ORIGINAL ARTICLE

Radiotherapy-Related Lymphopenia Affects Overall Survival in Patients With Lung Cancer

Azadeh Abravan, PhD,[a,b,*] Corinne Faivre-Finn, PhD,[a,b] Jason Kennedy, MSc,[b] Alan McWilliam, PhD,[a,b] Marcel van Herk, PhD[a,b]

[a]*Division of Cancer Sciences, School of Medical Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom*
[b]*Radiotherapy Related Research, The Christie NHS Foundation Trust, Manchester, United Kingdom*

Received 8 March 2020; revised 10 June 2020; accepted 11 June 2020
Available online - 14 June 2020

ABSTRACT

Introduction: Lymphopenia after radiotherapy has an adverse effect on the patient's outcome. However, the relationship between radiotherapy dose delivery and lymphopenia is not fully understood. This work used image-based data mining to identify anatomical regions where the received dose is correlated with severe lymphopenia.

Methods: A total of 901 patients with lung cancer were analyzed. A Cox model was used to assess prognostic factors of overall survival (OS). Two matched groups were defined—patients with lymphopenia of grade 3 or higher and patients without lymphopenia of grade 3—based on tumor volume, baseline lymphocytes, and prescribed dose. Then, data mining was used to identify regions where dose correlates significantly with lymphopenia of grade 3 or higher. For this, dose matrices were aligned using registration of the computed tomography images to one reference patient. Mean dose distributions were obtained for the two groups, and organs of significance were detected. Dosimetric parameters from the identified organs that had the highest correlation with lymphocytes at nadir were selected. Multivariable analysis was conducted for lymphopenia of grade 3 or higher on the full lung cohort, and the model was tested on 305 patients with esophageal cancer.

Results: Adjusted Cox regression revealed that lymphopenia of grade 3 or higher is an independent factor of OS. The anatomical regions identified were the heart, lung, and thoracic vertebrae. Dosimetric parameters for lymphopenia included thoracic vertebrae V_{20}, mean lung dose, and mean heart dose, which were further validated in the esophageal cancer cohort.

Conclusions: We report that severe lymphopenia during radiotherapy is a poor prognostic factor for OS in patients with lung cancer and could be mitigated by minimizing thoracic vertebrae V_{20}, mean lung dose, and mean heart dose to limit the irradiation of stem cells and blood pool.

Keywords: Image-based data mining; Lymphopenia; Radiotherapy; Chemoradiotherapy; Lung cancer; Esophageal cancer

Introduction

Lung cancer is one of the most common types of cancer and the leading cause of cancer-related deaths worldwide.[1,2] Radiotherapy with or without chemotherapy is the mainstay treatment for lung cancer as most patients are not suitable for operation. Survival rates remain poor with 5-year overall survival (OS) rates of 15% to 30% for nonmetastatic SCLC and NSCLC.[3,4] These rates, however, have recently improved with the addition of immunotherapy in patients with locally advanced NSCLC, but only a subset of patients would benefit.[5]

The immune system plays a major role in cancer progression, suppression, and treatment outcome. Radiotherapy induces immune system activation

*Corresponding author.

Disclosure: The authors declare no conflict of interest.

Address for correspondence: Azadeh Abravan, PhD, Division of Cancer Sciences, School of Medical Sciences, Faculty of Biology, Medicine and Health/Radiotherapy Related Research, The University of Manchester, Department 58, Floor 2a, Wilmslow Road, Withington, Manchester M20 4BX, United Kingdom. E-mail: azadeh.abravan@manchester.ac.uk

ISSN: 1556-0864
https://doi.org/10.1016/j.jtho.2020.06.008

through the production of a local inflammatory response, cytokine signaling cascades, or tumor antigen release and tumor cell killing; leading to tumor regression both inside of the radiation field and outside, known as the abscopal effect.[6] However, radiotherapy can also be immunosuppressive through increasing immunosuppressive regulatory cells and stimulation of inhibitory cytokines. In addition, irradiation of primary lymphoid organs, including functional bone marrow and thymus, and secondary lymphoid organs, such as the spleen, may lead to immunosuppression.[7–9] Furthermore, radiotherapy may directly suppress immune function through the destruction of mature circulating lymphocytes. This is particularly problematic for patients with thoracic diseases, for whom radiation exposure of blood pool is substantial because the heart, large blood vessels, and lung are often included in the radiation field. Lymphocytes, circulating continuously between peripheral blood and tissue, account for 20% to 30% of total white blood cells and are of importance in the immune response to cancer.[10] They are one of the most radiosensitive cells within the human body, in which DNA fragmentation can occur at a radiation dose as low as 0.5 Gy.[11–13] It has been estimated that 99% of circulating blood receives at least 0.5 Gy in patients with glioma treated with 60 Gy in 2 Gy fractions using either intensity-modulated or three-dimensional conformal radiotherapy.[14]

Lymphopenia, a drop in lymphocyte counts, may occur after radiotherapy in patients with lung cancer and has been reported as a prognostic factor of poor OS.[15–17] In the literature, the incidence and severity of lymphopenia are associated with chemotherapy delivery, duration of radiotherapy, volume of irradiation field, and irradiation dose of lung, heart, or integral body.[16,18–21]

Despite the growing evidence on the impact of radiotherapy-related lymphopenia on lung cancer survival and response to immunotherapy consolidation, a comprehensive study identifying all regions that are responsible for severe lymphopenia after radiotherapy is still lacking. In this work, we aimed to identify the anatomical regions where the received dose is correlated with severe lymphopenia (grade 3 or higher; owing to its clinical importance) in patients with lung cancer treated with curative-intent radiotherapy (with or without chemotherapy). We used a data mining analysis using the whole radiotherapy dose distribution to identify all regions contributing to lymphopenia, without previous assumptions as to which organs are responsible.

Materials and Methods

Study Design

A retrospective analysis was performed for patients with lung cancer (as a development set), including both SCLC and NSCLC, and patients with esophageal cancer (as an independent validation set) treated with definitive radiotherapy at a single academic cancer center between 2005 and 2017. Patients receiving repeated radiotherapy or reirradiation to the thoracic region were excluded to avoid the complications associated with estimating the combined radiotherapy dose delivery.

Data were collected for patients who had full blood counts recorded up to 3 months before initiation of the radiotherapy. For patients with multiple recorded counts before radiotherapy, the value closest to the start of radiotherapy was collected and taken as the baseline value. Those patients with baseline lymphocyte counts of less than 0.5×10^9/liter were excluded. In addition, we collected full blood count during radiotherapy, where available. During radiotherapy, the lowest lymphocyte count was collected and taken as lymphocyte count at nadir. Thereafter, we only included patients with available counts both at baseline and during radiotherapy in the analyses ($n = 1032$; Supplementary Fig. 1). For these patients, planning computed tomography (CT) scans (Philips or Siemens), planned dose distributions, and radiotherapy planning contours were collected. Radiotherapy planning data were collected from the treatment planning system archive (Philips Pinnacle treatment planning system). All patients were scanned and treated in free breathing. For 131 patients, radiotherapy image collection or processing failed. This resulted in the records of 901 patients with lung cancer available for analyses (Supplementary Fig. 1). Patients' characteristics and demographic data were further collected. Chemotherapy delivery was manually checked for each patient using the hospital electronic patient record. The same procedure was performed for patients with esophageal cancer, which resulted in 305 patients suitable for the validation analysis. This project was approved by the UK Computer Aided Theragnostics Research Database Management Committee (research ethics committee reference no.: 17/NW/0060).

