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Orignal Article

The relationship of lymphocyte recovery and prognosis of esophageal cancer patients with severe radiation-induced lymphopenia after chemoradiation therapy

Wei Deng [a,b], Cai Xu [c], Amy Liu [d], Peter S.N. van Rossum [a,e], Weiye Deng [a], Zhongxing Liao [a], Albert C. Koong [a], Radhe Mohan [d], Steven H. Lin [a,*]

[a] Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, United States; [b] Department of Radiation Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing; [c] Department of Radiation Oncology, Tianjin Medical University Cancer Institute and Hospital, China; [d] Department of Radiation Physics, The University of Texas MD Anderson Cancer Center, Houston, United States; [e] Department of Radiation Oncology, University Medical Center Utrecht, The Netherlands

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ABSTRACT

Introduction: Radiation-induced lymphopenia (RIL) during therapy is associated with poor prognosis but is often temporary and resolves after treatment completion in esophageal cancer. How lymphocyte recovery contributes to prognosis is unknown.

Methods: We reviewed 755 patients with stage I-III esophageal carcinoma who received concurrent chemoradiation therapy (CRT) with or without surgery in 2004–2015. Complete blood counts were obtained before, during, and at first follow-up after CRT. Lymphopenia was graded per the Common Terminology Criteria for Adverse Events v4.03 during CRT (G) and as recovery after CRT (G_r). Clinical factors and lymphopenia grade were tested for association with survival in univariable and multivariable Cox proportional hazard regression analyses.

Results: During CRT, 294 patients (38.9%) had G4 lymphopenia; by the first follow-up, 406 patients (53.8%) had recovered (G_r0-1). Relative to patients with G0-3 lymphopenia during CRT, G4 lymphopenia independently predicted worse OS in multivariable analyses. However, lymphocyte recovery was not associated with a better prognosis. Patients with G4 lymphopenia during CRT and recovery (G_r0-1) afterward still had poorer 5-year OS rate than patients with G0-3 during CRT without recovery (G_r2-4) afterward (36.6% vs. 51.9%, HR = 1.40, 95% CI 1.04–1.89, $P = 0.027$). Moreover, the lymphocyte recovery ability (post-CRT ALC divided by pre-CRT ALC) was not affected by lymphopenia grade during CRT (0.66 in G0-3 vs. 0.65 in G4, $p = 0.473$). Among patients with G4 lymphopenia during treatment, lymphocyte recovery was only associated with pre-CRT lymphocyte counts.

Conclusion: Lymphocyte count recovery after CRT does not alter the poor long-term outcomes brought about by high-grade lymphopenia during CRT.

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Esophageal carcinoma is an often-fatal malignancy, with 455,800 new diagnoses and 400,200 deaths worldwide per year [1]. Chemoradiation therapy (CRT) with or without surgery is the standard of care for non-metastatic esophageal cancer [2,3]. However, radiation can suppress host immunity, manifesting as lymphopenia [4,5]. Lymphocytes are so radiosensitive that even relatively low doses delivered to the blood pool can result in significant depletion [6].

The immune system has an integral role in systemic anticancer activity. $CD8^+$ and $CD4^+$ tumor-infiltrating lymphocytes have been linked with favorable survival in esophageal cancer via their ability to directly destroy tumor cells or secrete cytokines that activate effector cells [7,8]. Radiation-induced lymphopenia (RIL), that is, a decline in the number of circulating lymphocytes during treatment, has been linked with poor outcomes in several types of cancer, including gliomas, head and neck cancer, lung cancer, rectal cancer, and pancreatic cancer [5,9–13]. These findings may reflect suppression of circulating lymphocytes in the blood, and as such RIL may be useful as a marker of tumor control and survival.

Low absolute lymphocyte counts (ALCs) during CRT is a strong predictor of poor outcomes and pathologic response rates in esophageal cancer [14–16]. However, RIL is temporary, and ALC can

* Corresponding author at: Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, United States.
E-mail address: SHLin@mdanderson.org (S.H. Lin).

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recover to near-normal levels after treatment is completed. Currently, little is known about how ALC recovery contributes to prognosis. In this study, we evaluated the relationship between ALC recovery and survival outcomes in patients who had CRT with or without surgery for esophageal cancer.

Methods and materials

Patient selection

Patients with stage I–III esophageal carcinoma who received CRT followed or not followed by surgery at a single institution from January 2004 to December 2015 were included. Induction chemotherapy was optional. Radiation was delivered at least 40 Gy by either intensity-modulated radiation therapy (IMRT) or proton therapy. Patients were selected if they had documentation of ALC before, during each week, and at the first follow-up visit after CRT. Those with stage IV disease or other diseases that might affect lymphocyte count (e.g., hematologic malignancies, severe infection or immunosuppression) or lack of follow-up information were excluded. This retrospective analysis was approved by the appropriate institutional review board.

