Clinical and Translational Radiation Oncology 36 (2022) 83–90

Contents lists available at ScienceDirect

Clinical and Translational Radiation Oncology

journal homepage: www.sciencedirect.com/journal/clinical-and-translational-radiation-oncology

Original Research Article

Splenic irradiation contributes to grade ≥ 3 lymphopenia after adjuvant chemoradiation for stomach cancer

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ARTICLE INFO

Keywords:
Gastric cancer
Survival
Postoperative chemoradiotherapy
Normal tissue toxicity
Spleen

ABSTRACT

Introduction: Adjuvant chemoradiation therapy (CRT) in gastric cancer inevitably results in an unintentional spleen radiation dose. We aimed to determine the association between the spleen radiation dose and the observed severity of lymphopenia which may affect the clinical outcomes (survival time and infection risk).

Methods: Patients who received adjuvant CRT for gastric cancer between January 2015 and December 2020 were analyzed. The splenic dose-volume histogram (DVH) parameters were reported as mean splenic dose (MSD) and percentage of splenic volume receiving at least × Gray (Gy). Peripheral blood counts were recorded pre- and post-CRT. The development of severe (Common Terminology Criteria for Adverse Events, version 5.0, grade ≥ 3) post-CRT lymphopenia (absolute lymphocyte count [ALC] < 0.5 K/μL) was assessed by multivariable logistic regression using patient and dosimetric factors. Overall survival (OS), recurrence-free survival (RFS), and cumulative incidence of infectious events were estimated and analyzed using the Cox model or competing risk analysis.

Results: Eighty-four patients with a median follow-up duration of 42 months were analyzed. Pre- and post-CRT median ALC values were 1.8 K/μL (0.9–3.1 K/μL) and 0.9 K/μL (0.0–4.9 K/μL), respectively ($P < 0.001$). MSD > 40 Gy (odds ratio [OR], 1.13; 95 % confidence interval [CI], 1.01–1.26; $P = 0.041$), sex (OR for male to female, 0.25; 95 % CI, 0.09–0.70; $P = 0.008$), and baseline absolute neutrophil count (OR per 1 unit increase, 1.61; 95 % CI, 1.02–2.58; $P = 0.040$) were associated with the development of severe post-CRT lymphopenia, which was a risk factor for poorer OS (hazard ratio [HR] = 2.47; 95 % CI, 1.24–4.92; $P = 0.010$) and RFS (HR = 2.27; 95 % CI, 1.16–4.46; $P = 0.017$). The cumulative incidence of infections was higher among severe post-CRT lymphopenia patients (2.53, 95 % CI, 1.03–6.23, $P = 0.043$).

Conclusion: High splenic radiation doses increase the odds of severe post-CRT lymphopenia, an independent predictor of lower OS and higher risks of recurrence and infections in gastric cancer patients receiving adjuvant CRT. Therefore, optimizing the splenic DVH parameters may decrease the risk of severe post-CRT lymphopenia.

Introduction

Immunosurveillance is the immune system's ability to detect and eliminate tumor cells before developing into a clinical malignancy [1]. It functions through "immunoediting" and plays an integral and contradictory role of promoting tumor growth and mediating disease eradication, depending on the interactions between immune effector, stromal, and tumor cells, and humoral factors [2,3]. Evidence indicates host immunity's importance in controlling cancer development and progression [1].

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https://doi.org/10.1016/j.ctro.2022.07.007
Received 2 May 2022; Received in revised form 7 July 2022; Accepted 15 July 2022
Available online 21 July 2022

Lymphocytes are highly radiosensitive. Radiotherapy (RT) can lead to lymphopenia via direct cytotoxic effects on circulating lymphocytes, as blood flows through the field or residing lymphocytes in lymphoid organs [4–12]. Studies suggest that lymphopenia after chemoradiation (CRT) is linked to a higher risk of complications and poorer prognosis of different types of solid tumors [13,14]. Lymphopenia risk can be related to the spleen dose because it is a secondary lymphoid organ containing more lymphocytes than those in the blood, at 15 % and 2 %, respectively [15]. A higher spleen dose has been associated with an increased risk of lymphopenia in patients with upper abdominal malignancies, such as esophageal, pancreatic, and liver cancers [6–9,11,12], although, in one study, a lower risk of hematologic toxicity with a higher spleen dose was observed in patients receiving CRT for esophageal cancer [10].

CRT for stomach cancer is unique, because the spleen is usually included in the field. However, it is not routinely considered an organ-at-risk (OAR) with dosimetric constraints. A better understanding of the spleen's role in acute hematologic toxicity and its clinical significance allows us to consider applying dose constraints to the spleen as an OAR. Therefore, this study aimed to identify the irradiated spleen's radiation dose and fractional volume as predictors of lymphopenia risk among gastric cancer patients receiving adjuvant CRT and the clinical consequences (survival time and infection risk).

Methods

Study design, data, and setting

We retrospectively identified consecutive patients with adenocarcinoma of the stomach in Tuen Mun Hospital, Hong Kong treated with radical total or subtotal gastrectomy for stage IB-III disease (i.e., T3–4, or N1–3 diseases) followed by adjuvant CRT with the 5-Fluorouracil (5-FU) regimen from 2015 to 2020 [16]. Patients who had primarily unresectable disease or distant metastases at presentation, did not complete the entire course of the CRT regimen or had splenectomy were excluded.