Patients with lung cancer were treated with standard fractionation regimes of either 45 Gy in 30 fractions (twice daily fractions), 50 to 55 Gy in 20 fractions, or 60 to 66 Gy in 30 or 33 fractions, with no, sequential, or concurrent chemotherapy. Patients with lung cancer included in this study did receive radiotherapy (with or without chemotherapy) as their primary treatment because they were deemed not eligible for operation for medical or technical reasons by the local multidisciplinary tumor board. Patients with esophageal cancer received either 50 Gy in 25 fractions or 55 Gy in 20 fractions, with or without concurrent chemotherapy.

Radiotherapy Dose Registration

Planning CT images were spatially registered to a randomly chosen reference patient by deformable registration of a region of interest covering the lung surface while disregarding the chest wall. Radiotherapy dose matrices were then deformed to the reference CT using the deformation vectors obtained from their corresponding planning CT. To account for fractionation, dose distributions were converted to biologically effective dose assuming α/β is equal to 10 for the lymphocyte counts and a conversion to equivalent dose of 2-Gy fractions.

Data Mining for Lymphopenia

Lymphopenia during radiotherapy was graded by the Common Terminology Criteria for Adverse Events, version 4.0, and lymphopenia of grade 3 or higher was based on the lymphocyte counts at nadir (lowest lymphocyte counts during radiotherapy $< 0.5 \times 10^9$/liter). Treatment field size, tumor effect, and integral dose depend on tumor volume, and is therefore a strong confounder in the dose-response analysis of lymphocytes during radiotherapy. To exclude this effect and improve the sensitivity of data mining and parameter selection, we defined two matched groups for patients who developed lymphopenia of grade 3 or higher during radiotherapy and patients without lymphopenia of grade 3; taken from the development set of lung cancer data. Matching was done taking planning target volume (PTV), baseline lymphocytes, and prescribed dose for the two groups into account. Propensity score matching was used without replacement using a ratio of 1:1 and a caliper distance of 0.1. After matching, image-based data mining was used to identify regions where dose correlates significantly with the incidence of lymphopenia of grade 3 or higher. Mean dose distributions were then obtained for the two groups, and regions of significance were identified. To test whether the dose difference between the two groups was statistically significant, permutation testing was performed (1000 permutations), and the maximum t values over the entire dose distribution were used to test the significance.[22] For the identified organs from image-based data mining, various dose parameters were collected, again for the matched groups, and those parameters having the most negative correlation with lymphocyte counts at nadir were selected for the analysis. Finally, the dose-response model was developed on the full group of 901 patients with lung cancer and tested on 305 patients with esophageal cancer.

Statistical Analysis

Factors affecting survival were assessed by generating Kaplan-Meier curves and conducting log-rank tests and multivariable Cox regression analyses. Univariate and multivariable logistic regression analyses were conducted to investigate the association between lymphopenia of grade 3 or higher and the identified dose parameters, along with nondosimetric parameters for the development set. For multivariable Cox and logistic regressions, models were created with backward elimination, based on a Wald chi-square, using a p value greater than 0.05 for removal of variables. Continuous variables were compared using the Student's t test. The goodness-of-fit was tested by Hosmer-Lemeshow test on the basis of deciles of risk. Discrimination was assessed by the area under the receiver operating characteristic curve (C-statistic). The 2-tailed p values less than 0.05 were considered to be significant. Statistical analyses were performed in R 3.6.1 (R Core Team, Vienna, Austria).

Results

Overall Survival

Data from 901 patients with lung cancer and 305 patients with esophageal cancer have been included in this study as development and validation sets, respectively. Patients' characteristics are listed in Table 1. Median follow-up for patients with lung cancer was 17.4 months (range 1.1–158.0 mo), and 751 deaths (83%) were recorded over the course of the follow-up. For the lung cancer cohort, the log-rank test revealed that lymphopenia of grade 3 or higher during radiotherapy is correlated with worse survival (log-rank hazard ratio [HR] = 1.25; $p = 0.002$) (Fig. 1*A*). For the esophageal cancer cohort, patients with lymphopenia of grade 3 or higher had worse OS, although this was not significant during radiotherapy (log-rank HR = 1.20; $p = 0.2$). However, grade 4 lymphopenia was significantly correlated with worse OS (log-rank HR = 1.82; $p < 0.001$) (Fig. 1*B*). Survival analyses were further carried out separately for patients with SCLC and NSCLC owing to the differences in the nature and anatomical distribution of these malignancies. Univariate and multivariable analyses of OS for SCLC and NSCLC are summarized in Table 2. Multivariable Cox analyses revealed that lymphopenia of grade 3 or higher, tumor volume, and chemotherapy delivery were significantly correlated with OS in both lung cancer types. Cox models for SCLC and NSCLC were further adjusted for age and prescribed dose, respectively (Fig. 1*C* and *D*). For patients with SCLC, median survival was 21 months for those without grade 3 lymphopenia compared with 15 months for those who developed grade 3 lymphopenia during radiotherapy (adjusted HR = 1.29; $p = 0.04$). For NSCLC, median survival was 22 months for patients without grade 3 lymphopenia compared with 17 months for those with grade

Table 1. Patient and Tumor Characteristics for Lung and Esophageal Cohorts Stratified Based on Lymphopenia Grade 3 During Radiotherapy