Treatment

Patients received definitive or neoadjuvant CRT to a median dose of 50.4 Gy (range, 41.4–66.0) by either IMRT or proton therapy. Concurrent chemotherapy regimens were generally doublets of a taxane, fluorouracil, or platinum-based compound (TF, PF, or TP). Chemotherapy was delivered weekly during radiation. Among those who had surgery, radical esophagectomy was performed at a median 56 days after CRT (interquartile range [IQR] 47–76 days). The most common surgical procedures were Ivor Lewis, transthoracic, and minimally invasive esophagectomy.

Complete blood counts (CBCs), which included lymphocyte, white blood cell (WBC), and neutrophil measurements, were obtained before initiation of CRT (or after induction chemotherapy, if applicable), each week during CRT, and at first follow-up after CRT (or before surgery, if applicable, usually 6–8 weeks after CRT). Patients were followed up every 3 or 4 months during the first 2 years, every 6 months during the third and fourth years, then annually until death.

Statistical analysis

Lymphopenia was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. The lowest count during CRT was identified as the nadir and was graded from G0 (>1000 cells/μL) to G4 (<200 cells/μL). Lymphocyte counts after CRT were defined separately as G_r0-4. Endpoints were overall survival (OS), progression-free survival (PFS), local-regional recurrence-free survival (LRFS), distant metastasis-free survival (DMFS), and disease-specific survival (DSS), which were defined from date of treatment completion until the events or censoring.

Kaplan–Meier's analysis with time-to-event curves were generated for endpoints in terms of lymphocyte nadirs during and after CRT. Univariable and multivariable Cox proportional hazard regression analyses were used to provide hazard ratios (HRs) with 95% confidence interval (CIs) for outcomes among different groups. Independent-samples *t* tests were used to compare lymphocyte recovery ability between patients with G0-3 or G4 lymphopenia during treatment. A binary logistic regression model was created to test factors potentially associated with lymphocyte recovery. All statistical tests were two-sided, and *P* values <0.05 were considered statistically significant. Analyses were done with R software, version 3.4.3.

Results

Baseline characteristics

A total of 755 patients were included (Table 1), most of them were male (84.5%); about one-third (33.9%) had an ECOG performance status score of 0; and the median age was 64 years. The median tumor length was 5 cm, most tumors (85.2%) were in the lower esophagus, most (81.8%) were adenocarcinomas, and a slight majority (55.8%) were poorly differentiated. Most patients (62.8%) had stage III disease. The proportion of patients who went on to undergo surgery (49.9%) was roughly the same as those who had definitive CRT (50.1%). More patients underwent IMRT (67.7%) rather than proton therapy (32.3%), 262 patients (34.7%) received induction chemotherapy, and most (87%) had TF, PF or TP as concurrent chemotherapy. The median ALC level before CRT was 1.48×10^3 cells/μL. The median interval from the end of CRT to the first follow-up visit was 1.3 months (IQR, 1.02–1.48).

Table 1
Baseline patient characteristics.

Characteristics	No. of patients (%)
Sex	
Male	638 (84.5)
Female	117 (15.5)
Age, years	
≤65	431 (57.1)
>65	324 (42.9)
Median (IQR)	64 (57–71)
ECOG Score	
0	256 (33.9)
1	451 (59.7)
2	48 (6.4)
Tumor length, cm	
≤5	443 (58.7)
>5	312 (41.3)
Median (IQR)	5 (3–7)
Tumor location	
Upper	46 (6.1)
Middle	66 (8.7)
Lower	643 (85.2)
Overall disease stage	
I	40 (5.3)
II	241 (31.9)
III	474 (62.8)
Tumor histology	
Squamous cell carcinoma	133 (17.6)
Adenocarcinoma	618 (81.8)
Other	4 (0.5)
Tumor differentiation	
Well to moderate	333 (44.2)
Poor	421 (55.8)
Radiation modality	
IMRT	511 (67.7)
Proton	244 (32.3)
Surgery	
Yes	377 (49.9)
No	378 (50.1)
Induction chemotherapy	262 (34.7)
Concurrent chemotherapy regimen	
Taxane/fluorouracil	379 (50.2)
Platinum/fluorouracil	228 (30.2)
Platinum/taxane	50 (6.6)
Other	98 (13.0)
Pre-CRT ALC, $\times 10^3$/μL	
<1.0	113 (15)
≥1.0	642 (85)
Median (IQR), $\times 10^3$/μL	1.48 (1.16–1.90)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; IMRT, intensity-modulated radiation therapy; CRT, concurrent chemoradiation therapy; ALC, absolute lymphocyte count.