Pretreatment evaluation included a complete history and physical examination, blood tests (complete blood count, liver, and renal function), and imaging (computed tomography or positron emission tomography-computed tomography). Stage was defined according to the American Joint Committee on Cancer (7th edition) criteria [17].

Treatment monitoring and blood count evaluation

The patients were reviewed weekly during RT. White blood cell count, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), hemoglobin, and platelet count were measured at weekly intervals throughout the treatment. Laboratory results were also recorded before adjuvant treatment (baseline) and up to 2 months after CRT to observe potential delayed counts changes. Hematologic toxicity was graded based on the Common Terminology Criteria for Adverse Events, version 5.0, as per the established cut-off values [18]. Grades 1 and 2 lymphopenia (ALC between 0.5 and 1 K/μL) and grade 3 and 4 lymphopenia (ALC $<$ 0.5 K/μL) were categorized as mild and severe, respectively.

Treatment planning and delivery

The patients were simulated and treated in a supine position with their arms up. RT was delivered using three-dimensional conformal or intensity-modulated RT at 45 Gy in 25 daily fractions over 5 weeks (1.8 Gy per fraction per day). The radiation dose constraints for the spinal cord were $<$ 45 Gy, kidney V15 $<$ 60 %, liver V30 $<$ 40 %, and heart V40 $<$ 30 %. No dose constraints were applied to the spleen or vertebrae.

The chemotherapy regimen consisted of intravenous drug delivery consisting of 5-FU 425 mg/m^2 per day and leucovorin 20 mg/m^2 per day for 5 days, followed by RT concurrent with 5-FU 400 mg/m^2 and leucovorin 20 mg/m^2 on the first four and last three days of RT. Four weeks after RT completion, two five-day cycles of 5-FU (425 mg/m^2 per day) plus leucovorin (20 mg/m^2 per day) were administered 4 weeks apart.

Dosimetric and volumetric parameters

The spleen was not contoured as an OAR in our institution; we delineated it after RT had been delivered for this study. For each patient, the entire spleen and spine (from the most cranial to the most caudal spinal level of the planning target volume [PTV]) were delineated on the treatment planning CT scan by a trained radiation oncologist or radiotherapist (Supplementary Figure S1). We contoured the spine as the vertebral body, transverse processes, and posterior elements to account for the potential immunosuppressive effect of bone marrow exposure to RT [19]. We retrieved the dose distribution to compute the dose-volume histogram (DVH) data using Pinnacle 9.1 (Philips Medical Systems, the Netherlands). The following data were recorded for the structures: volume, mean dose, and relative volume receiving at least 5 Gy (V5), 10 Gy (V10), 15 Gy (V15), 20 Gy (V20), 30 Gy (V30), 40 Gy (V40), and 45 Gy (V45). The physical radiation dose was not converted to the equivalent dose in 2 Gy fractions because the α/β of the spleen is uncertain.

Statistical analysis

We compared the occurrence of severe lymphopenia between the splenic dosimetric parameters using the Wilcoxon rank-sum test or the Fisher exact test, as appropriate. To assess the potential predictors of post-CRT ALC, we performed logistic regression with the development of severe lymphopenia as a dependent variable.

Overall survival (OS) and recurrence-free survival (RFS) were estimated by Kaplan–Meier analysis, and the differences between groups were compared using log-rank tests [20,21]. We counted survival events only if the outcomes occurred beyond a landmark period of 4 months after starting CRT because most patients would have completed adjuvant therapy by that time. The landmark analysis accounted for the lead-time bias between the start of treatment and the assessment of survival time. OS was defined as the time from the landmark to death from any cause or the last follow-up, whichever occurred first, whereas RFS was defined as the time from the landmark to cancer relapse, death, or the last follow-up, whichever occurred first. The patients were censored at the last follow-up visit. We fit semiparametric Cox proportional hazards models to evaluate the association between OS and RFS and post-CRT ALC, adjusted for other potential prognostic factors. Furthermore, we used a Fine-Gray competing risk model to analyze the competing risks of non-infection-related mortality and derive the subdistribution hazard ratios (HRs) for infections [22,23]. Factors with a $P < 0.1$ on univariable analysis were included in the multivariable model, and backward elimination was used to obtain the final model.

All analyses were performed using Stata (StataCorp. 2019. Stata statistical software: Release 16 College Station, TX, StataCorp LLC, USA). A two-tailed P of < 0.05 was considered statistically significant.

Results

Description of the cohort

The characteristics of the 84 patients with stomach cancer treated with gastrectomy followed by adjuvant CRT are detailed in Table 1 and Supplementary Table S1. The median age at diagnosis was 61 years (range, 34–77 years), and 64.0 % were males. The median follow-up duration from the start of CRT for alive patients was 42 months (range, 12–80 months). The median baseline ALC was 1.8 K/μL (range, 0.9–3.1 K/μL). The median ALC decreased to 0.9 K/μL (range, 0.0–4.9 K/μL) shortly after CRT and was significantly lower than the baseline ($P < 0.001$); the median ALC remained lower than the baseline at follow-up

Table 1
Patient, disease, and dosimetric factors of the gastric cancer patients who had adjuvant chemoradiation, 2015–2020 (N = 84).

