Prediction of Radiation-induced Lymphopenia following Exposure of the Thoracic Region and Associated Risk of Infections and Mortality


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Prediction of Radiation-induced Lymphopenia following Exposure of the Thoracic Region and Associated Risk of Infections and Mortality


* Centre of Excellence for Health, Immunity and Infections (CHIP), Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
† Department of Oncology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
‡ Department of Computer Science, University of Copenhagen, Copenhagen, Denmark

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Abstract

Aims: Large blood volumes are irradiated when the heart is exposed to radiation. The mean heart dose (MHD) may be a good surrogate for circulating lymphocytes exposure. We investigated the association between MHD and radiation-induced lymphopenia and explored the impact of the end-of-therapy (EoRT) lymphocyte count on clinical outcomes.

Materials and methods: In total, 915 patients were analysed: 303 patients with breast cancer and 612 with intrathoracic tumours: oesophageal cancer (291), non-small cell lung cancer (265) and small cell lung cancer (56). Heart contours were generated using an interactive deep learning delineation process and an individual dose volume histogram for each heart was obtained. A dose volume histogram for the body was extracted from the clinical systems. We compared different models analysing the effect of heart dosimetry on the EoRT lymphocyte count using multivariable linear regression and assessed goodness of fit. We published interactive nomograms for the best models. The association of the degree of EoRT lymphopenia with clinical outcomes (overall survival, cancer treatment failure and infection) was investigated.

Results: An increasing low dose bath to the body and MHD were associated with a low EoRT lymphocyte count. The best models for intrathoracic tumours included dosimetric parameters, age, gender, number of fractions, concomitant chemotherapy and pre-treatment lymphocyte count. Models for patients with breast cancer showed no improvement when adding dosimetric variables to the clinical predictors. EoRT lymphopenia grade /C213 was associated with decreased survival and increased risk of infections among patients with intrathoracic tumours.

Conclusion: Among patients with intrathoracic tumours, radiation exposure to the heart contributes to lymphopenia and low levels of peripheral lymphocytes after radiotherapy are associated with worse clinical outcomes.

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Key words: Heart irradiation; lung cancer; organs at risk; radiation-induced lymphopenia

Introduction

The recent successes in adding immunotherapy to anticancer treatment regimens puts renewed focus on the complex interaction between the host immune system, the cancer and the treatment effects. Despite positive trial results, for example when applying adjuvant durvalumab to chemoradiotherapy for lung cancer [1], the response to immunotherapy is limited to a subset of patients [2].

There is a need to identify methods to increase and expand the effect of immunotherapy. A radiotherapy and immunotherapy combination is a promising strategy as there is some evidence suggesting that radiotherapy can trigger the immune system and act like an in situ tumour vaccine, complementing the effects of immunotherapy [3]. However, radiotherapy has mostly been associated with immunosuppression rather than immune-stimulating effects [4], suggesting that radiotherapy is a double-edged sword and emphasising the need to increase our understanding of the immune effects of radiation.

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In a previous study, we described a negative association between the volume of the body exposed to at least 2 Gy (body V2) and the end-of-radiation-therapy (EoRT) lymphocyte count [5]. We proposed that the body V2 would be a good estimate for circulating lymphocytes within the irradiated volume. However, given that large blood volumes and, therefore, circulating blood cells (including lymphocytes) are irradiated when the heart is contained within the irradiated volume, it is a reasonable hypothesis that adding the cardiac dose as a predictor would improve our predictive model. Here, we aimed to assess the association of the body V2 and the mean heart dose (MHD) with the development of radiation-induced lymphopenia among patients with thoracic solid tumours. We also explored the association between radiation-induced lymphopenia with clinical outcomes among these patients.

Materials and Methods

We conducted a retrospective cohort study of patients who (i) received curative-intent external beam radiotherapy for their first cancer from 1 January 2009 to 31 December 2016 at Rigshospitalet, University of Copenhagen; (ii) had radiation-planning computed tomography scans available; (iii) were ≥ 18 years old; (iv) had both a pretreatment and an EoRT blood count; and (v) had a solid tumour diagnosis in the thoracic region [breast cancer, oesophageal cancer, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC)]. We excluded patients with HIV infection, organ transplant recipients, patients with in situ carcinomas or basal cell cutaneous carcinomas, patients with an unspecified or uncertain diagnosis and patients whose cancer follow-up was in another hospital. We further excluded patients who had received radiotherapy with more than two plans that could not be normalised and patients who did not receive radiation to the chest area.