Lymphopenia during and after CRT

Median ALC levels declined gradually at each week, from a baseline level of 1.48 $\times$ 10^3 cells/μL to 0.88 at week 1, 0.58 at week 2, 0.42 at week 3, 0.33 at week 4, and 0.28 $\times$ 10^3 cells/μL at week 5, followed by an increase to 0.86 $\times$ 10^3 cells/μL at the first follow-up visit (Fig. 1A). G0, G1, G2, G3, and G4 ALC nadir were seen in 4 (0.5%) patients, 5 (0.7%), 56 (7.4%), 395 (52.3%) and 294 (38.9%) patients. At the first follow-up, 406 patients (53.8%) had (nearly) normal ALCs (G_r0-1) (Fig. 1B). WBC and neutrophil counts were generally more stable, with only 3 patients (0.4%) experiencing G4 leukopenia and 3 patients (0.4%) G4 neutropenia during CRT.

Survival outcomes by lymphopenia during CRT

At a median follow-up time of 65.5 months (IQR, 39.1–86.6), 403 patients (53.4%) had died. The 5-year OS rate was 45.4% and median OS time was 45.6 months (95% CI 35.44–55.70). In subgroup analysis, OS was not different between patients with G3 ALC nadir during treatment and those with G0-2 during treatment (HR 1.38, 95% CI 0.91–2.09, $P = 0.132$). However, OS for G4 patients was significantly worse compared to G3 patients (HR 1.40, 95% CI 1.14–1.71, $P = 0.001$). Considering this difference and the clinical severity of G4 toxicity, we decided to compare patients with G4 nadir versus G0-3 nadir and found that G4 patients had poorer outcomes than G0-3 patients. Specifically, 5-year OS rates were 35.4% G4 vs. 51.8% G0-3 (HR 1.46, 95% CI 1.20–1.78, $P < 0.001$; Suppl. Fig. 1); 5-year PFS rates, 30.1% G4 vs. 40.7% G0-3 (HR 1.34, 95% CI 1.12–1.61, $P = 0.002$); 5-year LRFS rates, 31.9% G4 vs. 45.4% G0-3 (HR 1.36, 95% CI 1.13–1.65, $P = 0.001$); 5-year DMFS, 34.2% G4 vs. 46.3% G0-3 (HR 1.42, 95% CI 1.17–1.71, $P < 0.001$), and 5-year DSS, 42.3% G4 vs. 59.1% G0-3 (HR 1.59, 95% CI 1.28–1.98, $P < 0.001$).

After adjusting for risk factors on multivariable analysis, G4 ALC nadir during CRT (HR 1.28, 95% CI 1.04–1.58, $P = 0.021$), male patients (HR 1.60, 95% CI 1.16–2.21, $P = 0.004$), poorly differentiated tumor (HR 1.66, 95% CI 1.35–2.05, $P < 0.001$), stage III disease (HR 1.70, 95% CI 1.35–2.15, $P < 0.001$) and not receiving surgery (HR 1.80, 95% CI 1.43–2.26, $P < 0.001$) each independently predicted unfavorable OS. G4 ALC nadir during CRT was also significantly associated with LRFS (HR 1.23, 95% CI 1.00–1.50, $P = 0.047$), DMFS (HR 1.27, 95% CI 1.03–1.55, $P = 0.022$), and DSS (HR 1.39, 95% CI 1.10–1.76, $P = 0.006$), but not with PFS (HR 1.21, 95% CI 0.99–1.47, $P = 0.063$) (Suppl. Table S1).

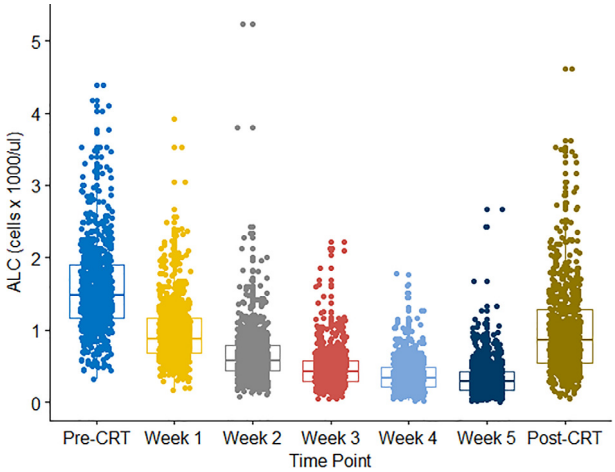

Fig. 1A. Distribution of absolute lymphocyte counts (ALC) before, during, and after concurrent chemoradiation therapy (CRT).

