lower post-RT ALC was also associated with poorer PFS in patients (24). Similar to the results reported in the literature, the results of this study showed that the median OS of patients in the pre-immunotherapy lymphopenia group was 6 months with a 1-year survival rate of 14.6% and the median OS in the non-lymphopenia group was 12 months with a 1-year survival rate of 51.6%, and these differences were statistically significant. These results suggested that

pre-immunotherapy lymphopenia was associated with poor prognosis in patients with recurrent metastatic EC treated with immunotherapy.

RT is a local treatment method that uses radiation to kill tumor cells so as to improve the effect of tumor treatment. Although it can kill tumor cells directly, it is vital to consider that lymphocytes are the most radiosensitive cells in the hematopoietic system, and a dose of 1 Gy is sufficient to kill 50% of circulating lymphocytes (D50), leading to impaired systemic tumor surveillance (25). Therefore, RT causes a significant decrease in lymphocytes, affecting all lymphocyte subsets ($CD4^+$, $CD8^+$ T cells, B cells, and NK cells, among others) (26).

Our study exploring the effect of RT-related parameters on lymphopenia found that a PTV volume >521.2 cm^3 was an independent risk factor affecting G4 lymphopenia during RT. The Min ALC after RT was significantly correlated with PTV volume as well as V5 and V10 of both lungs (P=0.002, P=0.005, and P=0.009, respectively). The Min ALC was correlated with V20, V30, and V40 of the heart; V20 of both lungs; and V10, V20, V30, and V40 of the mediastinum. Therefore, reducing the PTV volume and controlling the volume dose in the heart, both lungs, and the mediastinum may reduce the risk of lymphopenia.

This idea was also confirmed in a related study. Rudra *et al.* reported that compared with standard-field RT (T1 enhancement + surgical cavity + T2 abnormal + 1.3–2.5 cm margin), limited-field RT (T1 enhancement + surgical cavity + 1.8–2 cm margin) reduces the brain exposure volume, leading to a reduced risk of Min ALC reduction in patients with glioblastoma (27). Chin *et al.* suggested that the course of RT for squamous cell carcinoma of the head and neck is associated with the depletion of circulating lymphocytes and may attenuate tumor antigen presentation. Limiting the irradiation field to the primary tumor and the ipsilateral neck reduces the risk of reduced Min ALC while protecting the patients' immune function (28). Similarly, Saito *et al.* retrospectively analyzed various types of patients with lung, liver, and gastrointestinal tumors treated with palliative RT, defining a total of three organs at risk: the volume enclosed by the body contour (A), the volume remaining after exclusion of air, pleural effusion, ascites, bile, urine, and intestinal contents (B), and BM. Higher dose-volume parameters of A and B and a higher number of RT sessions predicted grade 3 Min ALC (29). Wang *et al.* suggested that the Min ALC during RT is associated with PTV volume in EC as well as V10 and V20 of the heart, and that larger PTV volume is an independent risk factor for the Min ALC during RT (19). Therefore, controlling the PTV volume and optimizing the normal dosimetric parameters of tissues may have a protective effect on lymphocytes.

In the era of immunotherapy, given the correlation between ALC and the therapeutic efficacy of immunotherapy, we usually need to consider the following two factors to optimize the RT regimen for EC: target area volume and target area dose. Currently, postoperative adjuvant RT and radical RT are widely used in the treatment of EC. Regardless of the type of RT, there is a controversy regarding large and small field irradiation, namely, whether clinicians should select lymphatic drainage area irradiation [elective nodal irradiation (ENI)] or involved field irradiation (IFI). Several retrospective and prospective studies have found that the main failure mode after IFI is still in-field recurrence and distant metastasis, rather than isolated out-of-field nodal recurrence only (30). ENI only improves local control but does not improve the long-term survival of patients with EC, and the efficacy of IFI and ENI is essentially similar (31,32). For ENI irradiation mode, the target area volume is not conducive to the protection of peripheral circulating lymphocytes because it covers many large blood vessels and lymphatic tissues in the cervicothoracic region. It is reasonable to assume that choosing IFI will reduce the target area volume, which will not only help to protect the endangered organs and reduce the side effects of RT, but also help to reduce the risk of peripheral circulating lymphopenia and better protect the immune function of patients.

