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ORIGINAL ARTICLE – THORACIC ONCOLOGY

Treatment-Related Lymphopenia Predicts Pathologic Complete Response and Recurrence in Esophageal Squamous Cell Carcinoma Undergoing Neoadjuvant Chemoradiotherapy

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ABSTRACT

Purpose. To investigate the relationship between treatment-related lymphopenia and pathologic response to neoadjuvant chemoradiotherapy (CRT) in patients with esophageal squamous cell carcinoma (ESCC).

Methods. Between 2002 and 2016, 220 ESCC patients treated with neoadjuvant CRT followed by surgery were retrospectively analyzed. Absolute lymphocyte count was determined before, during, and 1 month after neoadjuvant CRT. Treatment-related lymphopenia was graded using Common Terminology Criteria for Adverse Events version 4.0. Relationship between lymphopenia with pathologic complete response (pCR) and recurrence were evaluated.

Results. Ninety-five patients (43.2%) achieved a pCR after neoadjuvant CRT and 71 patients (32.3%) developed recurrences. The incidence of grade 0, 1, 2, 3, and 4 lymphopenia during CRT were 1.8%, 6.8%, 31.4%, 38.2% and 21.8%, respectively. Patients with grade 4 lymphopenia had a significantly lower pCR rate than those with grade 0-3 lymphopenia (22.9% vs. 48.8%, $P = 0.001$). Moreover, grade 4 lymphopenia was significantly associated with a higher risk of recurrences (45.8% vs. 28.5%, $P = 0.023$). Multivariable analysis identified that primary tumor length, tumor location, and radiation dose were independent predictors for grade 4 lymphopenia.

Conclusions. ESCC patients with grade 4 lymphopenia during neoadjuvant CRT were associated with a significantly lower pCR rate and a higher recurrence risk.

Neoadjuvant chemoradiotherapy (CRT) followed by esophagectomy has been the standard of treatment for locally advanced esophageal cancer (EC) in recent years.[1–3] Approximately 20–40% of EC could achieve pathologic complete response (pCR) after neoadjuvant CRT, which was associated with significantly favorable overall survival (OS) and a lower recurrence risk.[3–5] Of them, esophageal squamous cell carcinoma (ESCC) is more chemoradiosensitive than adenocarcinoma, which has been well documented by previous reports.[1,6] Although interest is growing in predicting pCR to neoadjuvant treatment using various clinical tools in recent years, there is still no accurate method to predict pathologic response preoperatively in EC.[7–10]

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As an essential component of host immunity, lymphocytes play a critical role in the prevention of tumor development and progression. Because lymphocytes are extremely sensitive to ionizing radiation, radiotherapy can destroy mature circulating lymphocytes directly and induce immunosuppression independently of the administration of systemic therapies.[11,12] Therefore, treatment-related lymphopenia is a rather common phenomenon in cancer patients undergoing CRT or radiotherapy alone. Previous studies have demonstrated that lymphopenia correlated with worse prognosis in several types of solid tumors, including pancreatic, lung, head and neck, and rectal cancer.[13–17] Similarly, in a recent large series of 504 EC patients (15% SCC and 85% adenocarcinoma) reported by Davuluri et al.[18] severe lymphopenia during CRT was strongly associated with poor OS and disease-specific survival. Moreover, this research group further found that a higher level of circulating absolute lymphocyte count (ALC) during neoadjuvant CRT was associated with a higher rate of pCR in esophageal adenocarcinoma.[19] Given the differences in radiobiological characteristics between esophageal adenocarcinoma and SCC, whether lymphopenia is predictive of pathologic response to neoadjuvant CRT in ESCC is unknown.

The primary objective of this study was to explore the correlation between treatment-related lymphopenia and pathologic response to neoadjuvant CRT in ESCC. Considering the high rate of locoregional recurrence and distant metastasis in this patient population, we also investigated whether lymphopenia during CRT is associated with an increased risk of disease recurrence.

PATIENTS AND METHODS

Patients

We retrospectively analyzed all ESCC patients who received neoadjuvant CRT and surgery from our prospectively maintained database between April 2002 and May 2016. Inclusion criteria included: pathologic confirmation of stage II/III ESCC according to the 7th TNM staging system of the American Joint Committee on Cancer, receipt of neoadjuvant CRT followed by esophagectomy with curative intent, completed radiotherapy, and documentation of at least three weekly laboratory screens during CRT.[20] Patients with prior or concomitant malignancy, those with macroscopically incomplete resection, and those who died before hospital discharge were excluded. This study was approved by the Institutional Review Boards of Sun Yat-sen University Cancer Center and informed consent was waived due to its retrospective nature.