	NSCLC Cohort (n = 584)		SCLC Cohort (n = 317)		Esophageal Cohort (n = 305)	
			Lymphopenia Grade 3			
Parameters	No	Yes	No	Yes	No	Yes
Sex						
Female	109 (41.4)	154 (58.6)	90 (56.2)	70 (43.8)	25 (23.6)	81 (76.4)
Male	125 (38.9)	196 (61.1)	84 (53.5)	73 (46.5)	59 (29.6)	140 (70.4)
Age, y						
Mean (SD)	67.6 (10.0)	64.0 (9.9)	62.9 (9.1)	64.9 (8.5)	66.4 (10.9)	70.6 (9.1)
ECOG PS						
0	44 (34.9)	82 (65.1)	42 (64.6)	23 (35.4)	16 (23.5)	52 (76.5)
1	106 (34.8)	199 (65.2)	87 (50.6)	85 (49.4)	46 (27.5)	121 (72.5)
2	50 (54.9)	41 (45.1)	33 (61.1)	21 (38.9)	19 (35.8)	34 (64.2)
3	10 (47.6)	11 (52.4)	5 (41.7)	7 (58.3)	2 (20.0)	8 (80.0)
NA	24 (60.0)	16 (40.0)	7 (50.0)	7 (50.0)	1 (16.7)	5 (83.3)
Histology						
Adenocarcinoma	100 (44.1)	127 (55.9)	—	—	31 (20.8)	118 (79.2)
Squamous cell carcinoma	85 (34.0)	165 (66.0)	—	—	50 (32.9)	102 (67.1)
Small cell carcinoma	—	—	173 (55.3)	140 (44.7)	—	—
NoS	21 (33.3)	42 (66.7)	1 (25.0)	3 (75.0)	2 (66.7)	1 (33.3)
NK	28 (63.6)	16 (36.4)	—	—	1 (100.0)	0 (0.0)
Tumor laterality						
Left	104 (40.8)	151 (59.2)	80 (55.6)	64 (44.4)	—	—
Right	130 (39.5)	199 (60.5)	94 (54.3)	79 (45.7)	—	—
T stage						
1	27 (58.7)	19 (41.3)	32 (72.7)	12 (27.3)	3 (18.8)	13 (81.2)
2	89 (46.8)	101 (53.2)	47 (53.4)	41 (46.6)	22 (29.7)	52 (70.3)
3	45 (33.8)	88 (66.2)	36 (61.0)	23 (39.0)	34 (23.6)	110 (76.4)
4	58 (32.2)	122 (67.8)	39 (48.8)	41 (51.2)	9 (33.3)	18 (66.7)
NA	15 (44.1)	19 (55.9)	20 (43.5)	26 (56.5)	16 (37.2)	27 (62.8)
N stage						
0	92 (54.4)	77 (45.6)	34 (60.7)	22 (39.3)	30 (28.3)	76 (71.7)
1	17 (34.0)	33 (66.0)	18 (62.1)	11 (37.9)	31 (24.6)	95 (75.4)
2	80 (31.2)	176 (68.8)	65 (53.3)	57 (46.7)	9 (30.0)	21 (70.0)
3	20 (34.5)	38 (65.5)	27 (61.4)	17 (38.6)	0 (0.0)	4 (100.0)
NA	25 (50.0)	25 (50.0)	30 (45.5)	36 (54.5)	14 (36.8)	24 (63.2)
Baseline lymphocyte, $\times 10^9$/liter						
Mean (SD)	2.4 (6.4)	1.7 (0.9)	2.1 (0.8)	1.8 (0.9)	2.1 (0.8)	1.5 (0.6)
Follow-up, mo						
Mean (SD)	30.7 (30.2)	25.0 (27.2)	30.7 (29.6)	27.1 (32.7)	34.9 (37.9)	28.5 (29.5)
Chemotherapy						
Concurrent	65 (22.3)	227 (77.7)	100 (53.2)	88 (46.8)	55 (26.1)	156 (73.9)
Radiotherapy only	86 (58.5)	61 (41.5)	24 (63.2)	14 (36.8)	29 (30.9)	65 (69.1)
Sequential	83 (57.2)	62 (42.8)	50 (54.9)	41 (45.1)	—	—
PTV, Ln						
Mean (SD)	5.8 (0.6)	6.1 (0.5)	5.8 (0.5)	5.9 (0.5)	5.7 (0.3)	5.8 (0.4)
Prescribed dose and fractions, Gy						
Mean (SD)	57.2 (4.7)	61.5 (5.2)	49.9 (5.8)	55.7 (8.2)	51.8 (2.4)	51.5 (2.3)
20	175 (60.8)	113 (39.2)	73 (56.2)	57 (43.8)	30 (30.9)	67 (69.1)
25	—	—	—	—	54 (26.0)	154 (74.0)
30	12 (21.1)	45 (78.9)	94 (70.1)	40 (29.9)	—	—
33	47 (19.7)	192 (80.3)	7 (13.2)	46 (86.8)	—	—
Twice daily fractions						
No	—	—	87 (43.7)	112 (56.3)	—	—
Yes	—	—	87 (73.7)	31 (26.3)	—	—
Radiotherapy duration, d						
Mean (SD)	30.9 (8.1)	38.8 (8.8)	23.6 (6.8)	33.1 (14.2)	31.6 (7.0)	31.0 (3.6)

Note: Values are n (%) unless indicated otherwise.
ECOG PS, Eastern Cooperative Oncology Group performance status; NA, not available; NoS, not otherwise specified; NK, not known; PTV, planning target volume.

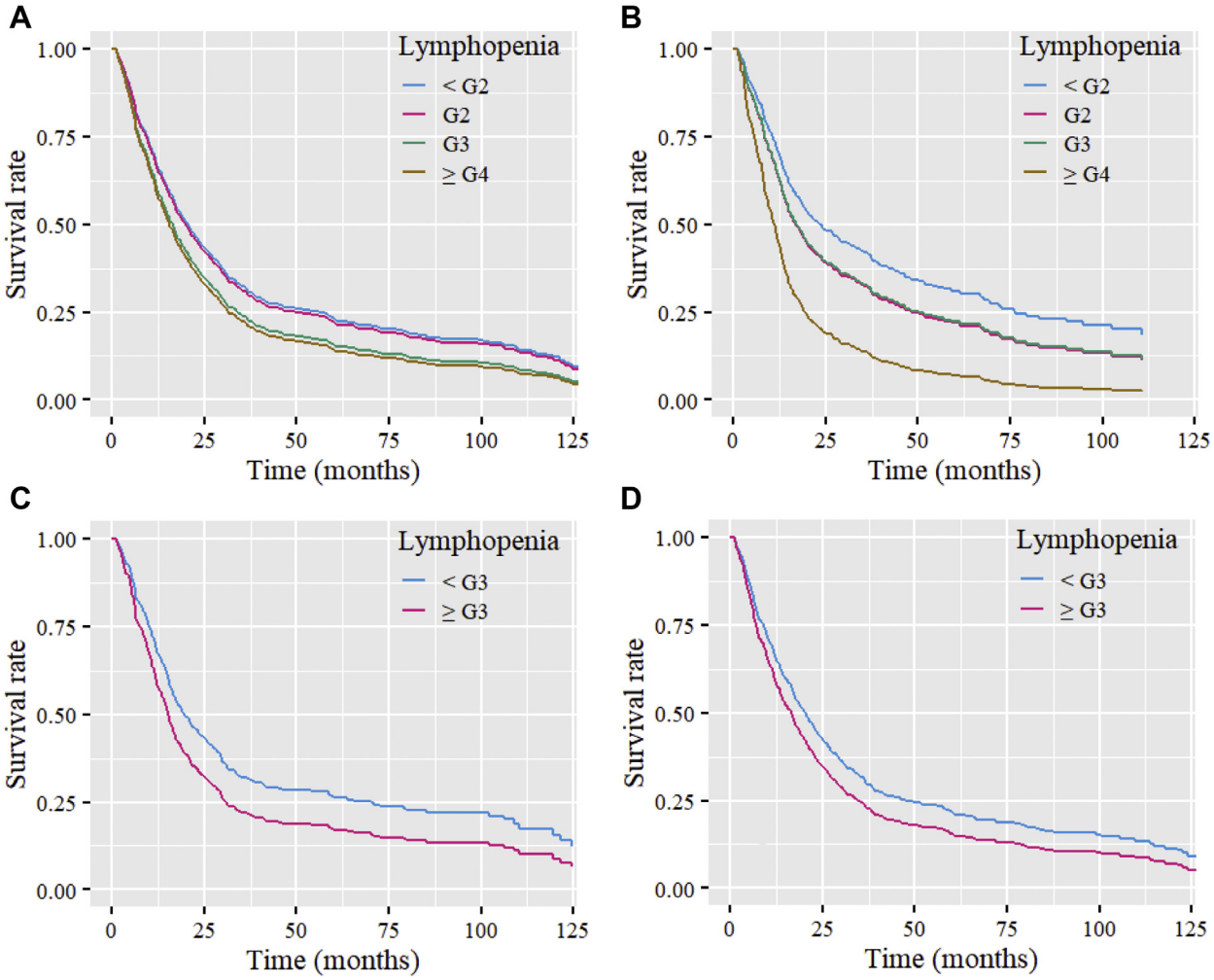

Figure 1. Kaplan-Meier (log-rank test) curves reveal overall survival in patients with (*A*) lung cancer and (*B*) esophageal cancer. Patients were stratified by lymphocytes at nadir according to the Common Terminology Criteria for Adverse Events V4 as less than grade 1, grade 2, grade 3, and grade 4 or higher. Multivariable (adjusted) Cox regression survival curves for the (*C*) SCLC and (*D*) NSCLC cohorts stratified by lymphopenia grade 3. For SCLC, the curves were adjusted for tumor volume, chemotherapy status, and age, whereas for NSCLC, the curves were adjusted for tumor volume, chemotherapy status, and prescribed dose. G, grade.

