Advances in Radiation Oncology (2021) **6**, 100545

advances
in radiation oncology

www.advancesradonc.org

Scientific Article

Patient-Specific Lymphocyte Loss Kinetics as Biomarker of Spleen Dose in Patients Undergoing Radiation Therapy for Upper Abdominal Malignancies

Anirudh Yalamanchali, MS, BS,[a] Hong Zhang, PhD, MS,[b] Ke Colin Huang, PhD,[b] Radhe Mohan, PhD,[c] Steven H. Lin, MD, PhD,[c] Cong Zhu, MPH,[d,e] Stuart A. Grossman, MD,[f] Jian-Yue Jin, PhD,[g] and Susannah G. Ellsworth, MD[h,*]

[a]Indiana University School of Medicine, Indianapolis, Indiana; [b]Department of Radiation Oncology, Indiana University School of Medicine, Indianapolis, Indiana; Departments of [c]Radiation Oncology and [d]Radiation Physics, The University of Texas MD Anderson Cancer Center, Houston, Texas; [e]Department of Epidemiology, Human Genetics, and Environmental Sciences, The University of Texas Health Science Center at Houston, Houston, Texas; [f]Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, Maryland; [g]Department of Radiation Oncology, Case Western Reserve University, Cleveland, Ohio; and [h]Department of Radiation Oncology, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania

Received 6 April 2020; revised 26 May 2020; accepted 3 August 2020

Abstract

Purpose: Radiation therapy (RT)−induced lymphopenia (RIL) is linked with inferior survival in esophageal and pancreatic cancers. Previous work has demonstrated a correlation between spleen dose and RIL risk. The present study correlates spleen dose-volume parameters with fractional lymphocyte loss rate (FLL) and total percent change in absolute lymphocyte count (%ΔALC) and suggests spleen dose constraints to reduce RIL risk.

Methods and Materials: This registry-based study included 140 patients who underwent RT for pancreatic (n = 67), gastroesophageal (n = 61), or biliary tract (n = 12) adenocarcinoma. Patient-specific parameters of lymphocyte loss kinetics, including FLL and %ΔALC, were calculated based on serial ALCs obtained during RT. Spearman's rho was used to correlate spleen dose-volume parameters with %ΔALC, end-treatment ALC, and FLL. Multivariable logistic regression was used to identify predictors of ≥grade 3 and grade 4 RIL.

Results: Spleen dose-volume parameters, including mean spleen dose (MSD), all correlated with %ΔALC, end-treatment ALC, and FLL. Controlling for baseline ALC and planning target volume (PTV), an increase in any spleen dose-volume parameter increased the odds of developing ≥grade 3 lymphopenia. Each 1-Gy increase in MSD increased the odds of ≥grade 3 RIL by 18.6%, and each 100-cm^3 increase in PTV increased the odds of ≥grade 3 lymphopenia by 20%. Patients with baseline ALC < 1500 cells/μL had a high risk of ≥grade 3 RIL regardless of MSD or PTV. FLL was an equally good predictor of ≥grade 3 lymphopenia as any spleen dose-volume parameter.

Sources of support: This research was supported by the NIH/NCATS Clinical and Translational Sciences Award (Grant #UL1TR002529), Indiana University Health Values Fund Grant (IUH-VF-458), and Indiana Clinical and Translational Sciences Institute Biomedical Research Grant (22-874-02).

Disclosures: Dr Lin reports grants from Genetech, Hitachi Chemicals, New River Labs, and Beyond Spring Pharmaceuticals as well as advisory board membership at Astra-Zeneca and honorarium status at Varian outside the submitted work.

Research data are stored in an institutional repository and will be shared upon request to the corresponding author.

* Corresponding author: Susannah G. Ellsworth, MD; E-mail: ellsworths3@upmc.edu

https://doi.org/10.1016/j.adro.2020.08.002

Conclusions: In patients undergoing RT for upper abdominal malignancies, higher spleen dose is associated with higher per-fraction lymphocyte loss rates, higher total %ΔALC, and increased odds of severe lymphopenia. Spleen dose constraints should be individualized based on baseline ALC and PTV size to minimize RIL risk, although our findings require validation in larger, ideally prospective data sets.