Characteristics	All patients (n = 84)	Patients categorized by post-CRT ALC		
		ALC < 0.5 K/μL (n = 27)	ALC ≥ 0.5 K/μL (n = 57)	*P*
Age, year				0.438
Median (range)	61 (54–66)	60 (53–65)	62 (55–66)	
Sex, n (%)				0.002
Male	54 (64.3)	11 (40.7)	43 (75.4)	
Female	30 (35.7)	16 (59.3)	14 (24.6)	
Stage, n (%)				0.608
I	2 (2.5)	0 (0.0)	2 (3.7)	
II	16 (18.5)	5 (18.5)	11 (19.3)	
III	66 (79.0)	22 (81.5)	44 (77.2)	
Extent of gastric surgery, n (%)				0.582
Total gastrectomy	41 (48.8)	12 (44.4)	29 (50.9)	
Partial gastrectomy	43 (51.2)	15 (55.6)	28 (49.1)	
Lymph node dissection, n (%)				0.506
D1	24 (28.6)	9 (33.3)	15 (26.3)	
D2	60 (71.4)	18 (66.7)	42 (73.7)	
Tumor grade, n (%)				0.137
Well/moderately differentiated	28 (33.3)	6 (22.2)	22 (38.6)	
Poorly differentiated	56 (66.7)	21 (77.8)	35 (61.4)	
Resection margin, n (%)				0.256
R0	76 (90.5)	23 (85.2)	53 (93.0)	
R1	8 (9.5)	4 (14.8)	4 (7.0)	
RCS co-morbidity scores, n (%)				0.154
0	63 (75.0)	19 (70.4)	44 (77.2)	
1	17 (20.2)	8 (29.6)	9 (15.8)	
≥2	4 (4.8)	0 (0.0)	4 (7.0)	
White cell count, median (range) (K/μL)				
Baseline	5.9 (1.6–12.3)	6.0 (1.6–7.7)	5.7 (2.7–12.3)	0.943
Before chemoradiation	5.1 (2.3–11.7)	5.1 (2.3–9.0)	5.1 (2.7–11.7)	0.620
After chemoradiation	3.9 (1.9–12.7)	3.5 (1.9–7.3)	4.1 (2.0–12.7)	0.1030
ALC, median (range) (K/μL)				
Baseline	1.8 (0.9–3.1)	2.0 (1.3–2.6)	1.7 (0.9–3.1)	0.590
Before chemoradiation	1.9 (0.7–2.8)	1.7 (0.7–2.4)	2.0 (0.8–2.8)	0.070
After chemoradiation	0.5 (0.0–3.5)	0.2 (0–0.4)	0.9 (0.1–3.5)	<0.001
ANC, median (range) (K/μL)				
Baseline	3.2 (0.2–9.1)	3.1 (0.2–5.0)	3.2 (1.1–9.1)	0.839
Before chemoradiation	2.5 (0.6–8.1)	2.7 (0.6–5.9)	2.4 (1.4–8.1)	0.126
After chemoradiation	2.2 (0.7–8.1)	2.4 (1.4–5.8)	2.1 (0.7–8.1)	0.171
Monocyte, median (range) (K/μL)				
Baseline	0.5 (0.1–1.4)	0.5 (0.1–1.1)	0.5 (0.2–1.4)	0.707
Before chemoradiation	0.5 (0.2–1.2)	0.5 (0.2–0.9)	0.5 (0.3–1.2)	0.810
After chemoradiation	0.6 (0.2–1.8)	0.6 (0.2–0.9)	0.6 (0.2–1.8)	0.910
Platelet, median (range) (K/μL)				
Baseline	230 (89–479)	242 (172–386)	230 (89–479)	0.875
Before chemoradiation	229 (115–511)	250 (143–401)	222 (115–511)	0.238
After chemoradiation	152 (52–381)	166 (65–381)	144 (52–299)	0.734
Hemoglobin, median (range) (g/dL)				
Baseline	11.6 (8.6–15.1)	11.8 (8.6–14.1)	11.6 (9.4–15.1)	0.877
Before chemoradiation	11.6 (8.7–15.0)	11.0 (8.9–15.0)	11.9 (8.7–15.0)	0.015
After chemoradiation	11.8 (9.1–15.4)	11.3 (9.1–14.3)	12.0 (9.3–15.4)	0.037
Total chemotherapy dose, median (range) (% of full dose)	100 (84–100)	100 (84–100)	89 (77–100)	0.225
Radiotherapy techniques, n (%)				0.750
3-dimensional conformal radiotherapy	61 (72.6)	19 (70.4)	42 (73.7)	
Intensity-modulated radiotherapy	23 (27.4)	8 (29.6)	15 (26.3)	
Mean spleen dose (Gy), median (range)	40.7 (25.0–48.1)	42.4 (27.0–48.1)	40.2 (25.0–47.6)	0.018
Spleen volume (cm^3), median (range)	117.6 (17.0–342.4)	94.5 (34.0–197.8)	127.7 (17.0–342.4)	0.094
Spleen V5 (%), median (range)	100.0 (100.0–100.0)	100.0 (100.0–100.0)	100.0 (100.0–100.0)	1.000
Spleen V10 (%), median (range)	100.0 (99.6–100.0)	100.0 (100.0–100.0)	100.0 (99.0–100.0)	0.068
Spleen V15 (%), median (range)	99.7 (94.9–100.0)	100.0 (99.5–100.0)	98.3 (93.6–100.0)	0.056
Spleen V20 (%), median (range)	95.8 (50.8–100.0)	99.5 (78.5–100.0)	93.9 (50.8–100.0)	0.009
Spleen V30 (%), median (range)	84.6 (29.8–100.0)	91.3 (29.8–100.0)	81.8 (40.3–100.0)	0.024
Spleen V40 (%), median (range)	69.4 (12.2–100.0)	72.9 (12.2–100.0)	65.3 (28.0–100.0)	0.039
Spleen V45 (%) median (range)	53.5 (4.1–100.0)	59.0 (4.1–100.0)	52.3 (16.8–96.0)	0.099
Mean spine dose (Gy), median (range)	29.5 (20.6–40.1)	30.4 (20.9–40.1)	28.6 (20.6–35.2)	0.112
Spine V5 (%), median (range)	100.0 (99.7–100.0)	100.0 (99.8–100.0)	100.0 (99.7–100.0)	0.318
Spine V10 (%), median (range)	98.3 (93.9–99.6)	99.3 (97.6–99.8)	97.6 (93.8–99.4)	0.046
Spine V15 (%), median (range)	92.6 (84.9–97.0)	95.8 (88.2–98.3)	90.2 (83.0–96.6)	0.063
Spine V20 (%), median (range)	82.2 (32.0–99.3)	83.9 (32.0–98.2)	80.1 (42.2–99.3)	0.048
Spine V30 (%), median (range)	42.6 (13.7–91.0)	48.0 (22.0–91.0)	39.1 (13.7–79.3)	0.225
Spine V40 (%), median (range)	19.8 (0.2–68.1)	21.9 (10.8–68.1)	19.4 (0.2–45.8)	0.050
Spine V45 (%), median (range)	9.0 (0.7–38.5)	10.2 (2.7–22.4)	8.7 (0.7–38.5)	0.124
PTV (cm^3), median (range)	1463 (754–2435)	1357 (754–2435)	1465 (933–2386)	0.225