3 lymphopenia during radiotherapy (adjusted HR = 1.50; $p < 0.001$).

Lymphopenia

To indicate that patients with blood test both before radiotherapy and during radiotherapy are not a selected group, we compared the counts from this group, at the corresponding time points, with those patients who had either baseline or during radiotherapy counts recorded. There were no significant differences in baseline lymphocyte count between those patients with lung cancer who had full blood counts recorded during radiotherapy (average: 1.96, SD: 3.31) and those who did not (average: 1.73, SD: 3.26) (*t* test; $p > 0.1$). This was also valid for lymphocytes at nadir between those patients who had full blood counts recorded before radiotherapy (average: 0.52, SD: 0.46) and those who did not (average: 0.54, SD: 0.78) (*t* test; $p > 0.6$).

For the lung cohort, 738 patients (82%) had lymphopenia of grade 2 or higher during radiotherapy, of which 399 patients (44%) developed grade 3 and 94 patients (10%) developed grade 4. A significant reduction was observed in lymphocyte counts during radiotherapy compared with baseline, irrespective of lung cancer type or chemotherapy delivery (paired *t* test; $p < 0.001$) (Supplementary Fig. 2). For the esophageal cohort, 277 patients (91%) had at least grade 2 lymphopenia, of which 172 patients (56%) developed grade 3 and 49 patients (16%) developed grade 4 during the course of radiotherapy.

Table 2. Univariate and Multivariable Cox Regression Analyses for SCLC and NSCLC Cohorts. Variables Included in the Multivariable Cox Regression Were Chosen After Backward Elimination

	SCLC Cohort (n = 317)			NSCLC Cohort (n = 584)		
Parameters		HR (Univariate)	Adjusted HR (Multivariable)		HR (Univariate)	Adjusted HR (Multivariable)
Sex						
Female	160 (50.5)	—	—	263 (45.0)	—	—
Male	157 (49.5)	1.18 (0.92-1.52, $p = 0.185$)	—	321 (55.0)	1.21 (1.01-1.44, **$p = 0.038$**)	—
Age, y						
Mean (SD)	63.8 (8.9)	1.02 (1.01-1.04, **$p = 0.005$**)	1.01 (1.00-1.03, $p = 0.144$)	65.4 (10.1)	1.01 (1.00-1.02, **$p = 0.033$**)	—
ECOG PS						
0	65 (20.5)	—	—	126 (21.6)	—	—
1	172 (54.3)	1.54 (1.08-2.18, **$p = 0.016$**)	—	305 (52.3)	0.99 (0.79-1.25, $p = 0.958$)	—
2	54 (17.0)	2.35 (1.55-3.57, **$p < 0.001$**)	—	91 (15.6)	1.11 (0.83-1.49, $p = 0.473$)	—
3	12 (3.8)	2.64 (1.35-5.14, **$p = 0.004$**)	—	21 (3.6)	1.46 (0.91-2.34, $p = 0.112$)	—
NA	14 (4.4)	2.12 (1.13-3.96, **$p = 0.019$**)	—	40 (6.9)	1.33 (0.92-1.93, $p = 0.131$)	—
Baseline lymphocytes, $\times 10^9$/liter						
Mean (SD)	2.0 (0.9)	0.80 (0.68-0.94, **$p = 0.007$**)	—	2.0 (4.1)	1.00 (0.98-1.02, $p = 0.932$)	—
Lymphopenia grade 3						
No	174 (54.9)	—	—	234 (40.1)	—	—
Yes	143 (45.1)	1.39 (1.08-1.78, **$p = 0.010$**)	1.29 (1.00-1.67, **$p = 0.044$**)	350 (59.9)	1.17 (0.98-1.40, **$p = 0.018$**)	1.50 (1.22-1.84, **$p < 0.001$**)
Chemotherapy						
Radiotherapy only	38 (12.0)	—	—	147 (25.2)	—	—
Concurrent	188 (59.3)	0.51 (0.35-0.73, **$p < 0.001$**)	0.46 (0.30-0.69, **$p < 0.001$**)	292 (50.0)	0.68 (0.55-0.84, **$p < 0.001$**)	0.61 (0.45-0.82, **$p = 0.001$**)
Sequential	91 (28.7)	1.24 (0.84-1.83, $p = 0.281$)	1.16 (0.77-1.74, $p = 0.477$)	145 (24.8)	1.22 (0.96-1.55, $p = 0.102$)	1.11 (0.87-1.42, $p = 0.396$)
PTV, ln						
Mean (SD)	5.9 (0.5)	1.90 (1.49-2.43, **$p < 0.001$**)	2.28 (1.73-3.00, **$p < 0.001$**)	6.0 (0.5)	1.40 (1.18-1.66, **$p < 0.001$**)	1.72 (1.43-2.07, **$p < 0.001$**)
Prescribed dose, Gy						
Mean (SD)	52.5 (7.5)	1.00 (0.99-1.02, $p = 0.987$)	—	59.8 (5.5)	0.96 (0.94-0.98, **$p < 0.001$**)	0.96 (0.94-0.99, **$p = 0.002$**)
Radiotherapy duration, d						
Mean (SD)	27.9 (11.8)	1.00 (0.99-1.01, $p = 0.719$)	—	35.6 (9.4)	0.98 (0.97-0.99, **$p < 0.001$**)	—
Twice daily fractions						
No	199 (62.8)	—	—	—	—	—
Yes	118 (37.2)	0.66 (0.50-0.86, **$p = 0.002$**)	—	—	—	—

Note: Values are n (%) unless indicated otherwise. *p*-values < 0.05 are in bold.
HR, hazard ratio; ECOG PS, Eastern Cooperative Oncology Group performance status; NA, not available; PTV, planning target volume.