As previously described, pretreatment assessment consisted of physical examination, standard laboratory tests, esophagogastroduodenoscopy (EGD) with endoscopic ultrasound and biopsies, chest/abdominal computed tomography (CT), and/or positron emission tomography (PET).[21] Before initiation of treatment, all patients were evaluated by a multidisciplinary team according to institutional practice guidelines.

Treatment Approaches

All patients received concurrent platinum-based chemotherapy during external-beam radiotherapy using three-dimensional conformal radiotherapy (3DCRT) or intensity-modulated radiotherapy (IMRT). Typical prescribed dose was 40.0–45.0 Gy in 20–25 fractions, 5 days per week. Patients underwent preoperative restaging approximately 4–6 weeks after the completion of neoadjuvant CRT. Surgical methods consisted of Ivor-Lewis esophagectomy, transhiatal esophagectomy, and three-field technique. Pathologic stage was evaluated by at least two experienced pathologists. The pCR was defined as no viable cancer cells in all layers of the esophagus and in the lymph nodes resected.[22]

Lymphocyte Count Examination

The ALC values were collected before, during (weekly), and 1 month after neoadjuvant CRT. Treatment-related lymphopenia was graded using Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. For patients with missing ALC at 1 month after CRT, the ALC values before surgery were collected instead.

Follow-Up and Recurrences

All patients underwent follow-up examinations every 3 months for the first year, then every 6 months for the next 2 years, and then annually until 5 years. The recurrence pattern was recorded according to the type of first recurrence, which was established on histologic, cytologic, or explicit radiologic proof. Locoregional recurrences were defined as recurrences within esophagus or regional lymph nodes, whereas distant recurrences were defined as nonregional lymph node metastases, distant organ metastases, or peritoneal carcinomatosis.

Statistical Analysis

The hematologic variables were grouped by the median value as a cutoff. Follow-up and survival times were defined from the date of surgery until event or censor.

Kaplan–Meier method was used to estimate OS, recurrence-free survival (RFS), locoregional failure-free survival (LRFFS), and distant metastasis-free survival (DMFS). Log-rank test was used to examine intergroup differences, and Cox proportional hazards regression model was used to analyze prognostic factors for tumor recurrence. Univariate and multivariable logistic regression models were performed to analyze possible predictors of pCR and severe treatment-related lymphopenia. Covariates with $P < 0.1$ in the univariate analysis were entered into the multivariable analysis (backward stepwise). Statistical analyses were performed using SPSS 22.0 software (SPSS Inc., Chicago, IL). $P < 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

Patient and treatment characteristics of 220 ESCC patients who met study criteria are shown in Table 1. Median age of the whole cohort was 56 years (range, 42–73 years), and the majority of the tumors were located in the upper/middle esophagus (77.3%). At baseline staging, 21.8% were stage II, and 78.2% were stage III. The majority of patients (66.8%) were treated with 3DCRT, and the rest with IMRT, with the median radiation dose of 40.0 Gy (range, 40.0–50.4 Gy). The most commonly used chemotherapy regimen was cisplatin/vinorelbine (57.3%) followed by cisplatin/taxane (29.5%) and cisplatin/fluorouracil (13.2%). Patients were treated with esophagectomy within a median interval of 6.2 weeks (range, 3.8–13.5 weeks) after neoadjuvant CRT.

Pathologic Response and Recurrences

After histopathological examination, 95 patients (43.2%) achieved a pCR. Median follow-up period was 24.8 months (range, 0.4–183.0 months) for the entire cohort and 43.5 months for survivors. At this analysis, 65 patients (29.5%) had died and 71 (32.3%) developed recurrences. Twenty-one patients (9.5%) had locoregional failure only, 35 (15.9%) had distant failure only, and 15 (6.8%) experienced concomitant locoregional and distant recurrences. The 3-year OS rate and 3-year RFS rate for the whole cohort were 70.1% and 67.3%, respectively.