Introduction

Lymphocytes are key mediators of the immune response to cancer. These cells recognize tumor antigens, leading to immune recruitment and activation.[1] Lymphocyte infiltration into tumors is associated with better survival,[2-6] and immunotherapies such as cytotoxic T-lymphocyte–associated protein 4 and programmed cell death-1/programmed cell death ligand-1 inhibitors depend on enhanced lymphocyte activity for their efficacy.[7,8] Response to immunotherapy appears to be at least partially dependent on the presence of adequate numbers of functional lymphocytes.[9,10] T-cells with genetically engineered chimeric antigen receptors can treat hematologic and solid malignancies by directing their antigen specificity.[11]

Radiation therapy (RT) enhances T-cell priming, tumor infiltration, tumor recognition, and tumor cell killing.[12-14] Additionally, clinical and preclinical evidence supports potential synergy between RT and checkpoint inhibitors via immune-mediated abscopal effects.[13,15] However, RT also causes immunosuppression, which has been exploited therapeutically in conditioning regimens for bone marrow transplants,[16] to prevent rejection in organ transplants[17] and treat autoimmune diseases.[18] Lymphocytes are particularly radiosensitive,[19,20] and acute lymphopenia commonly occurs during and after RT. Radiation-induced lymphopenia (RIL) was first identified as a negative prognostic factor in patients with glioma in 2011.[21] Subsequent research demonstrated a strong relationship between RIL and inferior survival in other tumor types,[22,23] including esophageal and pancreatic cancers,[24-28] prompting interest in identifying strategies to reduce RIL risk. Poorer survival from RIL persists even in individuals who quickly recover to near-normal lymphocyte counts after treatment.[29] Mechanisms implicated in RIL include direct toxicity to irradiated lymphocytes in the circulating blood and lymphoid organs. Although irradiation of circulating lymphocytes in the bloodstream is a major cause of RIL,[30] spleen dose is also related to lymphopenia risk, as the spleen is the largest secondary lymphoid organ. The spleen is estimated to contain 15% of lymphocytes, 7 times more than the circulating blood.[31] A relationship between higher spleen dose and increased lymphopenia risk has been described in patients with liver,[32] esophageal,[33] and pancreatic cancers.[26]

The present study aimed to build on prior work that identified splenic dose distribution as an important predictor of lymphopenia risk. We describe a novel approach to analyzing effects of spleen dose distribution on patient-specific lymphocyte loss kinetics, with consequent implications for lymphopenia risk estimation.

Methods and Materials

Data collection

This was an institutional review board–approved, registry-based, retrospective study. All patients had provided informed consent for RT. Clinical information, including absolute lymphocyte count (ALC) values, was obtained from the electronic medical record. Eligible patients were adults ($\geq$18 years) who received conventionally fractionated RT for pancreatic, hepatobiliary, or gastroesophageal (GE) cancers, with mean spleen dose (MSD) $>$ 0 Gy and baseline ALC $\geq$ 500 cells/μL. Patients with immune deficiencies or hematologic malignancies, or who had previously received RT, were excluded. Concurrent chemotherapy was administered in most patients (Table 1). Patients were treated between January 2008 and September 2018 with baseline ALC obtained $\leq$4 weeks before beginning RT. Final ALCs were obtained during the last week of RT or within a week of completion. ALCs and dosimetric data obtained during boost treatments were excluded to avoid confounding by changes in field size. Patients with $<$3 ALC measurements were excluded to permit accurate calculation of lymphocyte loss kinetics. Spleen dose-volume histograms (DVH) were generated using MIM Maestro (MIM Software, Cleveland, OH). The splenic volume (in cm^3) receiving 5, 10, 15, 20, and 25 Gy (V5-V25) was recorded, as was the MSD. Lymphopenia was graded according to the Common Toxicity Criteria for Adverse Events v.4.0; grade 3 lymphopenia was defined as ALC $<$ 500 cells/μL and grade 4 lymphopenia as ALC $<$ 200 cells/μL.