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; CRT, chemoradiation; PTV, planning target volume; RCS, Royal College of Surgeons.

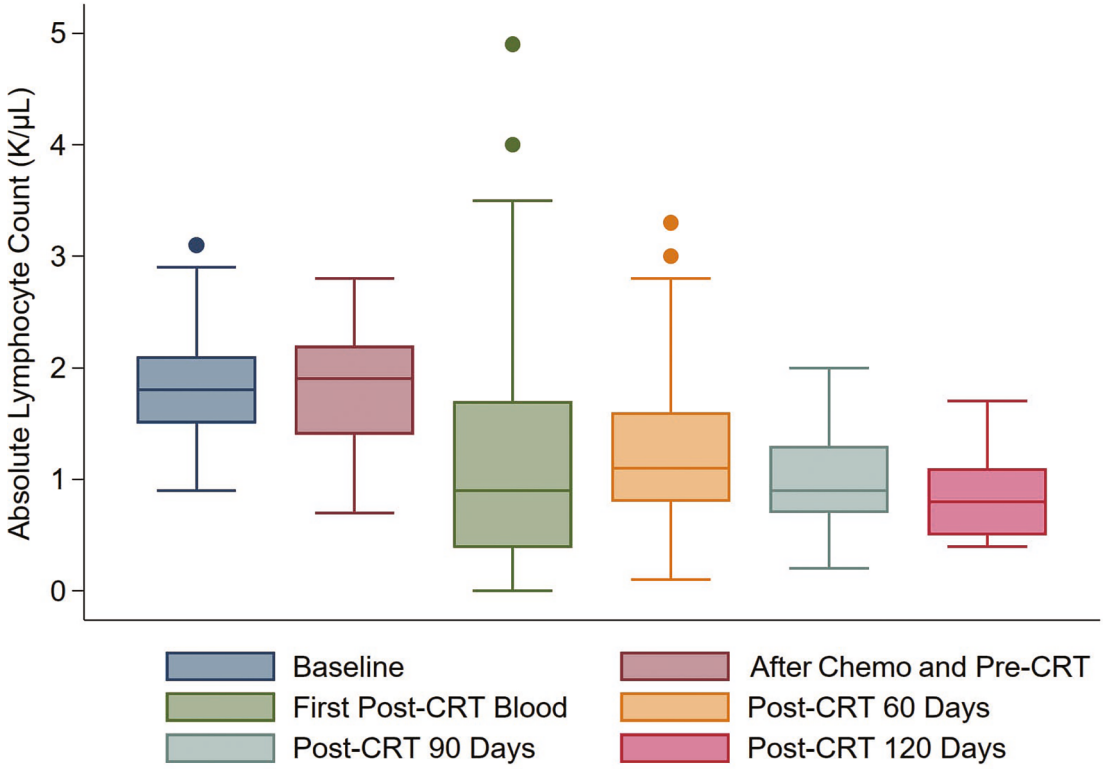

Fig. 1. Absolute lymphocyte count at baseline, before, and after adjuvant chemoradiation, and during follow-up. Abbreviations: chemo, chemotherapy; CRT, chemoradiation.