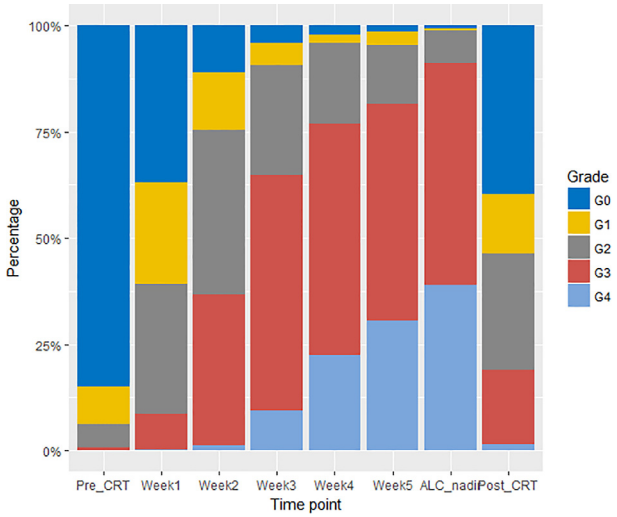

Fig.1B. Percentages of patients with grade 0 (G0), G1, G2, G3, and G4 ALC nadir before, during, and after CRT.

Survival outcomes by lymphocyte recovery after CRT

Lymphopenia in most patients had resolved by the first follow-up visit after CRT. At that time, 300 patients (39.7%) had G_r0, 106 (14.0%) G_r1, 206 (27.3%) G_r2, 131 (17.4%) G_r3, and only 12 patients (1.6%) had G_r4 lymphopenia (Fig. 1B). Among the 294 patients who had G4 ALC nadir during CRT, 102 (34.7%) had G_r0 after CRT, 33 (11.22%) G_r1, 76 (25.9%) G_r2, and 72 (24.5%) G_r3; 11 patients (3.7%) remained G_r4 lymphopenia after CRT.

Grouping patients with G_r0-1 lymphopenia ("recovered") versus G_r2-4 lymphopenia ("not recovered"), revealed no difference in OS between these two groups (5-year OS rates 46.9% vs. 43.7%, respectively; HR 0.91, 95% CI 0.75–1.11, $P = 0.355$;Fig. 2A). However, evaluating lymphocyte recovery in patients with G0-3 versus G4 ALC nadir during CRT, showed that 5-year OS rates were even worse for G4 patients who recovered to G_r0-1 after CRT as compared to G0-3 patients who did not recover (36.6% for G4 + G_r0-1 vs. 51.9% for G0-3 + G_r2-4; HR 1.40, 95% CI 1.04–1.89, $P = 0.027$). Regardless of whether lymphopenia resolved after CRT or not, the OS rates were similar; the 5-year OS rates were 36.6% for G4 + G_r0-1 vs. 34.2% for G4 + G_r2-4 (HR 0.90, 95% CI 0.67–1.20,

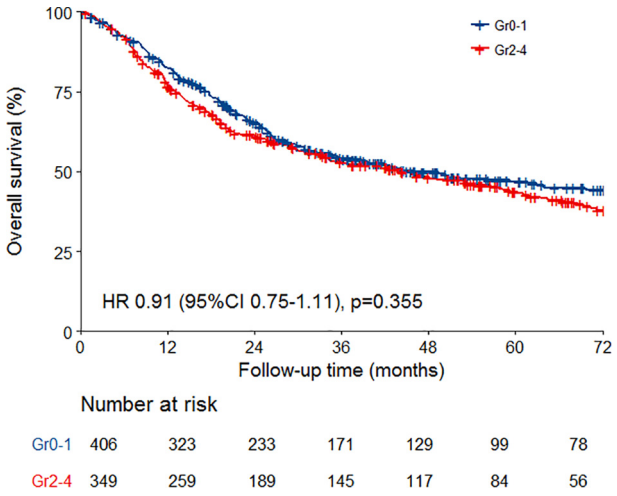

Fig. 2A. Overall survival did not differ according to whether absolute lymphocyte counts had recovered after treatment (blue line; Gr0-1) or did not recover after treatment (red line; Gr2-4). HR, hazard ratio; CI, confidence interval.

Table 2
Survival outcomes by combination of ALC nadir (during CRT) and lymphocyte recovery (after CRT).