Lymphopenia During Neoadjuvant CRT

The median baseline ALC for the entire cohort was 1.80×10^9/L and only 13 patients (5.9%) had ALC $< 1.0 \times 10^9$/L before CRT. As shown in Fig. 1, the ALC

TABLE 1 Patient characteristics

Characteristic	Total (n = 220) (%)
Age (year)	
< 56	103 (46.8)
≥ 56	117 (53.2)
Sex	
Male	187 (85.0)
Female	33 (15.0)
Smoking history	
Yes	145 (65.9)
No	75 (34.1)
Alcohol history	
Yes	76 (34.5)
No	144 (65.5)
ECOG performance status	
0	128 (58.2)
1–2	92 (41.8)
Weight loss	
< 10%	197 (89.5)
≥ 10%	23 (10.5)
Histologic grade	
G1-2	161 (73.2)
G3	59 (26.8)
Tumor location	
Upper/middle	170 (77.3)
Distal	50 (22.7)
Primary tumor length (cm)	
≤ 5	101 (45.9)
> 5	119 (54.1)
Clinical T stage	
T1-2	40 (18.2)
T3-4	180 (81.8)
Clinical N stage	
N0	16 (7.3)
N1-3	204 (92.7)
Clinical TNM stage	
II	48 (21.8)
III	172 (78.2)
Chemotherapy regimen	
Cisplatin/vinorelbine	126 (57.3)
Cisplatin/fluorouracil	29 (13.2)
Cisplatin/taxane	65 (29.5)
Total radiation dose (Gy)	
Median (range)	40.0 (40.0–50.4)
Dose per fraction (Gy)	
≤ 2.0	164 (74.5)
> 2.0	56 (25.5)
Radiotherapy modality	
3DCRT	147 (66.8)
IMRT	73 (33.2)

TABLE 1 continued

Characteristic	Total (n = 220) (%)
Interval to surgery (week)	
< 6.2	97 (44.1%)
≥ 6.2	123 (55.9%)
Pretreatment hemoglobin (g/L)	
Median (IQR)	138 (129–146)
Pretreatment platelets ($\times 10^9$/L)	
Median (IQR)	225 (190–266)
Pretreatment ALC ($\times 10^9$/L)	
Median (IQR)	1.8 (1.4–2.3)

ECOG Eastern Cooperative Oncology Group, *3DCRT* three-dimensional conformal radiation therapy, *IMRT* intensity-modulated radiation therapy, *IQR* interquartile range, *ALC* absolute lymphocyte count

values declined steeply during early CRT and generally reached a nadir at approximately week 3. The median ALC declined to 1.0, 0.70, 0.55, 0.46, and 0.50 $\times$ 10^9/L from weeks 1 to 5, and then it recovered to 1.1 $\times$ 10^9/L at 1 month after CRT. The cumulative incidence of grade 0, 1, 2, 3, and 4 lymphopenia during CRT were 1.8%, 6.8%, 31.4%, 38.2% and 21.8%, respectively.

Factors Associated with pCR

We identified patient and treatment characteristics associated with pCR (Table 2). Comparing patients with and without a pCR, there were no significant differences in age, sex, smoking history, alcohol history, performance status, weight loss, tumor characteristics, radiation dose, or radiation modality between the two groups. For patients with grade 0–2, 3, and 4 lymphopenia during CRT, the pCR rates were 51.1%, 46.4% and 22.9%, respectively (P = 0.005), suggesting that patients with grade 4 lymphopenia had a significantly lower pCR rate (grade 0–2 vs. grade 3, P = 0.537; grade 0–2 vs. grade 4, P = 0.001; and grade 3 vs. grade 4, P = 0.007). Considering the comparable pCR rates between grade 0–2 and grade 3, we therefore defined grade 4 lymphopenia as clinically significant lymphopenia. Multivariable analysis revealed that grade 4 lymphopenia was the only adverse independent factor associated with pCR (odds ratio 0.319, P = 0.003). Although the chemotherapy regimen of cisplatin/vinorelbine was more likely to achieve pCR than the other two regimens on univariate analysis (P = 0.036), this difference was not statistically significant on multivariable analysis.

Lymphopenia is Associated with Recurrences

Patients with grade 4 lymphopenia demonstrated a significantly higher recurrence rate than those without (45.8% vs. 28.5%, P = 0.023). In terms of survival endpoints, the grade 4 lymphopenia group showed significantly worse OS, RFS, LRFFS, and DMFS than did the grade 0-3 lymphopenia group (P < 0.05 for all; Fig. 2). On multivariable analysis, age, sex, clinical TNM stage, chemotherapy regimen, pathologic response, and grade 4 lymphopenia were independent prognostic factors for developing recurrences (Table 3).