ALC loss rate calculation

ALC loss during the initial phase (fractions 0-15) of partial-body RT is well described by pure exponential decay, permitting calculation of patient-specific ALC loss

Table 1 Patient and treatment characteristics, stratified by site

Median or count	PB (n = 79)	GE (n = 61)	Sig
Age	63 (33-83)	65 (36-84)	0.27
Treatment completed at last ALC (%)	89.3% (60%-100%)	92.0% (70.0%-100%)	0.763
Dose/Fx (cGy)	200 (180-216)	180 (180-202)	<0.001
Number of Fx	25 (22-30)	25 (23-30)	0.980
Treatment duration (days)	38 (30-52)	37 (33-51)	0.659
Chemo	Gem (54), 5-FU (24)	Pac/Plat (34), 5-FU/Plat (24)	-
Stage			-
1	13	8	-
2	35	21	-
3	17	19	-
4	1	10	-
Baseline ALC	1400 (500-4700)	1500 (900-3400)	0.216
Last ALC	300 (0-1300)	200 (100-900)	0.008
%ΔALC	−80.8 (−100.0 to −30.77)	−88.9 (−97.7 to −66.7)	<0.001
≥Grade 3 lymphopenia	62 (78.5%)	57 (93.4%)	0.014
PTV	595.9 (72.2-1366.5)	639.1 (164.0-1510.2)	0.262
FLL (n = 73, 53)	9.9 (3.2-41.9)	12.2 (6.2-25.9)	0.002
Spleen size	248.6 (41.4-1016.0)	231.0 (59.3-651.1)	0.261
MSD	7.5 (0.7-27.1)	16.1 (0.6-43.3)	<0.001
V5	151.7 (1.7-544.3)	155.6 (5.3-569.8)	0.832
V10	50.0 (0-523.4)	142.1 (1.3-514.2)	<0.001
V15	28.7 (0-476.3)	107.7 (0-498.6)	<0.001
V20	14.1 (0-430.7)	70.2 (0-424.9)	<0.001
V25	0.7 (0-373.8)	40.9 (0-322.7)	<0.001

Abbreviations: 5-FU = 5-fluorouracil; ALC = absolute lymphocyte count; FLL = fractional lymphocyte loss; GE = gastroesophageal; Gem = gemcitabine; MSD = mean spleen dose; Pac = paclitaxel; PB = pancreaticobiliary; Plat = cisplatin or carboplatin; PTV = planning target volume.

kinetics.[34] Briefly, ALCs collected during the first 15 fractions were plotted against fraction number for each patient individually. The curve fitting tool in MatLab v.R2018 (Mathworks, Natick, MA) was used to fit individual ALC curves to the equation $ALC(x) = ae^{-bx}$, where x is the number of fractions and a and b are fit parameters corresponding to baseline ALC and lymphocyte loss rate, respectively. Initial percent-per-fraction lymphocyte loss (FLL) is then calculated as $FLL = 100 * (1\text{-}e^{-b})$.

Statistical analysis

The relationship between spleen DVH data and relative change in ALC from baseline during treatment, lymphocyte loss rates, and the last measured ALC during treatment was described using Spearman's rank. The Wilcoxon rank sum test was used to compare treatment parameters between patients with pancreaticobiliary (PB) and GE cancers, as well as median fraction-matched normalized ALCs throughout treatment. The χ^2 test of association was used to compare the incidence of ≥grade 3 lymphopenia between patients with PB and GE cancer. Treatment- and patient-specific parameters between individuals who developed ≥grade 3 lymphopenia and those who did not were also compared using the Wilcoxon rank sum test. With the entire data set pooled together, multiple variable logistic regression was used to identify predictors of ≥grade 3 and grade 4 lymphopenia, using the Box-Tidwell test to assess linearity between continuous predictors and the log-odds of the outcomes. The concordance index (c-index) from the receiver operating curves for the probabilities generated from the logistic regression was used to evaluate goodness-of-fit. All statistical analyses were performed in Statistical Package for the Social Sciences v.25 (IBM, Armonk, NY). The 2-tailed significance level was specified as $\alpha = 0.05$.