	G0-3 + G_r0-1	G0-3 + G_r2-4	G4 + G_r0-1	G4 + G_r2-4	*P* value (G0-3 + G_r2-4 vs. G4 + G_r0-1)
Median OS time (months)	63.9	61.1	35.9	27.4	0.027
5-year OS Rate (%)	52.0	51.9	36.6	34.2	
Median PFS time (months)	26.5	22.5	15.9	10.6	0.110
5-year PFS Rate (%)	44.1	36.3	28.8	30.9	
Median LRFS time (months)	35.6	42.3	25.1	17.3	0.035
5-year LRFS Rate (%)	46.6	43.9	28.9	33.7	
Median DMFS time (months)	45.5	46.7	22.2	11.7	0.108
5-year DMFS Rate (%)	48.6	43.5	37.4	31.6	
Median DSS time (months)	–	119.2	44.2	33.5	0.006
5-year DSS Rate (%)	57.9	60.9	42.0	42.3	

P = 0.461), and 51.9% for G0-3 + G_r0-1 vs. 52.0% for G0-3 + G_r2-4 (HR 0.99, 95% CI 0.76–1.30, *P* = 0.946) (Table 2, Fig. 2B). These findings also held true for 5-year LRFS (28.9% for G4 + G_r0-1 vs. 43.9% for G0-3 + G_r2-4; HR 1.35, 95% CI 1.02–1.80, *P* = 0.035) (Table 2, Fig. 2C) and DSS (42.0% for G4 + G_r0-1 vs. 60.9% for G0-3 + G_r2-4; HR 1.60, 95% CI 1.14–2.24, *P* = 0.006) (Table 2, Fig. 2D), and revealed a trend for 5-year PFS (28.8% for G4 + G_r0-1 vs. 36.3% for G0-3 + G_r2-4; HR 1.25, 95% CI 0.95–1.64, *P* = 0.110) (Table 2, Fig. 2E) or DMFS (37.4% for G4 + G_r0-1 vs. 43.5% for G0-3 + G_r2-4; HR 1.27, 95% CI 0.95–1.69, *P* = 0.108) (Table 2,Fig. 2F).

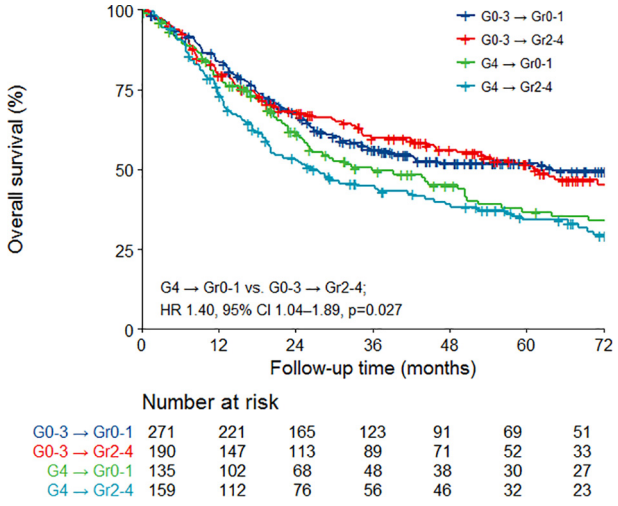

Fig. 2B. Overall survival curves for patients with G0-3 lymphopenia during treatment and lymphocyte recovery (Gr0-1) after treatment (dark blue); patients with G0-3 lymphopenia during treatment and no lymphocyte recovery (Gr2-4) after treatment (red); patients with G4 lymphopenia during treatment with recovery (Gr0-1) after treatment (green); and patients with G4 lymphopenia during treatment without recovery (Gr2-4) after treatment (blue).

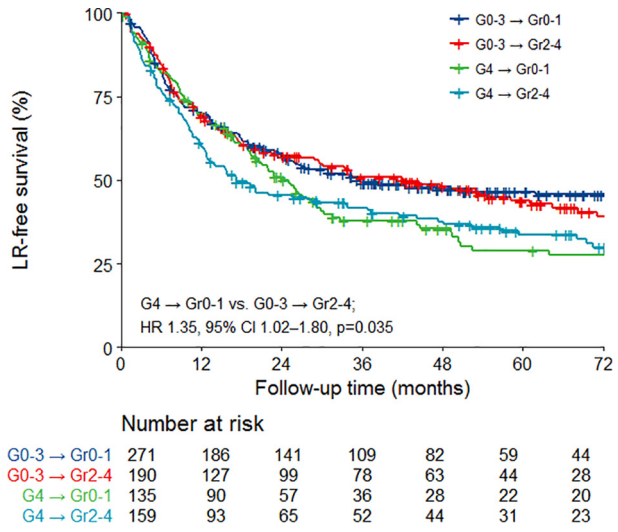

Fig. 2C. Progression-free survival (PFS) curves for the same groups of patients.