In addition, a recent study by Ellsworth *et al.* showed that there was an exponential decline in lymphocyte counts in the first 3 weeks of routine fractionation RT; the faster the decline in lymphocytes in the first 3 weeks, the more obvious the decrease in total lymphocyte counts. This can be used to evaluate the rate of decline in the lymphocyte count of patients in the early stage of RT to identify patients at a high risk of severe lymphopenia (33). We speculate that for high-risk patients with lymphopenia during RT, administration of a certain amount of cytokines to increase the number of lymphocytes, a better prognosis can be obtained. Further prospective studies are needed to confirm this hypothesis.

In conclusion, the present study showed that OS was significantly lower in patients with pre-immunotherapy lymphopenia than in the non-lymphopenia group (median OS: 6 *vs.* 12 months, P=0.002). Patients with G4 Min ALC during prior RT were more likely to develop baseline phase lymphopenia. A PTV volume >521.2 cm^3 was an

independent risk factor affecting G4 Min ALC during RT. In other words, the volume of previous RT exposure affects the Min ALC and indirectly impacts the therapeutic effect of immunotherapy for EC after recurrent metastasis.

This study is one of the first to report that the volume of previous RT exposure in patients with advanced EC indirectly impacts the therapeutic effect of immunotherapy by affecting the ALC. Therefore, we need to control the volume of RT exposure to help protect lymphocytes and thus maintain the robust immune function of patients. The limitations of this study include its retrospective design as well as the small number of included cases. Therefore, prospective studies are still required to confirm our findings. Also, this study did not analyze the influence of different lymphocyte subtypes on the prognosis of immunotherapy. In addition, this study did not evaluate patients who recover quickly from lymphopenia; a previous study has shown that patients who recover quickly are associated with a good prognosis compared with those who cannot recover (34).

In summary, lymphopenia is associated with previous RT (postoperative adjuvant RT and radical RT) and is a poor prognostic factor for patients with EC treated with immunotherapy. The standard treatment modality for recurrent metastatic EC is immune combination therapy. In the era of immunotherapy, it is necessary to explore factors including the field, dose, and normal tissue limits of previous RT. Ensuring the efficacy of RT, reducing the irradiated volume, optimizing the technical parameters, and defining stricter normal tissue limits will reduce the risk of Min ALC decline and protect the sound immune function of patients.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-2669/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-2669/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2669/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethical Committee of the First Affiliated Hospital of Soochow University [(2021) No. 329]. Individual consent for this retrospective analysis was waived.

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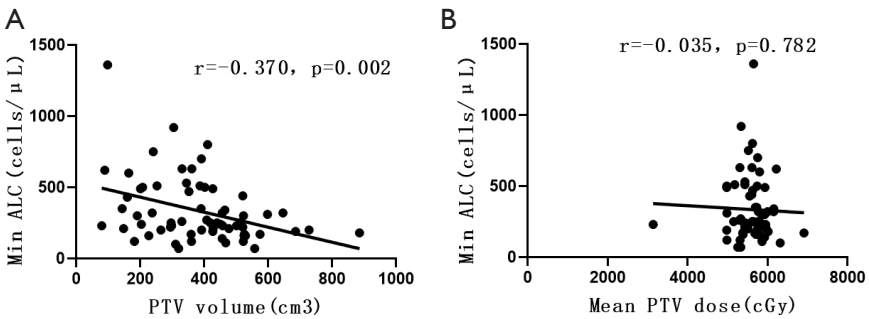

Figure S1 Correlation between the minimum absolute lymphocytes (Min ALC) of peripheral blood during radiotherapy and the PTV volume (A), and the mean PTV dose (B). ALC, absolute lymphocyte count; Min ALC, minimal ALC value; PTV, planning target volume.