Results

Comparison of treatment parameters and loss rates

The analysis included 140 patients who received definitive RT, including 67 (47.9%) with pancreatic cancer, 61 (43.6%) with GE cancer, and 12 (8.6%) with cholangiocarcinoma (Table 1). Baseline clinical parameters related to lymphopenia risk, including baseline ALC and planning target volume (PTV), were similar between patients with PB and GE cancer (Table 2). However, median FLL was 12.2% in patients with GE cancer versus 9.9% in patients with PB cancer ($P = .002$). Patients with GE cancer also had a higher incidence of ≥grade 3 lymphopenia (93.4% vs 78.5%; $P = .014$) and a

Table 2 Median values for patient characteristics and dose-volume parameters in those who developed ≥grade 3 lymphopenia versus those who did not

All upper abdominal			
Median	≥ Grade 3 (n = 119)	< Grade 3 (n = 21)	Sig
Age	64 (33-84)	64 (48-79)	0.998
Treatment completed at last ALC (%)	88.00%	92.00%	0.187
Dose/Fx (cGy)	180 (180-216)	180 (180-216)	0.840
Number of Fx	25 (22-30)	27 (25-30)	0.033
Treatment duration	37 (30-52)	39 (33-47)	0.059
Baseline ALC	1400 (500-3300)	1900 (1200-4700)	<0.001
Change in ALC (%)	−70.8% (−50.0% to −100.0%)	−87.5% (−30.8% to −80.8%)	<0.001
PTV	614.0 (92.8-1510.2)	533.8 (72.2-820.6)	0.041
FLL (n = 108, 18)	11.8 (4.3-41.9)	7.6 (3.12-12.7)	<0.001
Spleen size	239.5 (41.4-1016.0)	220.1 (80.9-579.3)	0.317
MSD	11.1 (0.64-43.4)	7.0 (0.7-12.2)	0.003
V5	159.5 (1.7-569.8)	107.4 (5.78-272.0)	0.007
V10	95.4 (0-523.4)	24.8 (0-144.3)	0.001
V15	60.6 (0-498.6)	12.6 (0-122.5)	0.002
V20	42.2 (0-430.7)	5.1 (0-85.4)	0.002
V25	19.0 (0-373.8)	0.1 (0-45.9)	<0.001

Abbreviations: ALC = absolute lymphocyte count; FLL = fractional lymphocyte loss; MSD = mean spleen dose; PTV = planning target volume. Medians compared via Wilcoxon sum ranks test. Proportions compared via the χ^2 test for association.

significantly lower median last ALC (200 vs 300 cells/μL; $P = .008$) than patients with PB cancer. Higher lymphocyte loss rates were reflected in lower median normalized ALCs at various time points during RT in patients with GE cancer (Fig E1). Spleen doses (MSD, V10, V15, V20, and V25) were also significantly higher in patients with GE cancer. Among all patients, those who developed ≥grade 3 lymphopenia had significantly lower baseline ALC, higher FLL, and larger PTVs, as well as higher spleen doses for all measured parameters. There was no significant difference in spleen size between the 2 groups (Table 2).

Relationship between spleen dosimetry, lymphopenia, and lymphocyte loss kinetics

Because there was a nonlinear relationship between large spleen dose-volume parameters and percent change in ALC during treatment (Fig E2), Spearman's rho was used to describe the relationship for all dose levels. There was a statistically significant negative correlation between percent change in ALC and each dosimetric parameter. Significant correlations were also seen between spleen dose and FLL/last ALC (Fig 1).