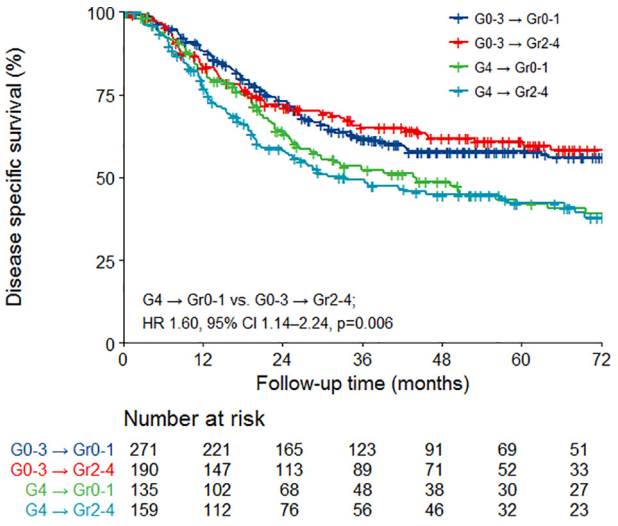

Fig. 2D. Local recurrence (LR)-free survival curves for the same groups of patients.

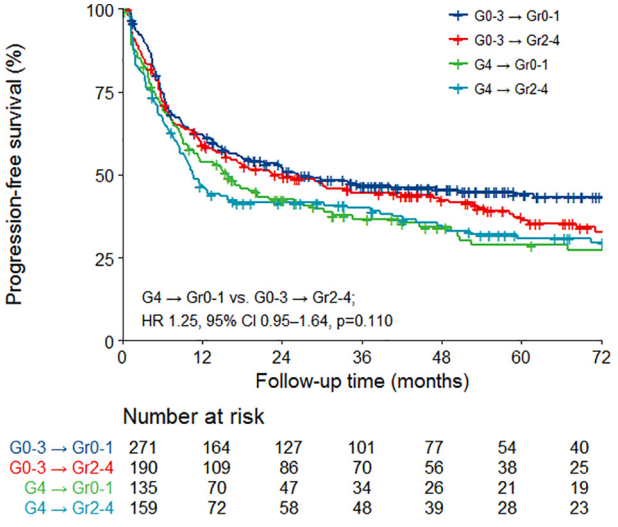

Fig. 2E. Distant metastasis (DM)-free survival curves for the same groups of patients.

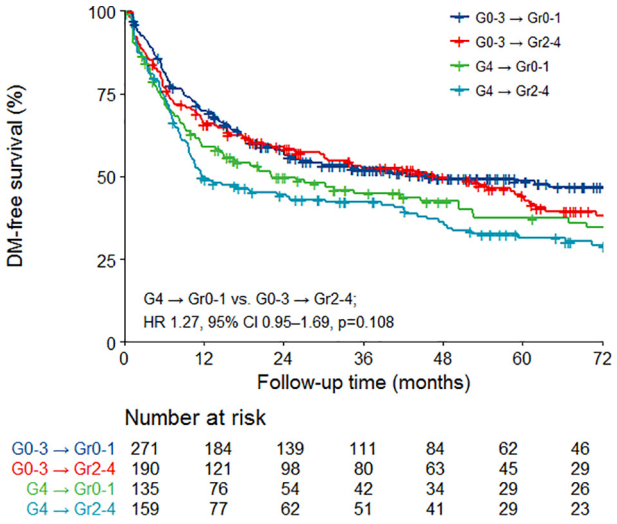

Fig. 2F. Disease-specific survival curves for the same group of patients.

Predictors of lymphocyte recovery

Among the 457 patients who had G0-3 ALC nadir during CRT, 271 (58.8%) recovered to G_r0-1 after CRT, as compared with 135 of 294 patients (45.9%) who had G4 ALC nadir during CRT. Notably, the G0-3-during-CRT group had more patients who had G0-1 ALC level at baseline (447 of 457, 97.8%) than did the G4-during-CRT group (261 of 294, 88.8%) ($P < 0.001$; Suppl. Fig. 2A and B). We further examined the ALC ratio (post-CRT ALC divided by pre-CRT ALC) to evaluate the relative ability of these two groups to recover from lymphopenia after CRT. For the G0-3-during-CRT group, the mean ALC ratio was 0.66 (SD, 0.39); for the G4-during-CRT group, it was 0.65 (SD, 0.41) ($P = 0.743$), which suggested that the ability to recover from lymphopenia after CRT was not associated with the ALC nadir during CRT.