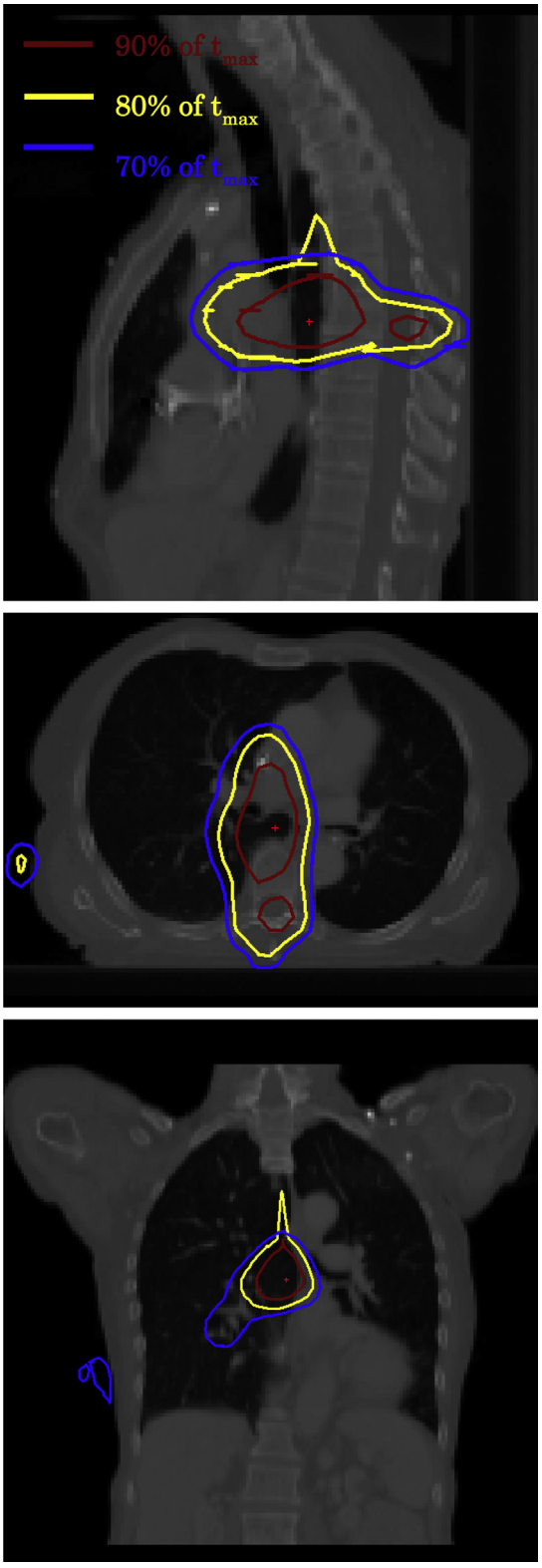

Figure 2. From top to bottom: sagittal, axial, and coronal views of the planning computed tomography of the reference patient. Different contours reveal the statistical significance from the 1000 permutation testing ($p < 0.01$). This reveals that excess dose to the heart, lung, and thoracic vertebrae is highly correlated with the incidence of grade 3 lymphopenia.

After the matching process, 386 patients were eligible for data mining (Supplementary Fig. 3). Data mining revealed the regions in the heart, lung, and thoracic vertebrae where the difference in dose between the matched groups, with and without grade lymphopenia of grade 3 or higher, was significant (Fig. 2). On the basis of this finding, the total heart, lungs, and thoracic vertebrae were chosen as organs at risk. Mean dose to the heart and lung and thoracic vertebrae V_{20} (volume receiving > 20 Gy) had the most negative correlations with lymphocyte counts at nadir in the matched set (Fig. 3). The full logistic regression model on the development cohort included sex, age, histology, N stage, performance status, baseline lymphocytes, concurrent chemoradiotherapy, prescribed dose, prescribed fractionation, radiotherapy duration (overall radiotherapy treatment time), twice-daily fractionation, mean heart dose, mean lung dose, and thoracic vertebrae V_{20}. A model including age (adjusted OR $= 1.02$; $p = 0.03$), baseline lymphocytes (adjusted OR $= 0.58$; $p < 0.001$), concurrent chemoradiotherapy (adjusted OR $= 1.54$; $p = 0.02$), radiotherapy duration (adjusted OR $= 1.09$; $p < 0.001$), mean heart dose (adjusted OR $= 1.03$; $p = 0.04$), mean lung dose (adjusted OR $= 1.05$; $p = 0.04$), and thoracic vertebrae V_{20} (adjusted OR $= 1.01$; $p = 0.02$) was chosen after backward elimination (Table 3). The Hosmer-Lemeshow test indicated that the model was a good fit ($p = 0.2$). The model was tested in an independent cohort of 305 patients with esophageal cancer. For patients with esophageal cancer, adjusted ORs from multivariable logistic model were as follows: age (adjusted OR $= 1.03$; $p = 0.09$), baseline lymphocytes (adjusted OR $= 0.26$; $p < 0.001$), concurrent chemoradiotherapy (adjusted OR $= 2.71$; $p = 0.01$), radiotherapy duration (adjusted OR $= 0.98$; $p = 0.5$), mean heart dose (adjusted OR $= 1.05$; $p = 0.01$), mean lung dose (adjusted OR $= 1.03$; $p = 0.04$), and thoracic vertebrae V_{20} (adjusted OR $= 1.04$; $p = 0.005$). C-statistics of the model in the development set was 0.81 and in the validation set was 0.78.

Discussion

Our study revealed that lymphopenia of grade 3 or higher during radiotherapy is a prognostic factor for OS in lung cancer in addition to tumor volume and concurrent chemotherapy delivery (which is likely to reflect the fitness of the patients at the time of diagnosis and severity of the cancer). For the esophageal cancer cohort, grade 4 lymphopenia seemed to have the largest impact on OS in our study, which is in line with the literature.[23,24] To our knowledge, this study is the first to use a matched group analysis in image-based data mining and dose-response modeling, which allows minimizing

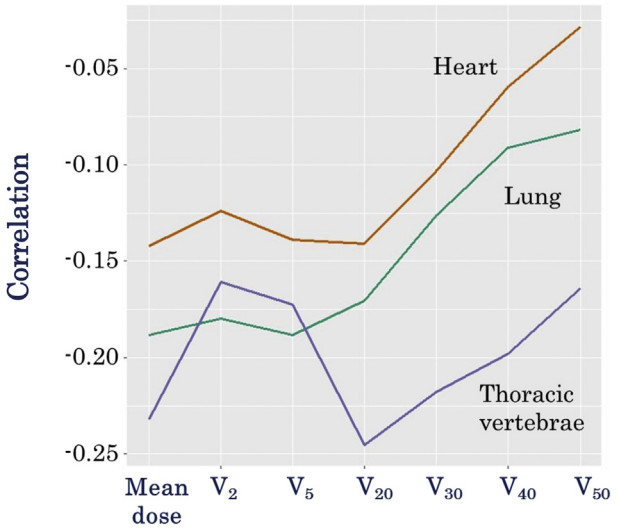

Figure 3. Correlation between lymphocyte counts at nadir and dosimetric parameters from heart, lung, and thoracic vertebrae reveals the highest level of correlations (negative) for mean heart, mean lung, and thoracic vertebrae V_{20}. V_{20}, volume receiving 20 Gy or higher.

the impact of confounders. The data mining approach detected regions in the thoracic vertebrae, heart, and lung, where the dose difference between the incidence of lymphopenia (grade $\geq$ 3: yes versus no) was significantly higher. The shape of the *t* map was subsequently affected by the tumor location and the regions identified were influenced by statistical variability, which resulted in only parts of the lung, heart, and thoracic vertebrae being identified after data mining. Therefore, we subsequently chose to include the entire organ in the analysis because we do not currently have sufficient data on sensitive substructures to select part of the organs. Heart and lung dosimetric parameters were correlated. For instance, mean heart dose was significantly associated with mean lung dose ($r = 0.56$, $p < 0.001$). Because of this correlation, both variables were included in multivariable modeling. A higher level of correlation was seen between PTV and mean lung dose ($r = 0.40$, $p < 0.001$) than mean heart dose ($r = 0.26$, $p < 0.001$), which is to be expected.