Factors Associated with Grade 4 Lymphopenia

We further identified clinical characteristics correlated with the development of grade 4 lymphopenia during CRT (Supplementary Table 1). Comparing patients with and without grade 4 lymphopenia, no statistical differences in sex, smoking history, performance status, weight loss, histologic grade, chemotherapy regimen, pretreatment hemoglobin, or pretreatment platelets between groups were observed. Multivariable analysis demonstrated that patients

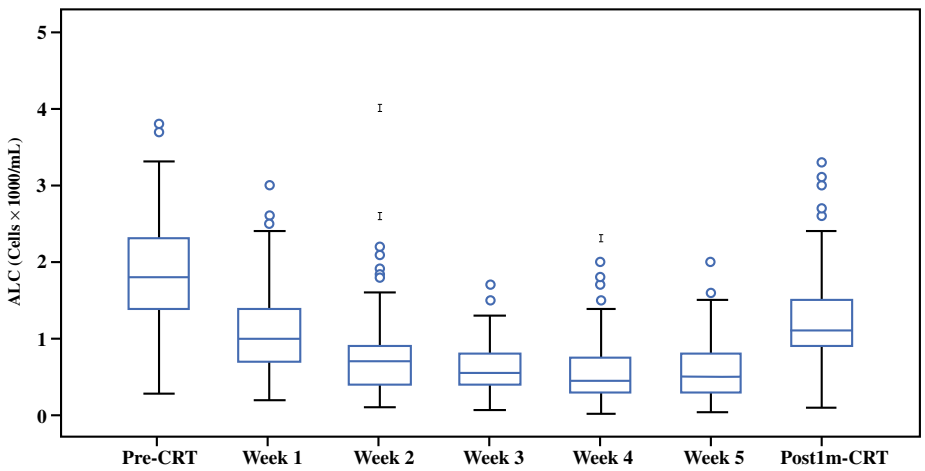

FIG. 1 Distribution of absolute lymphocyte count over time

TABLE 2 Univariate and multivariable analyses for variables associated with pathologic complete response

Variable	Univariate		Multivariable	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Age (< 56 vs. ≥ 56 yr)	0.965 (0.565–1.647)	0.896		
Sex (female vs. male)	1.714 (0.814–3.609)	0.156		
Smoking history (yes vs. no)	0.807 (0.460–1.414)	0.453		
Alcohol history (yes vs. no)	0.670 (0.379–1.185)	0.169		
ECOG performance status (0 vs. 1–2)	0.841 (0.490–1.444)	0.531		
Weight loss (< 10% vs. ≥ 10%)	0.811 (0.341–1.927)	0.635		
Histologic grade (G1-2 vs. G3)	0.952 (0.522–1.737)	0.872		
Tumor location (upper/middle vs. distal)	1.319 (0.691–2.517)	0.401		
Primary tumor length (≤ 5 vs. > 5 cm)	1.029 (0.603–1.758)	0.916		
Clinical TNM stage (II vs. III)	1.584 (0.833–3.012)	0.161		
Chemotherapy regimen[a]				
2 versus 1	0.350 (0.140–0.878)	0.025		
3 versus 1	0.420 (0.157–1.123)	0.084		
Total radiation dose (40 vs. > 40 Gy)	0.885 (0.493–1.590)	0.683		
Dose per fraction (≤ 2.0 vs. > 2.0 Gy)	1.093 (0.051–23.401)	0.955		
Radiotherapy modality (3DCRT vs. IMRT)	1.045 (0.592–1.842)	0.880		
Interval to surgery (< 6.2 vs. ≥ 6.2 weeks)	0.549 (0.318–0.948)	0.031		
Pretreatment hemoglobin (< 138 vs. ≥ 138 g/L)	1.017 (0.596–1.735)	0.951		
Pretreatment platelets (< 225 vs. ≥ 225 × 10^9/L)	1.750 (1.021–3.000)	0.042		
Pretreatment ALC (< 1.8 vs. ≥ 1.8 × 10^9/L)	1.231 (0.721–2.100)	0.447		
Post1m-ALC (< 1.1 vs. ≥ 1.1 × 10^9/L)	0.988 (0.576–1.696)	0.965		
Grade 4 lymphopenia (yes vs. no)	0.311 (0.149–0.651)	0.002	0.319 (0.151–0.674)	0.003