Finally, we tested whether clinical factors may have influenced lymphocyte recovery (G_r0-1 vs. G_r2-4) in patients who had G4 ALC nadir during CRT, and both univariable and multivariable analyses identified baseline ALC as the only factor related to lymphocyte recovery (odds ratio 3.77, 95% CI 2.00–7.14, $P < 0.001$; Table 3). We further assessed whether radiation modality affected the extent of change in ALC during the course of CRT. Lymphocyte counts were not different before CRT for patients treated with IMRT versus proton therapy. However, patients who had proton therapy had significantly higher ALC during CRT (Suppl. Fig. 3A), which was consistent with better OS (Suppl. Fig. 3B). Lymphocyte counts after CRT were also similar in the IMRT and proton groups (Suppl. Fig. 3A).

Discussion

In this study, we demonstrated that patients with esophageal cancer experienced significant lymphopenia during the course of CRT, that G4 lymphopenia during CRT was an independent predictor of survival outcomes, and that recovery to (near) normal levels after CRT could not mitigate this poor survival. Moreover, recovery from lymphopenia was associated only with baseline lymphocyte level and not with other factors, and the ability to recover was no different for patients who had G4 vs. G0-3 lymphopenia during CRT.

Lymphopenia, as a manifestation of immunosuppression, has been shown by several groups to predict poor clinical outcomes. In one study, patients with pancreatic adenocarcinoma and total lymphocyte counts of <500 cells/μL at 2 months after initiating CRT had significantly inferior median survival time (8.7 months vs. 13.3 months for those with ≥500 cells/μL at that time) [12]. Another group combined independent data from patients with malignant glioma, pancreatic cancer, and non-small-cell-lung-cancer and found that severe and persistent treatment-related lymphopenia was independently associated with shorter survival regardless of pathologic findings or chemotherapy regimen [13]. Other studies have also shown that patients with low ALC during CRT were less likely to achieve a pathologic or clinical complete response [11,16]. Notably, in esophageal cancer patients, G4 lymphopenia during CRT was reported independently predicting poor OS and disease-specific outcomes. [14].

The prevalence of RIL is reflected by the capability of radiation doses as low as 2 Gy to inactivate some 50% of circulating lymphocytes within the radiation field [17,18]. In a model of malignant glioma, 60 Gy, delivered at 2 Gy per fraction to the brain, resulted a mean dose to circulating lymphocytes of 2 Gy, and nearly all of the circulating blood received at least 0.5 Gy [6]. Esophageal tumors, especially distal and gastroesophageal junction tumors, are near the heart, and so presumably even minimal scattered radiation could be delivered quickly to a large, fast-flowing blood pool

Table 3
Univariable and multivariable analysis of factors associated with lymphocyte recovery (G_r0-1 vs. G_r2-4) among patients with G4 lymphopenia during CRT.

Characteristics	Univariable analysis			Multivariable analysis		
	Odds ratio	95% CI	*P* value	Odds ratio	95% CI	*P* value
Pre-CRT ALC (<1.0 vs. ≥1.0 × 10^3/μL)	4.00	2.13–7.51	<0.001	3.77	2.00–7.14	<0.001
Sex (male vs. female)	0.96	0.49–1.87	0.900	–	–	–
Age (>65 vs. ≤65)	0.91	0.57–1.43	0.674	–	–	–
ECOG (1–2 vs. 0)	0.88	0.53–1.46	0.617	–	–	–
Tumor length (>5 cm vs. ≤5 cm)	1.05	0.66–1.67	0.831	–	–	–
Tumor location	–	–	0.859	–	–	–
Upper vs. lower	1.44	0.34–6.16	0.621	–	–	–
Middle vs. lower	1.13	0.48–2.66	0.788	–	–	–
Histology (SCC and others vs. adenocarcinoma)	1.79	0.91–3.49	0.091	1.44	0.71–2.92	0.311
Grade (poor vs. well to moderate)	0.81	0.51–1.29	0.372	–	–	–
Stage (III vs. I–II)	1.20	0.74–1.95	0.467	–	–	–
Induction chemo (no vs. yes)	1.23	0.76–1.97	0.399	–	–	–
Surgery (no vs. yes)	1.13	0.71–1.79	0.612	–	–	–
RT modality (IMRT vs. proton)	0.53	0.28–1.00	0.050	0.57	0.29–1.09	0.089
Concurrent chemotherapy regimen	–	–	0.707	–	–	–
PF vs. TF	1.15	0.65–2.03	0.624	–	–	–
TP vs. TF	0.62	0.24–1.63	0.334	–	–	–
Others vs. TF	1.06	0.52–2.15	0.874	–	–	–

Abbreviations: CRT, concurrent chemoradiation therapy; CI, confidence interval.

and could easily result in severe lymphopenia. The spleen V5 (that is, the percentage volume of spleen receiving ≥5 Gy) and mean spleen dose have been correlated with ALC nadir during radiotherapy for hepatocellular carcinoma or pancreatic cancer [19,20]. Because the spleen is an important immune organ to which many lymphocytes migrate, irradiation of tumors in the lower esophagus could well affect lymphocytes in the spleen.