Multivariable logistic regression analysis revealed that thoracic vertebrae V_{20} and mean heart and lung dose were significantly associated with the incidence of lymphopenia of grade 3 or higher, which was validated in a cohort of esophageal cancer. Mean dose to the lung and heart had the highest correlation with lymphocytes at nadir indicating that the effect of local irradiation of circulating lymphocytes in the blood pool may be the most important mechanism of lymphopenia. Because irradiation dose of the lung and heart is a surrogate for the dose received by circulating lymphocytes, and lymphocytes in blood circulate at a high speed ($\sim$1 cycle/min), the dose to the lymphocytes is not equal to that for the heart and lung. Moreover, owing to the rapid movement of circulating lymphocytes, continuous mixing of blood occurs. As a result, we expect the blood pool to receive a uniform dose that is equal to the mean dose of all blood-filled compartments in the body. Given the small blood volume in the heart and lungs compared with the entire blood pool, even the low reported dose for lethality of lymphocytes ($\sim$2 Gy)[25] may not be reached for significant mean heart and lung doses. Thus, in the case of lymphopenia, in which circulating lymphocytes behave as *mobile* organs at risk, the mean dose would be a better proxy over volumetric dose parameters for the heart and lungs, which is in line with our results. The higher mean dose to the heart and the lung may not only generate clinically apparent toxicities to these organs but also have a large impact on circulating lymphocytes owing to their radiosensitivity and, hence, negatively affect the outcome.

Lymphocytes at nadir were further affected by the volume of thoracic vertebrae, which received 20 Gy or higher, suggesting a threshold dose effect on the functional thoracic bone marrow. Bone marrow contains both stem cells and circulating lymphocytes. The lymphocytes in the bone marrow circulate at a few hours per cycle and are therefore considered *static* during each radiotherapy fraction but mobile between fractions. So, although the fraction dose to the lymphocytes equals that of the bone marrow, the total dose for lymphocytes is much lower because they are less likely to be irradiated at multiple fractions. Therefore, we expect to find a higher dose threshold, which is consistent with our observation. Moreover, after irradiation of the stem cell pool, compensatory effects are expected from the unirradiated bone marrow, where progenitor cell population is increased to maintain hematopoiesis, which can also affect the threshold dose.

Hematopoietic suppression is a common occurrence in malignancies, in which both the cancer and the treatment can affect hematopoiesis. Hematologic toxicity, including lymphopenia, resulting from therapy-induced suppression of blood cells and bone marrow is known to affect the outcome of patients with cancer.[15,26,27] Lymphopenia has been reported to have a negative impact on survival possibly owing to the impact on the immune system leading to reduced treatment efficacy, early tumor progression, and development of infections, especially opportunistic infections, such as *Pneumocystis jirovecii* pneumonia, which often lead to death.[28–30] However, such opportunistic infections are difficult to diagnose in patients with lung cancer treated with radiotherapy due to a number of alternative differential diagnoses (such as radiation pneumonitis or

Table 3. Univariable and Multivariable Logistic Regression Results for Parameters Associated With Lymphopenia of Grade 3 or Higher in Lung Cancer Cohort

	Lymphopenia G3			
Parameters	No (n = 408)	Yes (n = 493)	OR (Univariable)	adjusted OR (Multivariable)
Sex				
Female	199 (48.8)	224 (45.4)	–	–
Male	209 (51.2)	269 (54.6)	1.14 (0.88-1.49, $p = 0.318$)	–
Age, y				
Mean (SD)	65.6 (9.9)	64.2 (9.5)	0.99 (0.97-1.00, **$p = 0.031$**)	1.02 (1.00-1.04, **$p = 0.031$**)
ECOG PS				
0	86 (21.1)	105 (21.3)	–	–
1	193 (47.3)	284 (57.7)	1.21 (0.86-1.69, $p = 0.280$)	–
2	83 (20.3)	62 (12.6)	0.61 (0.39-0.94, **$p = 0.027$**)	–
3	15 (3.7)	18 (3.7)	0.98 (0.47-2.09, $p = 0.964$)	–
NA	31 (7.6)	23 (4.7)	0.61 (0.33-1.11, $p = 0.110$)	–
Histology				
Adenocarcinoma	100 (24.5)	127 (25.8)	–	–
Squamous cell carcinoma	85 (20.8)	165 (33.5)	1.53 (1.06-2.22, **$p = 0.025$**)	–
NoS	22 (5.4)	45 (9.1)	1.61 (0.92-2.90, $p = 0.103$)	–
Not known	28 (6.9)	16 (3.2)	0.45 (0.23-0.87, **$p = 0.019$**)	–
Small cell carcinoma	173 (42.4)	140 (28.4)	0.64 (0.45-0.90, **$p = 0.010$**)	–
PTV, ln				
Mean (SD)	5.8 (0.6)	6.1 (0.5)	2.80 (2.14-3.70, **$p < 0.001$**)	–
Tumor laterality				
Left	184 (45.1)	215 (43.6)	–	–
Right	224 (54.9)	278 (56.4)	1.06 (0.82-1.38, $p = 0.655$)	–
N stage				
0	126 (30.9)	99 (20.1)	–	–
1	35 (8.6)	44 (8.9)	1.60 (0.96-2.69, $p = 0.074$)	–
2	145 (35.5)	233 (47.4)	2.05 (1.46-2.86, **$p < 0.001$**)	–
3	47 (11.5)	55 (11.2)	1.49 (0.93-2.39, $p = 0.097$)	–
NA	55 (13.5)	61 (12.4)	1.41 (0.90-2.22, $p = 0.133$)	–
Baseline lymphocytes, $\times 10^9$/liter				
Mean (SD)	2.2 (4.9)	1.7 (0.9)	0.73 (0.62-0.85, **$p < 0.001$**)	0.58 (0.47-0.71, **$p < 0.001$**)
Concurrent chemoradiotherapy				
No	243 (59.6)	178 (36.1)	–	–
Yes	165 (40.4)	315 (63.9)	2.61 (1.99-3.42, **$p < 0.001$**)	1.54 (1.07-2.23, **$p = 0.020$**)
Prescribed dose and fractions, Gy				
Mean (SD)	54.1 (6.3)	59.9 (6.8)	1.13 (1.11-1.16, **$p < 0.001$**)	–
20	248 (60.8)	170 (34.5)	–	–
30	106 (26.0)	85 (17.2)	1.17 (0.83-1.65, $p = 0.374$)	–
33	54 (13.2)	238 (48.3)	6.43 (4.54-9.23, **$p < 0.001$**)	–
Radiotherapy duration, d				
Mean (SD)	27.8 (8.4)	37.1 (10.9)	1.11 (1.09-1.12, **$p < 0.001$**)	1.09 (1.07-1.11, **$p < 0.001$**)
Twice daily fractions				
No	321 (78.7)	462 (93.7)	–	–
Yes	87 (21.3)	31 (6.3)	0.25 (0.16-0.38, **$p < 0.001$**)	–
Mean heart dose (EQD2), Gy				
Mean (SD)	10.9 (7.0)	14.6 (7.8)	1.07 (1.05-1.09, **$p < 0.001$**)	1.03 (1.00-1.06, **$p = 0.038$**)
Mean lung dose (EQD2), Gy				
Mean (SD)	14.2 (4.8)	17.7 (5.1)	1.15 (1.12-1.19, **$p < 0.001$**)	1.05 (1.00-1.09, **$p = 0.041$**)
V_{20} thoracic vertebrae, %				
Mean (SD)	26.4 (18.0)	36.9 (17.0)	1.03 (1.03-1.04, **$p < 0.001$**)	1.01 (1.00-1.03, **$p = 0.018$**)

Note: Values are n (%) unless indicated otherwise. p values < 0.05 are in bold.
ECOG PS, Eastern Cooperative Oncology Group performance status; NA, not available; PTV, planning target volume; EQD2, equivalent dose at 2 Gy per fraction; $\alpha/\beta = 10$; V_{20}, volume receiving 20 Gy or higher.