(Fig. 1). The incidence of severe lymphopenia at the first post-CRT blood test (the median time was 3 weeks after completion of adjuvant CRT, with interquartile range 1–4 weeks) was 32.1 %. Most of these patients had recovery of their ALC during follow-up. Nine (10.7 %), 7 (8.3 %), and 7 (8.3 %) patients had persistent severe lymphopenia at 60-, 90-, and 120-days post-CRT, respectively. Most patients had the spine contoured from T9 or 10 to L3. Sensitivity analyses did not detect statistically significant difference in dosimetric parameters by spinal levels contoured (Supplementary Table S2).

Prediction of post-CRT severe lymphopenia

The median mean spleen dose (MSD) was 40.7 Gy (range, 25.0–48.1 Gy) for the whole cohort. The median MSD among patients with severe lymphopenia (ALC < 0.5 K/μL) was higher than those without (42.4 Gy vs 40.2 Gy; $P = 0.018$). We also detected a dose–response relationship between MSD and the severity of lymphopenia, with the median MSD for patients with normal ALC (ALC > 1.0 K/μL), mild (ALC 0.5–1.0 K/μL), and severe lymphopenia of 39.4 Gy, 40.7 Gy, and 42.4 Gy, respectively ($P = 0.044$). Univariable logistic regression analysis showed that MSD as a binary variable (cut-off using integer closest to the median and round to the nearest multiple of 5) was significantly associated with severe lymphopenia (odds ratio [OR] for > 40 Gy vs ≤ 40 Gy, 2.76; 95 %CI, 1.01–7.54; $P = 0.048$). However, MSD as a continuous variable showed a weaker association (OR per Gy increase, 1.11; 95 %CI, 1.00–1.23; $P = 0.060$) with post-CRT lymphopenia. Spine V40 (OR per % increase, 1.06; 95 %CI, 1.01–1.11; $P = 0.024$) was also found to be statistically significant in the univariable analysis and was selected for the multivariable analysis. We present the incidence of post-CRT grade ≥ 3 lymphopenia in different MSD binary cut-off values (Supplementary Table S3).

The median spleen V20 (99.5 % vs 93.9 %; $P = 0.009$), V30 (91.3 % vs 81.8 %; $P = 0.024$), and V40 (72.9 % vs 65.3 %; $P = 0.039$) were significantly higher in patients with severe lymphopenia than in those without it (Table 1). Furthermore, a greater proportion of patients with severe lymphopenia had V40 > 70 % ($P = 0.024$). Similar trends were obtained at 60 % and 80 % for V30, V40, and V45, respectively, but did not reach statistical significance ($P = 0.055$–0.403).

In the stepwise multivariable logistic regression model with backward elimination, MSD dichotomized into a median of 40 Gy (OR > 40 vs ≤ 40 Gy, 1.13; 95 %CI, 1.01–1.26, $P = 0.041$), sex (OR for male vs female, 0.25; 95 %CI, 0.09–0.70; $P = 0.008$), and baseline ANC (OR per 1 unit increase, 1.62; 95 %CI, 1.02–2.58; $P = 0.040$) were significant predictors of the development of post-CRT severe lymphopenia (Table 2). Multivariable model analyzing MSD as a continuous variable also showed significant results (OR per Gy increase, 1.15; 95 %CI, 1.02–1.30; $P = 0.022$). None of the spine dosimetric factors or spine volume was found to be significant in the multivariable analysis.

Survival outcomes and risk of infection

OS

Two patients who died within 4 months after the start of CRT were excluded, and 82 patients were included in the analysis. The median follow-up duration for alive patients was 3.2 years. The median OS at the landmark time point for the whole cohort was 4.9 years (95 %CI, 2.1–not reached). The 1-year, 2-year, and 5-year OS were 80.5 % (95 %CI, 70.0–87.5 %), 64.7 % (95 %CI, 52.8–74.4 %), and 46.9 % (95 %CI, 30.9–61.3 %), respectively. Patients who had post-CRT severe lymphopenia had a shorter median OS (2.0 years, 95 %CI, 0.7–3.9 years), while the median OS for those without was not reached (Fig. 2). In multivariable analysis, we found that post-CRT severe lymphopenia (HR, 2.47; 95 %CI, 1.24–4.92; $P = 0.010$), and total chemotherapy dose (HR per 1 % increase, 0.97; 95 %CI, 0.96–0.99; $P < 0.001$) were associated with OS (Table 3). Patients with persistent severe lymphopenia at 60-, 90-, and 120-days post-CRT had no significant difference in OS when compared with those without (log-rank $P = 0.224$–0.884) (Supplementary Figures S2–4).

Table 2
Univariable and multivariable analyses of predictors of developing post-chemoradiation severe lymphopenia, 2015–2020 (N = 84).