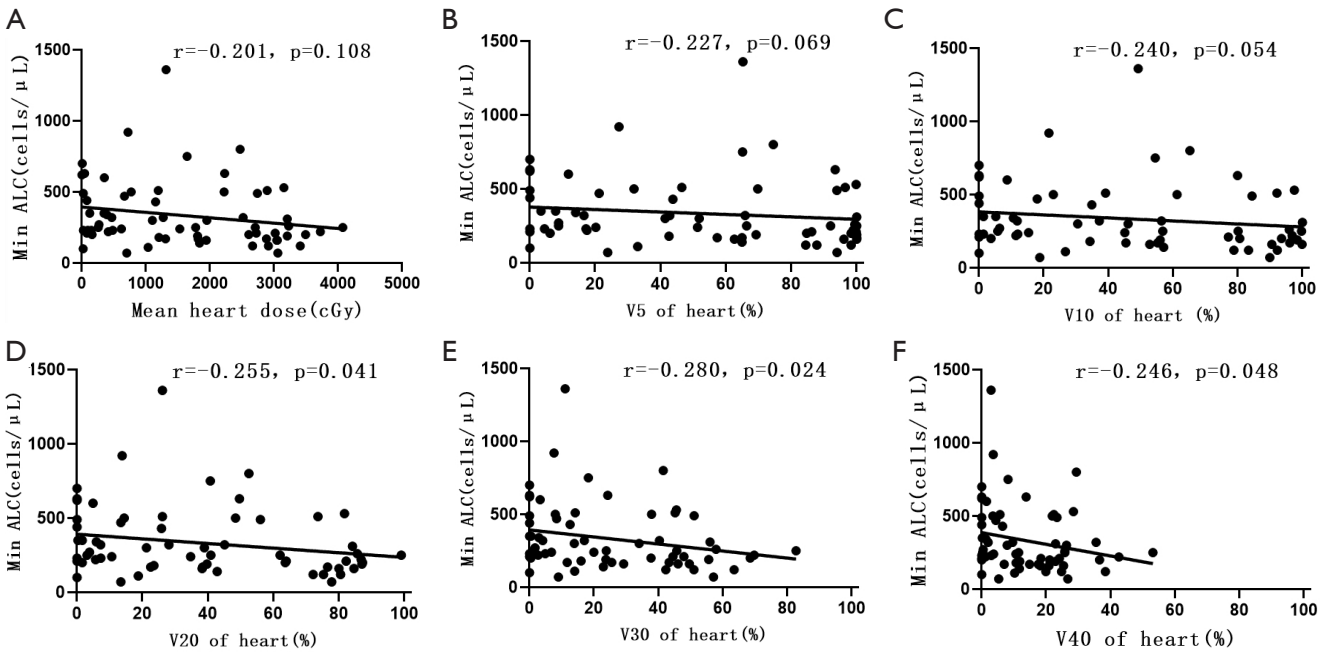

Figure S2 Correlation between the Min ALC of peripheral blood during radiotherapy and the mean heart dose (A), V5 of the heart (B), V10 of the heart (C), V20 of the heart (D), V30 of the heart (E), and V40 of the heart (F). Vn of heart: the percentage of heart receiving n Gy. ALC, absolute lymphocyte count; Min ALC, minimal ALC value.

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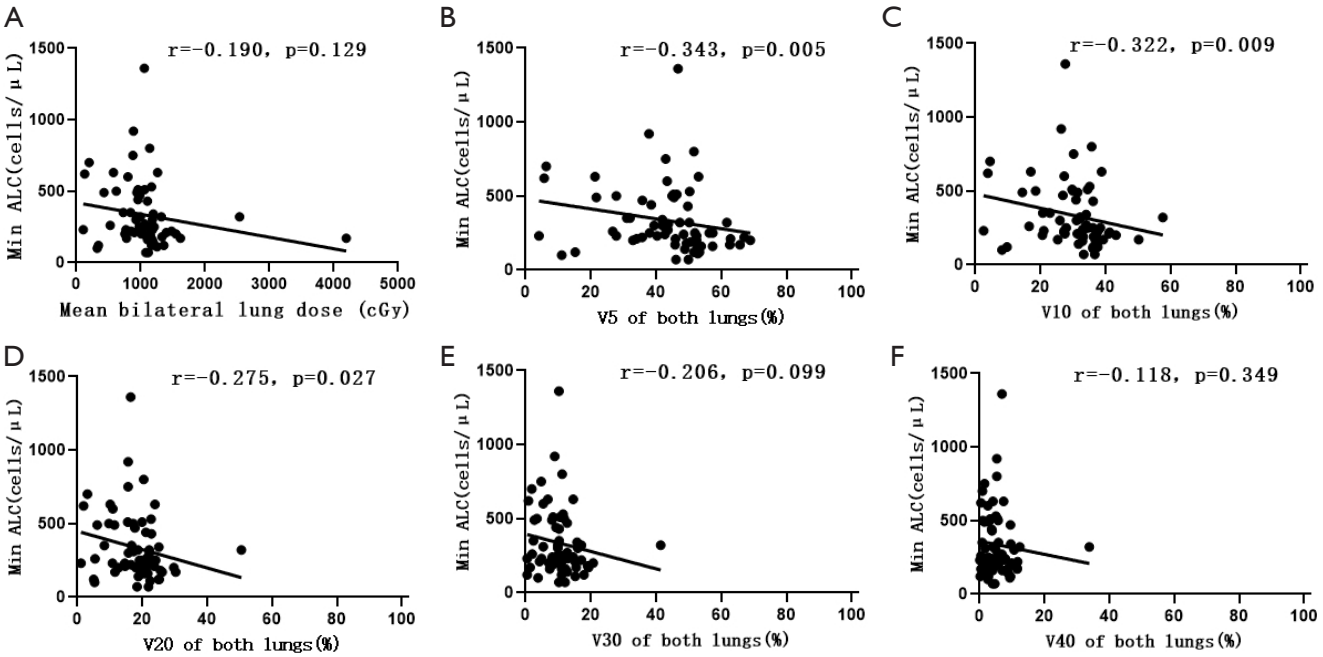