exacerbation of comorbidities). This leads to the development of infections, for example, opportunistic infections caused by severe lymphopenia being underreported in routine practice and clinical trials. Furthermore, patients with lung cancer may die at home of opportunistic infections or other underlying causes although the cause of death is generally recorded as "lung cancer." The recent PACIFIC study (A Global Study to Assess the Effects of MEDI4736 Following Concurrent Chemoradiation in Patients With Stage III Unresectable Non-Small Cell Lung Cancer) has revealed a significant improvement of survival with consolidation of durvalumab in locally advanced NSCLC.[31] In this context, radiotherapy-induced lymphopenia is likely to be of clinical significance as lymphopenia can negatively affect the efficacy of immunotherapy.[32]

Our result revealed that concurrent chemotherapy has a positive effect on OS even though it causes lymphopenia that has a negative correlation with survival. This would point to the fact that better tumor control outweighs the risk of induced lymphopenia, which is in line with the observed HRs (Table 2). Even though the impact of systemic treatment on hematologic toxicity is well established, this is not the case for radiotherapy. A number of studies have identified clinical and dosimetric factors associated with severe lymphopenia.[16,18] However, our study used novel image-based data mining methodology to allow identification of all organs involved with severe lymphopenia in a large patient cohort. We further used a matching cohort for data mining to reduce bias owing to confounding, which allowed us to sensitively detect regions with radiotherapy dose differences for patients who developed lymphopenia of grade 3 or higher during radiotherapy and patients who did not. The advantage of image-based data mining is that it indicates areas of the anatomy where the observed dose difference is related to a statistically significant difference for the incidence of severe lymphopenia. Therefore, it does not require making previous assumptions regarding the organ(s) that are responsible for the effect or time-consuming delineations of organs at risks by oncologists.

Deek et al.[26] reported that leukopenia of grade 3 or higher was associated with thoracic vertebrae V_{20} and V_{30} in patients with NSCLC treated with definitive chemoradiotherapy. However, the association between radiotherapy dose to the thoracic vertebrae and the risk of developing lymphopenia was not reported. Moreover, it has been reported that heart V_{50} greater than 25% is associated with higher neutrophil-to-lymphocyte ratio 4 months after radiotherapy in locally advanced NSCLC, but lymphocyte counts during radiotherapy were not analyzed.[33] A recent study reported that higher radiotherapy dose to the "host immune system," defined as a function of mean heart dose, mean lung dose, mean body dose, and number of fractions, was associated with OS in 117 patients with stage III NSCLC.[18,34] However, radiation-induced bone marrow damage was not directly included in the model. In our study, thoracic vertebrae dose also had a detrimental effect on the lymphocyte counts.

There are a number of limitations in our analysis, mainly owing to its retrospective nature. First, one can argue that those patients with recorded full blood count both before and during radiotherapy were in fact frailer and that was the reason that their blood had been monitored over time. To investigate this further, we compared the average of lymphocyte counts during radiotherapy for those patients who had full blood counts recorded before and those who had not. Similarly, the comparison was done for the average of lymphocyte counts before radiotherapy for those patients who had full blood counts recorded during radiotherapy and those who had not. This analysis revealed no significant differences between the two groups; hence, it is likely that the two groups are in fact from the same population and the recording of blood counts is more or less a random process at our institution for this cohort. In addition, due to the lack of full blood counts being systematically recorded for the patients at our institution throughout the follow-up, the duration of lymphopenia (transient or persistent) after treatment and its effect on the outcome could not be evaluated. Here, we have chosen to model severe lymphopenia during radiotherapy because of the fact that recovery of lymphocytes during treatment when nadir is reached, is unlikely compared with post radiotherapy. Hence, unless having available lymphocyte counts at precise time interval at post radiotherapy, analyzing lymphocytes over a large time interval would introduce pitfalls in the modelling. Another limitation of this study is the unavailability of data on the use of immunosuppressive agents, such as corticosteroids, which are known to reduce T lymphocyte counts[35,36] or prophylactic antibiotics. In addition, owing to the inclusion of patient data from a rather large time span (12 years), there may have been a significant variation in diagnostic work-up and heterogeneity in radiotherapy planning techniques, which may affect the results. However, the use of a wide range of planning techniques increases dose variability and therewith improves the statistical power of the applied modeling. Moreover, even though tumor laterality did not have an impact on lymphopenia in the lung cohort (Table 3), the incidence and severity of lymphopenia for centrally located tumors might not be the same for peripheral tumors or tumors located in the upper or lower parts of the lung. Furthermore, image-based data mining is not a sensitive methodology in selecting locations where the

dose variability is small. Hence, we cannot exclude the possibility that there might be other sensitive regions where dose impact is large on the incidence of lymphopenia (eg spleen). The presented model for lymhopenia is validated in the cohort of esophageal cancer, however, further validation in an external lung cancer cohort is warranted.

Our study suggested novel dose constraints for thoracic organs at risk to mitigate severe lymphopenia as long as sufficient tumor coverage is achieved. If dose constraints to the identified organs cannot be met, more frequent monitoring of lymphocyte counts during therapy and the use of prophylactic antibiotics are recommended. The results suggest that reducing radiotherapy dose to the heart, lung, and thoracic vertebrae can decrease the severity of lymphopenia, and thus, radiotherapy to these organs should be minimized as much as possible. The feasibility of reducing dose to the organs identified in this study needs to be investigated in planning studies in which the impact of optimized plan on OS can be estimated and compared with the original plan. Reducing the dose to the identified organs can be achieved by (1) reducing total prescribed dose, which, however, counteracts the benefits of higher radiotherapy doses to increase tumor control; and (2) reducing PTV or clinical target volume margins while maintaining the prescribed dose at the same level, which can be achieved by optimizing motion management. In addition, advanced forms of radiotherapy, including proton therapy, may help reduce radiotherapy-related lymphopenia by reducing the integral dose. This hypothesis will need to be tested in prospective clinical trials. Furthermore, our study suggests that the duration of radiotherapy has a negative effect on the incidence of lymphopenia; hence, hypofractionated regimens can potentially mitigate severe lymphopenia. Such management can also be used for patients with esophageal cancer treated with curative-intent radiotherapy because similar visceral organs are being irradiated.

In addition, for medically operable patients with lung cancer, operation with minimal injury, for instance, video-assisted thoracoscopic surgery resection, is the standard of care, and this treatment could be associated with a reduced risk of lymphopenia compared with radiotherapy. More biological insight could be obtained by comparing the presence and impact of lymphopenia in patients who have undergone operation and those treated with radiotherapy.

In conclusion, lymphopenia of grade 3 or higher during radiotherapy is a significant prognostic factor for OS in patients with lung cancer. Dose to organs at risk, including thoracic vertebrae, lung, and heart, should be kept *as low as possible* during the planning process to avoid severe lymphopenia as long as the target coverage is not compromised.