	Univariable Analysis		Multivariable Analysis*		Multivariable Analysis*#	
Variables	OR (95 % CI)	*P*	OR (95 % CI)	*P*	OR (95 % CI)	*P*
Dosimetric factors						
Mean spleen dose, Gy (>40 vs ≤ 40)	2.76 (1.01–7.54)	0.048	1.13 (1.01–1.26)	0.041	–	–
Mean spleen dose, Gy (per unit increase)	1.11 (1.00–1.23)	0.060	–	–	1.15 (1.02–1.30)	0.022
Spleen V5, % (per unit increase)	1.09 (0.70–1.69)	0.698				
Spleen V10, % (per unit increase)	1.12 (0.92–1.38)	0.262				
Spleen V15, % (per unit increase)	1.10 (0.99–1.23)	0.080				
Spleen V20, % (per unit increase)	1.09 (1.00–1.17)	0.072				
Spleen V30, % (per unit increase)	1.03 (1.00–1.07)	0.075				
Spleen V40, % (per unit increase)	1.02 (1.00–1.05)	0.105				
Spleen V45, % (per unit increase)	1.02 (1.00–1.04)	0.109				
Spleen volume, cm^3 (per unit increase)	0.99 (0.98–1.00)	0.095				
Mean spine dose, Gy (per unit increase)	1.13 (1.00–1.27)	0.713				
Spine V5, % (per unit increase)	1.10 (0.68–1.78)	0.687				
Spine V10, % (per unit increase)	1.10 (0.94–1.29)	0.219				
Spine V15, % (per unit increase)	1.04 (0.98–1.10)	0.191				
Spine V20, % (per unit increase)	1.03 (1.00–1.07)	0.084				
Spine V30, % (per unit increase)	1.02 (0.99–1.05)	0.125				
Spine V40, % (per unit increase)	1.06 (1.01–1.11)	0.024				
Spine V45, % (per unit increase)	1.06 (0.98–1.14)	0.171				
Spine volume, cm^3 (per unit increase)	1.00 (0.99–1.00)	0.195				
PTV, cm^3 (per unit increase)	1.00 (0.99–1.00)	0.218				
Patient factors						
Age, year (≥60 vs < 60)	0.78 (0.31–1.97)	0.603				
Sex (male vs female)	0.22 (0.08–0.59)	0.003	0.25 (0.09–0.70)	0.008	0.29 (0.10–0.85)	0.024
Comorbidity (yes vs no)	1.43 (0.51–4.00)	0.501				
Baseline white blood cell, K/μL (per unit increase)	1.09 (0.79–1.49)	0.605				
Baseline ALC, K/μL (per unit increase)	0.41 (0.15–1.10)	0.078			0.33 (0.10–1.07)	0.064
Baseline ANC, K/μL (per unit increase)	1.34 (0.91–1.97)	0.141	1.62 (1.02–2.58)	0.040	1.79 (1.09–2.94)	0.021
Baseline hemoglobin, K/μL (per unit increase)	0.68 (0.48–0.98)	0.036				
Baseline platelet, K/μL (per unit increase)	1.00 (1.00–1.01)	0.423				
Total chemotherapy dose, % (per unit increase)	0.98 (0.96–1.01)	0.144				

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; CI, confidence interval; CRT, chemoradiation; Gy, Gray; OR, odds ratio; PTV, planning target volume; Vx, volume of spleen receiving × Gy of radiation.

* Due to the collinearity between MSD and other spleen dosimetric parameters and between mean spine dose and other spine dosimetric parameters, the only dosimetric parameters included in the multivariable analysis were mean spleen dose and spine V40.

Mean spleen dose was analyzed in multivariable model as a continuous variable.

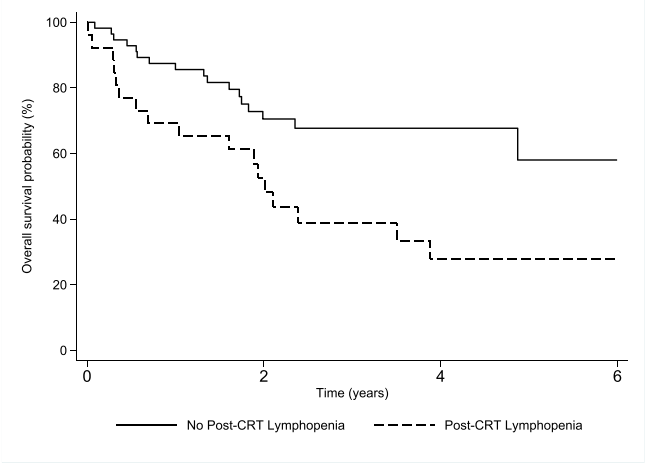

Fig. 2. Kaplan-Meier estimates of overall survival based on landmark analysis in patients with gastric cancer categorized by severe post-chemoradiation lymphopenia (absolute lymphocyte counts < 0.5 K/μL). The time 0 is 4 months after the start of CRT. Abbreviation: CRT, chemoradiation.

RFS

A total of 77 patients were included in the analysis. The 1-year, 2-year, and 5-year RFS for the cohort were 75.0 % (95 %CI, 63.7–83.3 %), 59.6 % (95 %CI, 47.1–70.1 %), and 52.4 % (95 %CI, 38.9–64.2 %), respectively. Patients who had post-CRT severe lymphopenia had a shorter median RFS (1.8 years, 95 %CI, 0.9–3.7 years), whereas the median RFS for those without it was not reached. Fig. 3 shows the cumulative incidence of recurrence between patients with and without post-CRT severe lymphopenia. Post-CRT severe lymphopenia (HR, 2.27; 95 %CI, 1.16–4.46; $P = 0.017$) and higher cancer stage (HR stage III vs I/II, 3.17; 95 %CI, 1.01–9.92; $P = 0.048$) were associated with RFS in multivariable analysis (Table 3).