CI confidence interval, *ECOG* Eastern Cooperative Oncology Group, *3DCRT* three-dimensional conformal radiation therapy, *IMRT* intensity-modulated radiation therapy, *ALC* absolute lymphocyte count

[a]Chemotherapy regimen: 1, cisplatin/vinorelbine; 2, cisplatin/fluorouracil; 3, cisplatin/taxane

with distal esophageal tumor, those with tumor length > 5 cm, and those treated with higher radiation dose (> 40 Gy) were more likely to develop grade 4 lymphopenia during treatment.

DISCUSSION

There is a paucity of published reports on the predictive value of treatment-related lymphopenia for pathologic response and recurrences in ESCC treated with neoadjuvant CRT. Based on a large cohort of patients, we have demonstrated that grade 4 lymphopenia during neoadjuvant CRT was strongly associated with a lower pCR rate and a higher recurrence risk. Therefore, treatment-related lymphopenia is an effective predictor for pathologic response and disease recurrences in ESCC. Individualized treatment decisions following CRT and postoperative surveillance strategies could be adjusted according to the development of severe lymphopenia.

To identify which patients may safely avoid or defer subsequent esophagectomy, several statistical models have been developed to predict pathologic response to neoadjuvant CRT by integrating various predictive factors, including clinical parameters, imaging tools, and molecular biomarkers.[7,9] Incorporating novel predictive factors will further improve the predictive ability of statistical models. Fang et al. reported that esophageal adenocarcinoma patients with high ALC nadir (≥ 0.35 × 10^9/L) had a higher pCR rate than those with low ALC nadir (< 0.35 × 10^9/L) during CRT (36.8% vs. 24.3%, $P = 0.023$).[19] For the better comparison across studies, we used the standard definition of ALC level according to CTCAE in the current study. Likewise, we found that ESCC patients with grade 4 lymphopenia had a notably lower pCR rate, suggesting that lymphopenia could be used as a marker along with other potential factors to form a predictive model.

There has been a general consensus that EC patients with pCR after neoadjuvant CRT have a significantly lower locoregional and distant recurrence rate than non-pCR