However, high-grade lymphopenia was observed to be recovered to some extent after CRT. Several potential reasons exist to explain the lack of lymphocyte recovery, especially T lymphocytes, in some patients. Radiation-induced changes in T lymphocytes can affect both their function and subtype. In one study of an animal model, irradiation led to a prompt reduction in blood $CD19^+$ B lymphocytes over the first few days but the counts increased quickly thereafter, whereas losses of $CD3^+$ T lymphocytes and $CD8^+$ T lymphocytes were more severe than reductions in $CD4^+$ T lymphocytes [21]. A study of patients with cervical cancer found that the extent of radiation-induced apoptosis of $CD8^+$ lymphocytes in the peripheral blood was an independent predictor of poor DSS outcomes [22]. Since the cytotoxic $CD8^+$ T lymphocytes act as the effector cells that directly kill aberrant cells and secrete proinflammatory cytokines [23], radiation-induced reductions in CD8+ T lymphocytes could have negative effects on cell-mediated immunity, even after recovery from CRT, because newly generated naïve T lymphocytes are not capable of antitumor effects. Regulatory T cells (Tregs), another T cell subtype, are known to participate in immune suppression [24]. Because Tregs are relatively resistant to radiation, one might assume that surviving Tregs could inhibit the induction of effector T cells during recovery from RIL [25]. Another animal study showed that whole-body irradiation to a dose of 2 Gy led to significant increases in ratios of $CD4^+$ $CD25^+$ Tregs and $CD4^+$-$CD25^+Fox3^+$ Tregs to $CD4^+$ T cells in peripheral blood [26]. Clinical findings also suggest that a high ratio of $CD8^+$ T cells/Tregs predicted better treatment response [27].

Lymphocyte diversity as well as absolute numbers are two indispensable dimensions for an efficient immune response. In one study of patients with metastatic breast cancer, low levels of T-cell receptor (TCR) diversity was associated with shorter OS times (9.7 months versus 21.7 months for those with higher diversity levels). Moreover, the combination of lymphopenia and low levels of TCR diversity was associated with a 2.52-fold increase in HR for death [28]. TCR repertoire diversity has also been identified as prognostic factor for patients with advanced colorectal cancer and gastric cancer [29,30]. The reconstitution of lymphocyte diversity can occur by either the production of progenitors by thymopoiesis or the peripheral expansion of mature lymphocytes via antigenic stimulation [31]. The thymic-dependent pathway is typically impaired in older patients, resulting in a lack of T-cell progenitors and important cytokines such as interleukin-7. The homeostatic peripheral expansion pathway can skew toward oligoclonality, with persistent and impaired repertoire diversity [32]. Attempts to maintain T cell development and diversity have included reinfusion of diverse mature T lymphocytes and immunotherapy with interleukin-7; preliminary results in patients with various types of cancer have shown increased numbers of $CD8^+$ and $CD4^+$ T lymphocytes [33–35].

Our study did have some limitations. First, the ALC data were obtained from clinical samples that had been tested for complete blood counts; we did not use flow cytometry to identify lymphocyte subtypes of changes therein over the course of treatment. However, our findings were fully examined through appropriate statistical analysis, correlated well with others' findings, and can be easily and simply applied in clinical settings. Second, because this was a retrospective review, we could not account for all of the factors that could affect lymphocyte counts during CRT. However, we believe that the possibility of bias from such factors was limited because we excluded patients with hematologic disease or those who discontinued therapy because of infection. Also, it is known that lymphocyte count was strongly influenced by different radiation modalities (IMRT vs. protons) [14,15], which could be a potential confounder, so that we did not include radiation modality into multivariable analysis of survival outcomes (Suppl. Table S1).

Despite these limitations, our study did demonstrate that severe RIL during CRT predicted poor survival for patients with esophageal cancer, and that lymphocyte recovery did not mitigate the poor survival outcomes. Although the extent of lymphocyte depletion during CRT seems to have been compensated for in some patients, one aspect of anti-cancer immunity was impaired and could not be recovered. Our findings, and this speculation, need confirmation in prospective trials. Means of avoiding severe lymphopenia during CRT could be a potential therapy in future applications.

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Conflict of interest statement

The authors have no conflicts of interest relevant to this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2018.12.002.

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