Data were retrieved from electronic health records stored in the Centre of Excellence for Personalised Medicine of Infectious Complications in Immune Deficiency (PERSIMUNE) data lake [5,6]. Study closure was 31 December 2016.

Tumours that were irradiated because of oesophageal cancer, NSCLC or SCLC were called intrathoracic tumours. Patients with breast cancer were analysed separately due to inherent different characteristics of radiotherapy. We reviewed fractionation schemes to separate those that denote curative from palliative intent.

We used an open-source corrective-annotation software application (RootPainter3D) that uses a deep-learning model for image segmentation of chest computed tomography scans and contouring of the whole heart structure [7]. Predictions were corrected by a trained physician according to Danish guidelines for whole heart delineation [8]. We then extracted the corresponding MHD information. We also extracted data on the radiotherapy fractionation schemes and the body V2. The body structure refers to the patient outline on the treatment planning scan as defined by the dose planner at the time of treatment, which is required to perform the clinical dose calculation. The volume of the body exposed to more than 2 Gy is measured in litres (absolute volume) as this is insensitive to the extent of the body included in the outline. The number of fractions were grouped in tertiles, except for patients with NSCLC, where data only allowed two groups (< 15 and ≥ 15 fractions) [9]. The body V2 was grouped in quintiles and the MHD in tertiles [5]. Categories were calculated for all patients and for each cancer diagnosis. We preferred these groups as they avoid assumptions of linear dependence with a continuous predictor.

Pre-treatment peripheral lymphocyte count was the closest measurement to radiation start, collected within 1 year before radiation. EoRT peripheral lymphocyte count was the closest measurement to radiation end, collected between 2 weeks before and 6 months after radiotherapy ended. We grouped the EoRT lymphocyte counts in grade ≥ 3, grade 1–2 and no lymphopenia, according to the Common Terminology Criteria of Adverse Events (version 5.0) [10].

Concomitant chemotherapy was defined as any chemotherapy administered between the day before the start of radiotherapy and the end date of radiotherapy and categorised according to the Anatomical, Therapeutic, Chemical (ATC) [11] classification system. We dichotomised this variable for analyses purposes.

We calculated the estimated dose of radiation to immune cells (EDRIC) according to the model proposed by Ladbury et al. [12] in a subset of patients with NSCLC with all required dosimetric parameters available. The EDRIC approximates the radiation dose to immune cells to the radiation dose to circulating blood volume. The dosimetric parameters required are the mean lung dose, the MHD and the mean body dose; as well as the number of fractions (see Supplementary Material). The formula assigns percentages to the blood volume received in the lungs, in the heart (based on the cardiac output) and in the rest of the body (based on estimations for irradiated blood with a single fraction and for the total number of fractions).

Outcomes

The primary outcome of this study was the EoRT lymphocyte count as a continuous variable. Additionally, we investigated the association of the degree of EoRT lymphopenia with four clinical outcomes as secondary outcomes: overall survival, cancer treatment failure, infection of any category and definite infection.

As we did not have access to relapse data, we defined cancer treatment failure if the patient received chemotherapy, radiotherapy or had a biopsy with a malignant morphology (excluding basal cell carcinomas) after a non-treatment period of 2 months.

Infections were identified according to an algorithm that combined diagnosis, microbiology and medication data, and they were classified as possible, probable or definite [13]. Infections met the definite category if the patient had an International Classification of Diseases, 10th revision
Statistical Analysis

We analysed factors associated with the square root transformed EoRT lymphocyte count using multivariable linear regression for the whole cohort of intrathoracic tumours and for each cancer group. We chose the square root transformation because it showed the best statistical distribution for the EoRT lymphocyte count for the model assumptions. Several models were built and compared, as presented in Supplementary Table S1 and in Table 1. We assessed goodness of fit of models by looking at $R^2$, which describes how much of the variability of the outcome can be explained by the model. We also used likelihood ratio tests to compare the different models. As some patients received chemotherapy between blood count measurements and the radiation therapy course, we carried out sensitivity analyses where we excluded these patients.