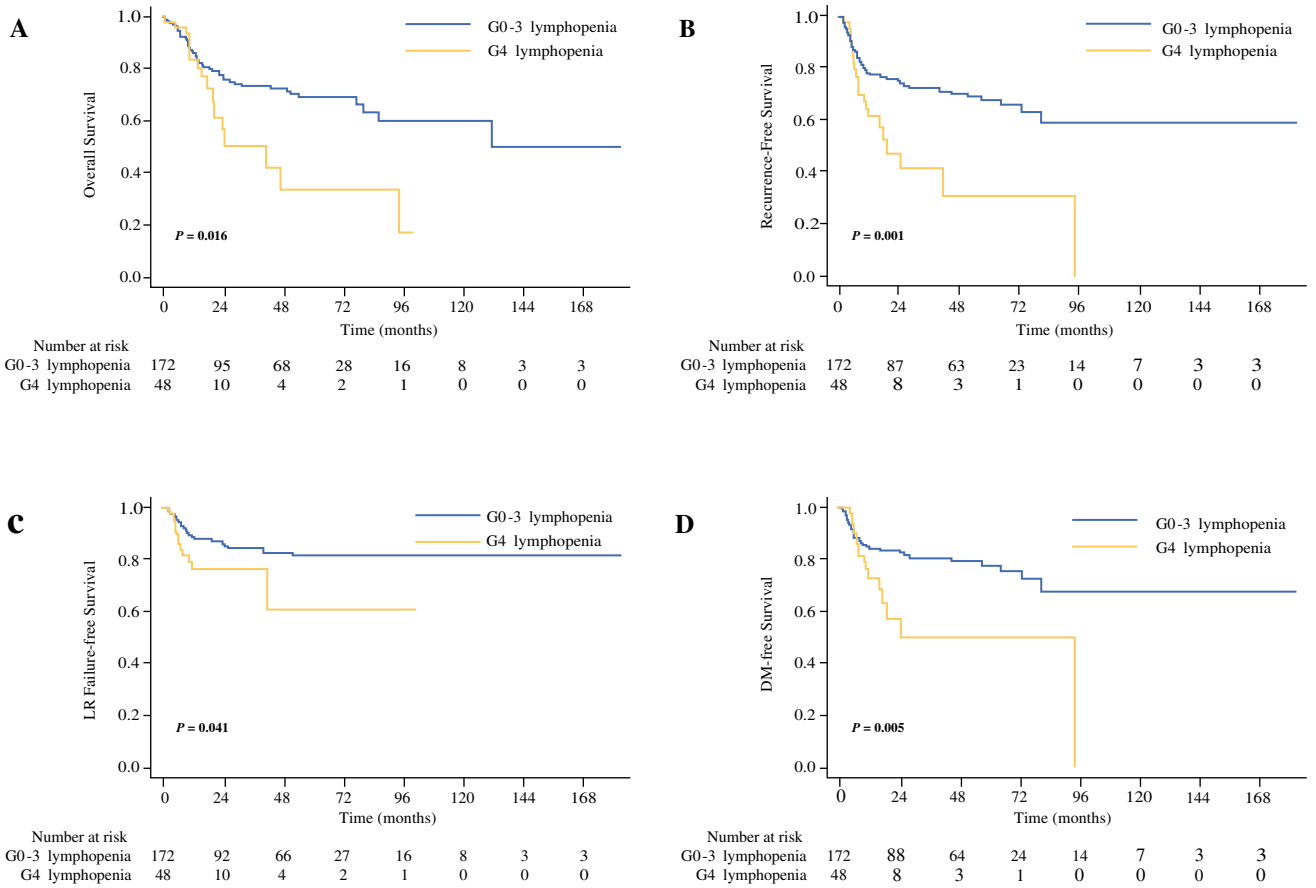

FIG. 2 Comparison of overall survival (**a**), recurrence-free survival (**b**), locoregional failure-free survival (**c**), and distant metastasis-free survival (**d**) between patients with and without grade 4 lymphopenia

patients.[4–7] Aside from pathologic response, our study demonstrated that grade 4 lymphopenia also was an independent prognostic factor for recurrences in ESCC. Therefore, recurrence risk stratification could be proposed based on pathologic response and lymphopenia for this patient population. For non-pCR patients with grade 4 lymphopenia during CRT, more efforts should be made to reduce the high risk of recurrences. In a retrospective study by Sun et al., the addition of consolidation chemotherapy demonstrated improved progression-free survival in ESCC after trimodality therapy, especially for males and non-pCR patients.[23] Similarly, Burt et al. found that adjuvant chemotherapy after neoadjuvant CRT was associated with an approximately 30% lower risk of death among EC patients with residual nodal disease.[24] Prospective studies are needed to confirm the role of adjuvant systemic therapy among high-risk ESCC patients.

Because treatment-related lymphopenia appears to be a negative predictor for pathologic response and recurrences, it is important to identify potential factors related to the development of lymphopenia. Yovino et al.[25] reported that the most important factors influencing circulating cell exposure were irradiated tumor volumes and the number of radiation fractions. Likewise, Tang et al.[14] demonstrated that larger gross tumor volumes were significantly associated with lymphocyte nadir regardless of concurrent chemotherapy receipt. Similar to these results, we found that patients with tumor length > 5 cm and those who received a higher radiation dose had a notably higher risk of severe lymphopenia during treatment. Consistent with the results reported by Davuluri et al.[18] we also found that distal esophageal tumor predicted for a high risk of grade 4 lymphopenia compared with upper/middle esophageal tumor. We speculate that this is mainly due to the relatively close distance between distal esophagus and heart, which is a large pool of lymphocytes in the body. Given the high radiosensitivity of circulating lymphocytes, the irradiating of heart would lead to increased risk of lymphopenia. On the other hand, radiation modality also may affect lymphopenia risk. Shiraishi et al.[26] compared the relative risk of lymphopenia between IMRT and proton beam therapy (PBT) in EC patients undergoing neoadjuvant CRT and found that a greater portion of IMRT patients developed grade 4 lymphopenia than PBT patients (40.4% vs. 17.6%,