Multivariable logistic regression was used to determine whether FLL and spleen dose-volume parameters were associated with risk of ≥grade 3 or grade 4 lymphopenia while controlling for baseline ALC and PTV. Each spleen dose parameter was analyzed individually, as these variables were strongly collinear. Because baseline ALC was not linearly related to the log-odds of developing ≥grade 3 lymphopenia, it was treated as a dichotomous variable, with a baseline ALC ≥ the median (1500 cells/μL) as the reference category. Although there was some variation in the level of significance and the resulting odds ratio (OR), depending on which spleen dose parameter was used as a covariate, baseline ALC < 1500 was significantly associated with increased odds of developing both ≥grade 3 and grade 4 lymphopenia. All dosimetric parameters were

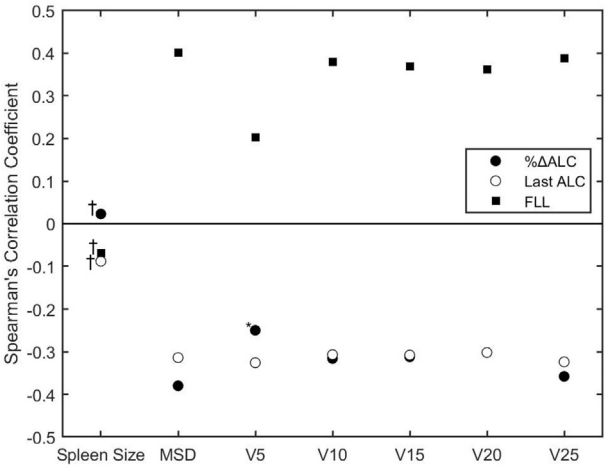

Figure 1 Spearman's correlation coefficient (r_s) for relationship between spleen size/spleen dose parameter and either percent change in absolute lymphocyte count (ALC) (%ΔALC, filled circles), last ALC (open circles), or initial per-fraction loss rate (fractional lymphocyte loss rate, filled squares). All relationships were significant at $\alpha < 0.01$, with the exception of those marked by * (significant at $\alpha < 0.05$) or † (not significant).

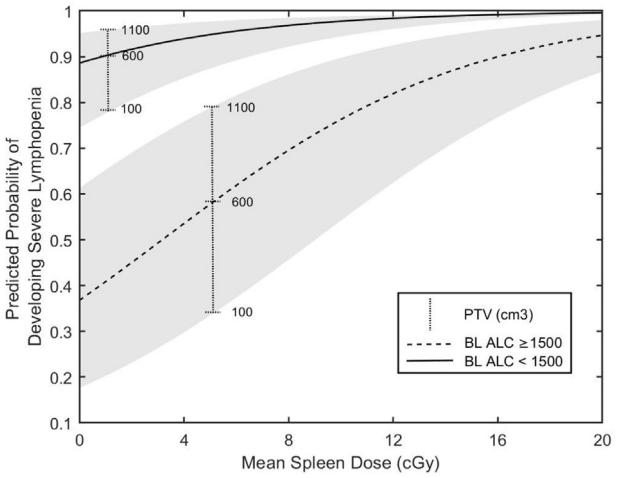

Figure 2 Predicted probability, controlled for baseline (BL) absolute lymphocyte count (ALC), of developing ≥grade 3 lymphopenia based on mean spleen dose (MSD) and planning target volume size (PTV; panel A), with corresponding receiver operating characteristic curve (panel B), illustrating dependence of lymphopenia risk on both PTV size and BL ALC. In panel A, solid and dashed lines represent BL ALC of < 1500 or ≥ 1500 cells/μL, respectively, for PTV of 600 cm^3. The shaded area around each curve represents the family of potential regression curves depending on PTV, with the top and bottom of each area corresponding to PTVs of 1100 and 100 cm^3, respectively. Regression results: logit (developing ≥grade 3 lymphopenia) = 2.59 (if BL ALC < 1500 cells/μL) + 0.002 × (PTV) + 0.171 × (MSD) − 1.742.

significant predictors of ≥grade 3 lymphopenia, with MSD having the strongest relationship (OR, 1.186; confidence interval [CI], 1.056-1.332). Although V5 was a significant predictor of grade 4 lymphopenia, all other parameters were borderline insignificant. FLL was a significant predictor of both ≥grade 3 (OR, 2.276; CI, 1.477-3.50) and grade 4 lymphopenia (OR, 1.124; CI, 1.025-1.233).