Figure S3 Correlation between the Min ALC of peripheral blood during radiotherapy and the mean bilateral lung dose (A), V5 of both lungs (B), V10 of both lungs (C), V20 of both lungs (D), V30 of both lungs (E), V40 of both lungs (F). Vn of double lung: the percentage of both lungs receiving n Gy. ALC, absolute lymphocyte count; Min ALC, minimal ALC value.

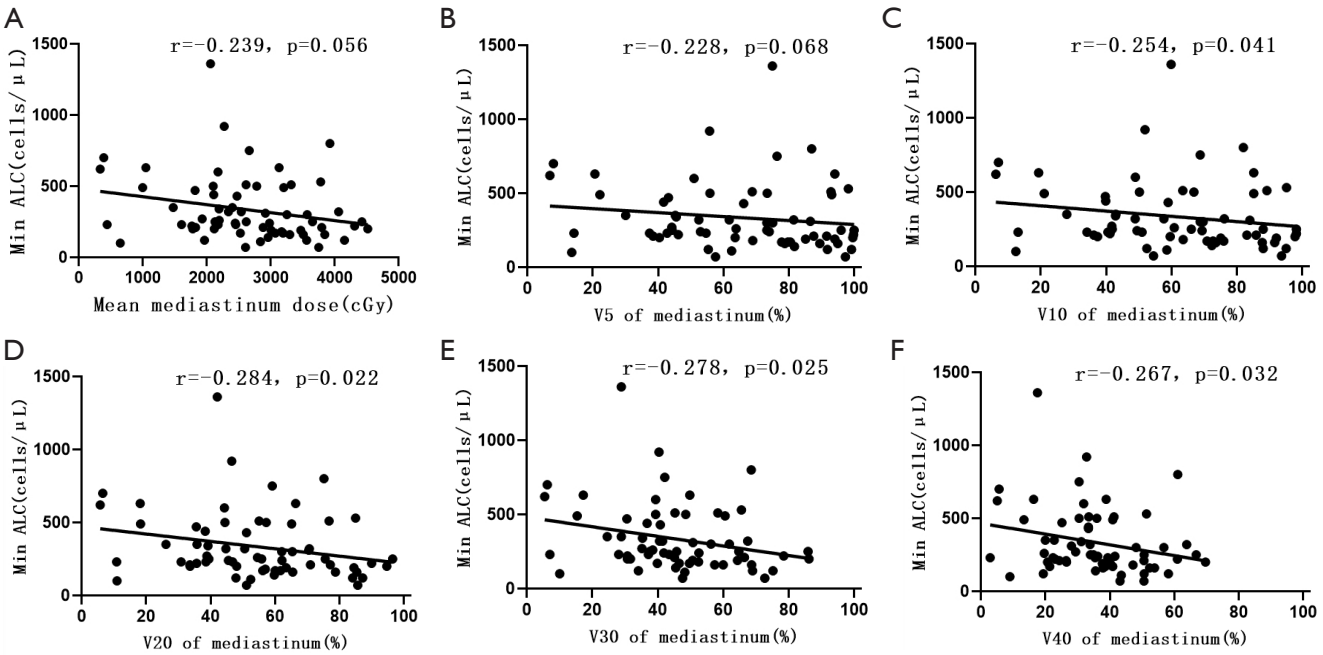

Figure S4 Correlation between the minimum absolute lymphocytes (Min ALC) of peripheral blood during radiotherapy and the mean mediastinal dose (A), V5 of the mediastinum (B), V10 of the mediastinum (C), V20 of the mediastinum (D), V30 of the mediastinum (E), and V40 of the mediastinum (F). Vn of mediastinum: the percentage of the mediastinum receiving n Gy. ALC, absolute lymphocyte count; Min ALC, minimal ALC value.

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