TABLE 3 Univariate and multivariable analyses for recurrences

Variable	Univariate		Multivariable	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age (< 56 vs. ≥ 56 yr)	1.819 (1.130–2.928)	0.014	2.201 (1.354–3.576)	0.001
Sex (female vs. male)	0.293 (0.107–0.805)	0.017	0.346 (0.125–0.957)	0.041
Smoking history (yes vs. no)	1.034 (0.628–1.703)	0.895		
Alcohol history (yes vs. no)	1.398 (0.870–2.245)	0.166		
ECOG performance status (0 vs. 1–2)	1.097 (0.686–1.753)	0.699		
Weight loss (< 10% vs. ≥ 10%)	0.871 (0.399–1.901)	0.728		
Histologic grade (G1-2 vs. G3)	1.438 (0.813–2.543)	0.212		
Tumor location (upper/middle vs. distal)	0.958 (0.549–1.673)	0.880		
Primary tumor length (≤ 5 vs. > 5 cm)	0.626 (0.387–1.013)	0.056		
Clinical TNM stage (II vs. III)	0.467 (0.232–0.940)	0.033	0.476 (0.234–0.969)	0.041
Chemotherapy regimen[a]				
1 versus 2	0.414 (0.226–0.759)	0.004	0.420 (0.227–0.779)	0.006
1 versus 3	0.662 (0.333–1.317)	0.240		
Total radiation dose (40 vs. > 40 Gy)	1.148 (0.617–2.135)	0.664		
Dose per fraction (≤ 2.0 vs. > 2.0 Gy)	1.032 (0.042–25.378)	0.985		
Radiotherapy modality (3DCRT vs. IMRT)	1.268 (0.692–2.322)	0.443		
Interval to surgery (< 6.2 vs. ≥ 6.2 weeks)	1.542 (0.958–2.4481)	0.075		
Pretreatment hemoglobin (< 138vs. ≥ 138 g/L)	1.057 (0.663–1.687)	0.815		
Pretreatment platelets (< 225 vs. ≥ 225 × 10^9/L)	0.764 (0.478–1.219)	0.259		
Pretreatment ALC (< 1.8 vs. ≥ 1.8 × 10^9/L)	0.939 (0.588–1.497)	0.790		
Post1m-ALC (< 1.1 vs. ≥ 1.1 × 10^9/L)	0.674 (0.414–1.100)	0.114		
Grade 4 lymphopenia (yes vs. no)	2.341 (1.397–3.925)	0.001	2.016 (1.161–3.502)	0.013
Pathologic response (pCR vs. non-pCR)	0.434 (0.259–0.729)	0.002	0.530 (0.311–0.902)	0.019

CI confidence interval, *ECOG* Eastern Cooperative Oncology Group, *3DCRT* three-dimensional conformal radiation therapy, *IMRT* intensity-modulated radiation therapy, *ALC* absolute lymphocyte count, *pCR* pathologic complete response

[a]Chemotherapy regimen: 1, cisplatin/vinorelbine; 2, cisplatin/fluorouracil; 3, cisplatin/taxane

$P < 0.001$). In our study, patients treated with IMRT had a greater risk of severe lymphopenia compared with those undergoing 3DCRT on univariate analysis. However, this difference was not statistically significant on multivariable analysis. These data indicated that PBT, reduced irradiated volumes, and appropriate radiation dose may decrease lymphopenia risk and could lead to less immune suppression.

Consistent with previous reports, the strong link between lymphopenia and clinical outcomes has been documented by our study, suggesting immune preservation is very important in cancer control. In an intent-to-treat analysis, Besser et al.[27] reported that adoptive transfer of tumor-infiltrating lymphocytes (TILs) could yield durable and complete responses in patients with refractory melanoma. Also, adoptive cell therapy using expanded TILs following CRT showed promising results in patients with locoregionally advanced nasopharyngeal carcinoma.[28] Thus, adoptive immunotherapy may be a potential avenue for EC patients with treatment-related lymphopenia in future.

This study has several limitations. First, our results may be inevitably influenced by the selection bias due to its retrospective nature from a single institution; therefore, the results should be validated by multicenter studies prospectively. Second, we cannot account for all factors that could potentially affect the ALC values during treatment, such as the systemic use of corticosteroids and the occurrence of infections. Lastly, we were not able to analyze the correlation between lymphocyte subtypes and clinical outcomes.

CONCLUSIONS

ESCC patients with grade 4 lymphopenia during neoadjuvant CRT were associated with a significantly lower pCR rate and a higher risk of recurrence, suggesting immune preservation is important in disease control.

Further prospective study is warranted to confirm the predictive value of lymphopenia and to investigate the possible underlying mechanisms that link treatment-related lymphopenia to clinical outcomes in ESCC.

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