Results of the multiple variable logistic regression model based on MSD are shown in Figure 2A, which plots the predicted probability of ≥grade 3 lymphopenia versus mean spleen dose and PTV size, split by baseline ALC. Individual lymphopenia risk probabilities based on the combination of baseline ALC, mean spleen dose, and PTV size can be calculated from this curve. The model predicts that a 1-Gy increase in MSD increases ≥grade 3 lymphopenia risk by 18.6%. Additionally, each 100-cm^3 increase in PTV increases the risk of ≥grade 3 lymphopenia by 20%. A receiver operating characteristic curve was created from the MSD model to determine a cutoff for the model probability that would best balance specificity and sensitivity in identifying individuals who will develop ≥grade 3 lymphopenia, which was found to be at 81% (sensitivity of 82.4% and specificity of 85.7%; Fig 2B). Given this optimal cutoff of an 81% probability, these data can begin suggesting dose constraints for the spleen (Table 3). In patients with a baseline ALC < 1500 cells/μL, avoiding ≥grade 3 lymphopenia is difficult. Even if no dose was delivered to the spleen, a PTV < 301 cm^3 would be needed for a predicted probability <81%. For the median PTV size of 610.9 cm^3 in this study, that probability constraint cannot be met for any MSD. Patients with baseline ALC ≥ 1500 cells/μL can tolerate higher spleen doses. At the median PTV size of 610.9 cm^3, a patient could tolerate an MSD of up to 11.5 Gy to meet a predicted probability of 81%. For PTVs of 100 cm^3 and 1100 cm^3, MSD constraints of 17.5 Gy and 5.8 Gy would be needed, respectively.

Table 3 Cells contain MSD (Gy) necessary to keep the predicted probability of developing ≥grade 3 lymphopenia at various probabilities for different PTVs

	Predicted probability of ≥grade 3 lymphopenia if BL ALC ≥ 1500 cells/μL						
	81%	70%	60%	50%	40%	30%	20%
PTV (cm^3)							
100	17.5	14.0	11.4	9.0	6.6	4.1	0.9
350	14.6	11.0	8.5	6.1	3.7	1.1	*
600	11.6	8.1	5.5	3.2	0.8	*	*
850	8.7	5.2	2.6	0.2	*	*	*
1100	5.8	2.3	*	*	*	*	*

Abbreviations: ALC = absolute lymphocyte count; BL = baseline; MSD = mean spleen dose; PTV = planning target volume.
* Denotes combinations where any MSD > 0 would exceed the predicted probability.

Discussion

Our work adds to the growing body of literature implicating incidental spleen irradiation in the pathophysiology of lymphopenia. It shows that higher spleen doses are correlated with increased lymphocyte loss rate, which is at least as good a predictor of the odds of severe lymphopenia as spleen dose parameters, if not better. These findings are clinically significant in light of previous work implicating RIL as a risk factor for decreased overall survival in pancreatic and esophageal cancers. Although this group of patients was too heterogenous to perform a survival analysis, it provided a diverse cohort to analyze the predictive utility of spleen dose distribution and lymphocyte loss rates and showed that spleen dosimetry is associated with lymphopenia risk independent of treated site.

Our findings suggest that spleen dose constraints may need to be individualized based on baseline patient characteristics. Patients with higher baseline ALC can tolerate higher spleen doses than patients with lower baseline lymphocyte counts, assuming dose distribution is similar in the remainder of the body. In general, our findings concur with previous literature. Liu et al[32]

reported a negative correlation between nadir ALC and MSD in addition to spleen V5-V30 in hepatocellular carcinoma. The present findings are similar; we observed statistically significant negative relationships among all observed spleen dose-volume parameters in 5-Gy increments between V5 and V25 and percent ALC lost from baseline. In the present series, spleen V5-V25 values were also significantly correlated with the odds of developing ≥grade 3 lymphopenia after controlling for PTV and baseline ALC. Chadha et al[26] examined prognostic factors for lymphopenia (measured as nadir values 2-10 weeks post-RT) in individuals with locally advanced pancreatic cancer. They found that individuals who had higher MSD or higher V5-V20 relative to spleen size (dichotomized at the mean) had increased odds of developing ≥grade 3 lymphopenia, with MSD being the strongest predictor of lymphopenia risk. Similarly, Saito et al[33] reported that MSD and V5-V30 were linearly correlated with log-transformed nadir ALC during RT for esophageal cancer and noted that higher MSD was the only significant dosimetric predictor of grade 4 lymphopenia. In this study, higher values across all analyzed spleen dose parameters were associated with a significant increase in ≥grade 3 lymphopenia risk, whereas only spleen V5 was correlated with grade 4 lymphopenia. In addition to reinforcing the link between spleen dose and lymphopenia, the present study shows a biological gradient of the effects of individual spleen dose-volume parameters on lymphopenia because they were analyzed as continuous variables. Additionally, we found that spleen dose-volume parameters were correlated with the log odds of developing grade 4 and ≥grade 3 lymphopenia.