Risk of infection

A total of 18 infections (14 pneumonia and 4 sepsis cases) were recorded. Nine infectious events were fatal. The incidence and mortality rates of the infections were 84.7 (95 %CI, 53.4–134.5) and 39.1 (95 %CI, 20.3–75.1) per 1000 person-years, respectively. The cumulative incidence of infectious events was 9.5 % (95 %CI, 4.5–16.9 %), 12.2 % (95 %CI, 6.2–20.3 %), and 25.4 % (95 %CI, 15.3–36.8 %) at 1-year, 2-year, and 5-year follow-up, respectively. Supplementary Figure S5 shows the cumulative incidence of severe post-CRT lymphopenia. Only severe post-CRT lymphopenia (2.53; 95 %CI, 1.03–6.23; $P = 0.043$) and presence of comorbidity (2.89; 95 %CI, 1.74–4.81; $P < 0.001$) were associated with infection (Supplementary Table S4).

Discussion

To our knowledge, this study is the first to report the relationship between splenic irradiation and severe post-CRT lymphopenia and its association with clinical outcomes in gastric cancer. Our data suggest that severe lymphopenia is common after adjuvant CRT for stomach cancer. MSD, sex, and baseline ANC were significant predictors of post-

Table 3
Univariable and multivariable analyses of prognostic factors for overall survival and recurrence-free survival, 2015–2020 (N = 84).

	Overall Survival				Recurrence-Free Survival			
	Univariable Analysis		Multivariable Analysis		Univariable Analysis		Multivariable Analysis	
Variables	HR (95 % CI)	*P* value	HR (95 % CI)	*P*	HR (95 % CI)	*P*	HR (95 % CI)	*P*
Post-CRT severe lymphopenia (yes vs no)	2.50 (1.27–4.89)	0.008	2.47 (1.24–4.92)	0.010	2.14 (1.10–4.20)	0.026	2.27 (1.16–4.46)	0.017
Baseline white blood cell, K/μL (per unit increase)	1.20 (0.98–1.46)	0.077	1.23 (0.99–1.52)	0.060	1.22 (1.05–1.41)	0.008		
Baseline ALC, K/μL (per unit increase)	1.56 (0.73–3.30)	0.250			1.19 (0.59–2.40)	0.618		
Baseline ANC, K/μL (per unit increase)	1.25 (0.98–1.58)	0.069			1.29 (1.10–1.51)	0.002		
Baseline hemoglobin, K/μL (per unit increase)	0.92 (0.73–1.17)	0.496			0.98 (0.74–1.29)	0.865		
Baseline platelet, K/μL (per unit increase)	1.00 (0.99–1.00)	0.892			1.00 (0.99–1.00)	0.674		
Age, year (≥60 vs < 60)	0.73 (0.37–1.44)	0.369			0.78 (0.39–1.56)	0.479		
Sex (male vs female)	0.79 (0.40–1.57)	0.505			0.67 (0.34–1.32)	0.245		
Comorbidity (yes vs no)	1.57 (0.76–3.23)	0.225			1.17 (0.55–2.47)	0.689		
Cancer stage (III vs I/II)	1.87 (0.72–4.85)	0.197			3.49 (1.08–11.30)	0.037	3.17 (1.01–9.92)	0.048
Tumor grade (poorly vs well/moderately differentiated)	2.41 (1.05–5.55)	0.038			1.77 (0.79–3.95)	0.166		
Resection margin (R1 vs R0)	1.71 (0.60–4.88)	0.316			2.95 (1.30–6.69)	0.010		
Infection (yes vs no)	2.24 (1.12–4.48)	0.023			1.08 (0.51–2.28)	0.847		
Total chemotherapy dose, % (per unit increase)	0.97 (0.96–0.99)	<0.001	0.97 (0.96–0.99)	<0.001	1.00 (0.98–1.02)	0.755		

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; CI, confidence interval; CRT, chemoradiation; HR, hazard ratio.

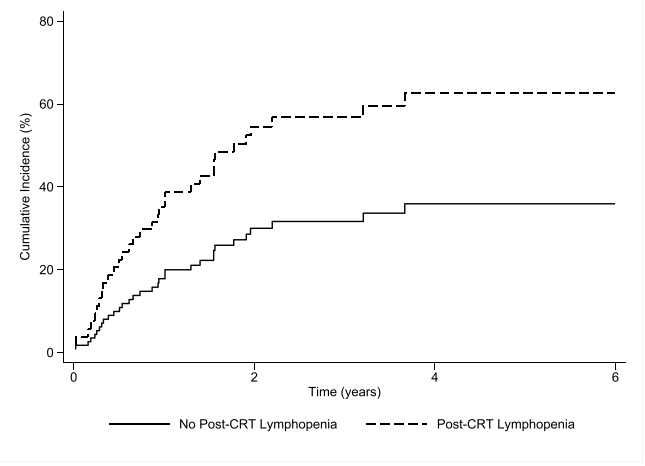

Fig. 3. Cumulative incidence of recurrence by competing-risk analysis among patients with gastric cancer with and without severe post-chemoradiation lymphopenia (absolute lymphocyte counts < 0.5 K/μL). The time 0 is 4 months after the start of CRT. Abbreviation: CRT, chemoradiation.

CRT severe lymphopenia, which in turn was an independent predictor of poorer OS and RFS. Patients with a higher post-CRT ALC and no comorbidities had lower infection risk during follow-up.