Acknowledgments

This work was supported by the Cancer Research UK RadNet Manchester [C1994/A28701]. Professor Corinne Faivre-Finn and Professor Marcel van Herk are supported by the National Institutes of Health Research of Manchester Biomedical Research Centre. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Parts of this work have been awarded as the highest scoring abstract under physics track and presented in the highlight session of the annual congress of the European SocieTy for Radiotherapy and Oncology (ESTRO) in 2019.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2020.06.008.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin*. 2017;67:7–30.
2. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136:E359–E386.
3. Früh M, De Ruysscher D, Popat S, et al. Small-cell lung cancer (SCLC): ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2013;24(suppl 6):vi99–vi105.
4. Crinò L, Weder W, van Meerbeeck J, Felip E, ESMO Guidelines Working Group. Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2010;21(suppl 5):v103–v115.
5. Antonia SJ, Villegas A, Daniel D, et al. Durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer. *N Engl J Med*. 2017;377:1919–1929.
6. Liu Y, Dong Y, Kong L, Shi F, Zhu H, Yu J. Abscopal effect of radiotherapy combined with immune checkpoint inhibitors. *J Hematol Oncol*. 2018;11:104.
7. Poulin JF, Viswanathan MN, Harris JM, et al. Direct evidence for thymic function in adult humans. *J Exp Med*. 1999;190:479–486.
8. Liu J, Zhao Q, Deng W, et al. Radiation-related lymphopenia is associated with spleen irradiation dose during radiotherapy in patients with hepatocellular carcinoma. *Radiat Oncol*. 2017;12:90.
9. Chadha AS, Liu G, Chen HC, et al. Does unintentional splenic radiation predict outcomes after pancreatic cancer radiation therapy? *Int J Radiat Oncol Biol Phys*. 2017;97:323–332.

10. Molon B, Calì B, Viola A. T cells and cancer: how metabolism shapes immunity. *Front Immunol*. 2016;7:20.
11. Yang TJ, Oh JH, Apte A, Son CH, Deasy JO, Goodman KA. Clinical and dosimetric predictors of acute hematologic toxicity in rectal cancer patients undergoing chemoradiotherapy. *Radiother Oncol*. 2014;113:29–34.
12. Heylmann D, Rödel F, Kindler T, Kaina B. Radiation sensitivity of human and murine peripheral blood lymphocytes, stem and progenitor cells. *Biochim Biophys Acta*. 2014;1846:121–129.
13. Sellins KS, Cohen JJ. Gene induction by gamma-irradiation leads to DNA fragmentation in lymphocytes. *J Immunol*. 1987;139:3199–3206.
14. Yovino S, Grossman SA. Severity, etiology and possible consequences of treatment-related lymphopenia in patients with newly diagnosed high-grade gliomas. *CNS Oncol*. 2012;1:149–154.
15. Campian JL, Ye X, Brock M, Grossman SA. Treatment-related lymphopenia in patients with stage III non-small-cell lung cancer. *Cancer Invest*. 2013;31:183–188.
16. Tang C, Liao Z, Gomez D, et al. Lymphopenia association with gross tumor volume and lung V5 and its effects on non-small cell lung cancer patient outcomes. *Int J Radiat Oncol Biol Phys*. 2014;89:1084–1091.
17. Cho O, Oh YT, Chun M, Noh OK, Lee HW. Radiation-related lymphopenia as a new prognostic factor in limited-stage small cell lung cancer. *Tumor Biol*. 2016;37:971–978.
18. Ladbury CJ, Rusthoven CG, Camidge DR, Kavanagh BD, Nath SK. Impact of radiation dose to the host immune system on tumor control and survival for stage III non-small cell lung cancer treated with definitive radiation therapy. *Int J Radiat Oncol Biol Phys*. 2019;105:346–355.
19. Joseph N, McWilliam A, Kennedy J, et al. Post-treatment lymphocytopaenia, integral body dose and overall survival in lung cancer patients treated with radical radiotherapy. *Radiother Oncol*. 2019;135:115–119.
20. Crocenzi T, Cottam B, Newell P, et al. A hypofractionated radiation regimen avoids the lymphopenia associated with neoadjuvant chemoradiation therapy of borderline resectable and locally advanced pancreatic adenocarcinoma. *J Immunother Cancer*. 2016;4:45.
21. Abravan A, Eide HA, Helland Å, Malinen E. Radiotherapy-related lymphopenia in patients with advanced non-small cell lung cancer receiving palliative radiotherapy. *Clin Transl Radiat Oncol*. 2020;22:15–21.
22. McWilliam A, Kennedy J, Hodgson C, Vasquez Osorio E, Faivre-Finn C, van Herk M. Radiation dose to heart base linked with poorer survival in lung cancer patients. *Eur J Cancer*. 2017;85:106–113.
23. Davuluri R, Jiang W, Fang P, et al. Lymphocyte nadir and esophageal cancer survival outcomes after chemoradiation therapy. *Int J Radiat Oncol Biol Phys*. 2017;99:128–135.
24. van Rossum PSN, Deng W, Routman DM, et al. Prediction of severe lymphopenia during chemoradiation therapy for esophageal cancer: development and validation of a pretreatment nomogram. *Pract Radiat Oncol*. 2020;10:e16–e26.
25. Nakamura N, Kusunoki Y, Akiyama M. Radiosensitivity of CD4 or CD8 positive human T-lymphocytes by an in vitro colony formation assay. *Radiat Res*. 1990;123:224–227.
26. Deek MP, Benenati B, Kim S, et al. Thoracic vertebral body irradiation contributes to acute hematologic toxicity during chemoradiation therapy for non-small cell lung cancer. *Int J Radiat Oncol Biol Phys*. 2016;94:147–154.
27. Venkatesulu BP, Mallick S, Lin SH, Krishnan S. A systematic review of the influence of radiation-induced lymphopenia on survival outcomes in solid tumors. *Crit Rev Oncol Hematol*. 2018;123:42–51.
28. McAleese J, Mooney L, Walls GM, Eakin RL, Harney J, Hanna GG. Risk of death from *Pneumocystis jirovecii* after curative-intent radiotherapy for lung cancer. *Clin Oncol (R Coll Radiol)*. 2018;30:e81–e82.
29. Lee EH, Kim EY, Lee SH, et al. Risk factors and clinical characteristics of *Pneumocystis jirovecii* pneumonia in lung cancer. *Sci Rep*. 2019;9:2094.
30. Warny M, Helby J, Nordestgaard BG, Birgens H, Bojesen SE. Lymphopenia and risk of infection and infection-related death in 98,344 individuals from a prospective Danish population-based study. *PLoS Med*. 2018;15:e1002685.
31. Antonia SJ, Villegas A, Daniel D, et al. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. *N Engl J Med*. 2018;379:2342–2350.
32. Pike LRG, Bang A, Mahal BA, et al. The impact of radiation therapy on lymphocyte count and survival in metastatic cancer patients receiving PD-1 immune checkpoint inhibitors. *Int J Radiat Oncol Biol Phys*. 2019;103:142–151.
33. Contreras JA, Lin AJ, Weiner A, et al. Cardiac dose is associated with immunosuppression and poor survival in locally advanced non-small cell lung cancer. *Radiother Oncol*. 2018;128:498–504.
34. Jin JY, Mereniuk T, Yalamanchali A, et al. A framework for modeling radiation induced lymphopenia in radiotherapy. *Radiother Oncol*. 2020;144:105–113.
35. Hughes MA, Parisi M, Grossman S, Kleinberg L. Primary brain tumors treated with steroids and radiotherapy: low CD4 counts and risk of infection. *Int J Radiat Oncol Biol Phys*. 2005;62:1423–1426.
36. Mansharamani NG, Balachandran D, Vernovsky I, Garland R, Koziel H. Peripheral blood CD4+ T-lymphocyte counts during *Pneumocystis carinii* pneumonia in immunocompromised patients without HIV infection. *Chest*. 2000;118:712–720.