After controlling for confounders, initial FLL was significantly correlated with ≥grade 3 or grade 4 lymphopenia risk. To the authors' knowledge, this study is the first to use per-fraction lymphocyte loss rate as a correlate of splenic dose distribution and a predictor of overall lymphopenia risk. This approach to the analysis extends the generalizability of the present findings to hypofractionated and conventionally fractionated plans, as per-fraction lymphocyte loss rate is independent of total treatment course duration.

It is important to note that spleen dosimetry alone cannot fully explain lymphocyte loss kinetics and lymphopenia risk in patients undergoing RT to the upper abdomen. Dose distributions in other lymphocyte-containing structures such as gut-associated lymphoid tissue, regional lymphatic ducts and lymph nodes, as well as the circulating blood itself, are not accounted for in the present analysis. The influence of these other structures on RIL is evident because there is a nonzero probability of developing ≥grade 3 lymphopenia even with zero dose to the spleen (Fig 2). However, a preliminary logistic regression model built with mean doses to the liver, heart, lungs, and spleen in patients with GE suggested that among solid organs, only dose to the spleen had a statistically significant effect on the likelihood of developing ≥grade 3 lymphopenia in this cohort. Furthermore, individual variations in lymphocyte radiosensitivity and lymphocyte repopulation after radiation exposure probably account for some of the observed differences in lymphocyte loss rates among patients with similar dose distributions. Additional study is needed to determine the extent to which these nondosimetric factors affect lymphocyte loss kinetics and lymphopenia risk.

Limitations of the present work include its retrospective nature and relatively small sample size, which precluded a survival analysis and inclusion of further patient characteristics, such as neoadjuvant or concurrent chemotherapy, in our regression models. The use of neoadjuvant chemotherapy has been shown to not affect baseline ALC in patients who go on to receive chemoradiation.[35] The effect of concurrent chemotherapy agents on lymphocyte loss kinetics in prior analyses appears to be relatively small, albeit statistically significant, and the present data set did not provide adequate statistical power to detect small effects of differing chemotherapy regimens on lymphocyte loss dynamics during RT.[34] Future analyses with larger sample sizes could further illuminate how chemotherapy backbone affects toxicity to circulating lymphocyte populations during concurrent treatment. For the logistic regression analyses, patients with GE and PB were combined for added power, despite potential differences in those 2 groups. Any negative results should be interpreted with caution given the relatively low power. Additionally, there may be a risk of overfitting in the logistic regression models due to the low number of patients who did not develop ≥grade 3 lymphopenia.

Despite these limitations, our data may prove useful in establishing a starting point for setting splenic dose constraints with the goal of minimizing the risk of severe RIL. Given the relationship between spleen dose and lymphopenia seen here and in other studies, spleen DVHs should be more regularly assessed during treatment planning, especially in patients with low baseline ALC. Because these constraints are based on parameters known before RT begins, including PTV size, MSD, and baseline ALC, this method can assist in identifying patients who are at high risk of RIL regardless of splenic dose. Such individuals might benefit from strategies to reduce RIL risk, including proton therapy or hypofractionation.[36,37] The splenic dose constraints suggested here should be validated prospectively; further research is also needed to determine whether spleen-sparing plans can lower RIL risk to a clinically acceptable level.

Supplementary Materials

Supplementary material for this article can be found at https://doi.org/10.1016/j.adro.2020.08.002.

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