RT is immune-stimulating and immune-suppressive, depending on dose fractionation, total dose, field size, and systemic treatment [19,24–26]. Its immunosuppressive effect is due to the depletion of circulating lymphocytes through the RT portal and the unintentional exposure of lymphopoiesis sites, like the bone marrow, spleen, and lymph nodes [19]. The spleen, being close to the treatment field, is also a reservoir of immune cells and a depot for clonal expansion of lymphocytes to specific antigens [27]. We observed a dose–response relationship between post-CRT ALC and MSD (as binary or continuous variable). This supports the postulation that splenic irradiation dose is a predictor for developing severe post-CRT lymphopenia. In contrast, the effects of lymphocyte depletion in the marrow and circulation were assessed by testing the association between PTV (as a surrogate for RT portal size) or spine DVH and severe post-CRT lymphopenia, respectively. The spine DVHs were found to be statistically insignificant factors, this could be related to the fact that the marrow irradiated was limited to several spinal levels and the rest of the spine and pelvis were spared.

Lymphopenia is predominantly a consequence of CRT, although patients receive chemotherapy followed by CRT. This was reflected in the lack of significant association between the total chemotherapy dose and post-CRT severe lymphopenia, and the normal median ALC after chemotherapy (before CRT), which was not significantly different from baseline values. No patient developed severe lymphopenia after chemotherapy, but this increased to 33 % after CRT. Furthermore, the lack of neutropenia accompanying lymphopenia was different from that observed with myelosuppression from chemotherapy [28,29]. Although higher baseline ANC was weakly associated with poorer OS and RFS, it was the opposite for severe post-CRT lymphopenia. This may reflect an underlying proinflammatory status that suppresses the activity and proliferation of immune cells, including lymphocytes [30–32].

The association between lymphopenia and inferior survival outcomes supports that the immune system contributes to tumor control [33]. Higher chemotherapy dose was associated with superior OS; these findings could be related to the better general health in some patients. Strategies to effectively kill all cancer stem cells using the correct combination and schedule of treatment, stimulating an immune response, and subverting immunosuppressive mechanisms should be developed [33]. However, efforts to combine systemic treatments and RT may be considerably hampered by lymphocyte depletion after CRT [34–38], this is supported by the finding that we did not detect significant association between chemotherapy dose and RFS. Considering the

spleen as an OAR for RT plan optimization and selection to minimize unintentional splenic irradiation to decrease the risk of RT-related lymphopenia may be a promising approach that deserves further investigation. Our results suggest that there may be an MSD threshold of approximately 40 Gy for severe lymphopenia.

We detected an association between infectious events and severe post-CRT lymphopenia and persistently low ALC for at least 6 months after CRT. Therefore, impaired immunity due to possible hyposplenia in our study cohort could be chronic and was further evidenced by the incidence and mortality rates of infections of 83.2 and 39.1 per 1000 person-years, respectively, and up to one-third of patients suffering from infections within 5 years. These figures are comparable to those observed in patients who underwent splenectomy, hyposplenia in other diseases, or after splenic irradiation for hematological diseases [27,39].

Although previous studies reported impaired splenic immunological function with peripheral blood microscopic changes and heightened risk of infection after approximately 10–40 Gy of splenic irradiation for a range of conditions [40–43], the tolerance dose of the spleen is uncertain. The radiation tolerance level of splenic immunological function may be lower than the dose administered to the spleen in many of our patients. Furthermore, consistent with our observation of increasing cumulative incidences years after CRT, a chronic pattern of hyposplenia after splenic irradiation was observed [41]. Routine use of antimicrobials against fatal infectious diseases, similar to hypo- and asplenia in other diseases, cannot be recommended due to the lack of evidence to guide their use in gastric RT. However, its use might be beneficial, especially when sparing the spleen is difficult and at the expense of other OARs, such as the kidneys, liver, and bowel.

Our study had several limitations and strengths. First, this was a retrospective study with inherent heterogeneity in the study population, and the effects of potential confounding and bias could not be eliminated. Second, lack of variation in the lower dose parameters limited the assessment of the RT dose–effect relationship of splenic irradiation. In general, the doses represented by the DVH parameters were higher than those reported in other studies [8,10–12,44]. This could be because of the high dose to the splenic hilum as the CTV included the splenic hilar lymph nodes [16,45,46]. We hypothesized that the location of the high radiation dose in the splenic hilum may induce vascular damage and changes in the white and red pulp, which was demonstrated in patients with Hodgkin's lymphoma receiving 40 Gy (which is the same as our median MSD) in 2 Gy daily fractions [40,47]. Finally, we only assessed gastric cancer and the results cannot be generalized to other treatment sites. However, because the spleen is close to other intra-abdominal organs like the pancreas, the frequent inclusion of parts of the spleen in the RT field may be similar to that in other upper abdominal cancers.

Conclusions

Severe lymphopenia occurs commonly after adjuvant CRT for gastric cancer and is an independent predictor of inferior OS and RFS and a higher infection risk. Furthermore, the risk of severe lymphopenia increased with higher MSD.

Funding

The author(s) received no specific funding for this work.

Ethics approval and consent to participate

The Research Ethics Committee, New Territories West Cluster, Hospital Authority, Hong Kong approved the study and waived patient consent requirement (reference no: NTWC/REC/16119). The research was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank King-Fung Tsang (Department of Clinical Oncology, Tuen Mun Hospital, Hospital Authority, Hong Kong) for clerical support and data retrieval.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ctro.2022.07.007.

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