We used time-to-event analyses to estimate hazard ratios for overall survival, cancer treatment failure, infection of any category and definite infection according to the degree of EoRT lymphopenia for all intrathoracic tumours and for each cancer group. We calculated the time from the start of radiotherapy. We censored time at 1 year after the start of radiotherapy, end of follow-up or death. For infection outcomes, we censored at cancer treatment failure. Due to non-proportional hazards, we split the follow-up time into 3-month intervals for the whole cohort of intrathoracic tumours and 6-month intervals for each cancer diagnosis. We adjusted for age, gender and Charlson comorbidity index, and stratified by cancer diagnosis in each interval.

This study was approved by the Danish Patient Safety Authority I-Suite no: 03605. Stata, version 15.1 (StataCorp, College Station, Texas, USA) and R, version 4.0.3 (R Core Team, 2014) was used for data analyses. Dynamic nomograms of multivariable linear regression models were created using DynNom [14].

Results

Patient Characteristics

We identified 915 patients who received radiotherapy to the chest area with curative intent and had both pre-treatment and EoRT lymphocyte counts (see Supplementary Figure S1). These included 303 patients with breast cancer. The remaining 612 patients received radiotherapy because of an intrathoracic tumour: oesophageal cancer (291 patients), NSCLC (265 patients) and SCLC (56 patients). Patient characteristics are shown in Table 2.

The median time from radiation therapy start to pre-treatment lymphocyte count was 17 days (interquartile range 4–40 days) for patients with intrathoracic tumours and slightly more prolonged for patients with breast cancer: 20 days (13–57 days). The median time from EoRT lymphocyte count to the end of radiotherapy was shorter: 3 days (–1 to 51 days) for patients with intrathoracic tumours, but again more prolonged for patients with breast cancer: 56 days (12–114 days). Over half the patients with intrathoracic tumours (66%) and breast cancer (64%) had a blood sample collected within 1 month before radiotherapy start. A similar proportion of patients with intrathoracic tumours (67%) had a blood sample within 1 month of the end of radiation therapy, whereas this proportion was reduced among patients with breast cancer (38%). The time from pre-treatment count to radiotherapy start, and from EoRT count to the end of radiotherapy is shown in Supplementary Figures S2 and S3.

One-third of patients (219; 36%) with intrathoracic tumours received chemotherapy between pre-treatment lymphocyte count and the start of radiotherapy, compared with one-half of patients with breast cancer (163; 54%).

Table 1.

Analyses adjusting for age, gender, number of fractions, concomitant chemotherapy and pre-treatment lymphocyte count (in splines). Stratified by cancer diagnosis for intrathoracic tumours. Outcome is the end-of-radiation-therapy lymphocyte count.

<table>
<thead>
<tr>
<th>Cancer diagnosis</th>
<th>$n$</th>
<th>Model 1 Without V2/MHD</th>
<th>R$^2$</th>
<th>p-value</th>
<th>Model 2 With V2</th>
<th>R$^2$</th>
<th>p-value</th>
<th>Model 3 With MHD</th>
<th>R$^2$</th>
<th>p-value</th>
<th>Model 4 With V2 and MHD</th>
<th>R$^2$</th>
<th>p-value</th>
<th>Model 5 With V2 and MHD as interaction</th>
<th>R$^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oesophageal cancer</td>
<td>291</td>
<td>0.11</td>
<td>0.15</td>
<td>0.015*</td>
<td>0.16</td>
<td>0.18</td>
<td>0.001*</td>
<td>0.18</td>
<td>0.002*</td>
<td>0.20</td>
<td>0.571*</td>
<td>0.60</td>
<td>0.015*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSCLC</td>
<td>265</td>
<td>0.24</td>
<td>0.27</td>
<td>0.164*</td>
<td>0.29</td>
<td>0.32</td>
<td>0.008*</td>
<td>0.33</td>
<td>0.029*</td>
<td>0.34</td>
<td>0.137*</td>
<td>0.59</td>
<td>0.001*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCLC</td>
<td>56</td>
<td>0.17</td>
<td>0.27</td>
<td>0.081*</td>
<td>0.28</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>0.31</td>
<td>&lt;0.001</td>
<td>0.31</td>
<td>0.625</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrathoracic tumours</td>
<td>612</td>
<td>0.25</td>
<td>0.28</td>
<td>&lt;0.001</td>
<td>0.28</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>0.31</td>
<td>0.625</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td>303</td>
<td>No model fitted an association.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MHD, mean heart dose (in tertiles); NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; V2, volume of the body exposed to 2 Gy (in quintiles). Bold values denote statistical significance at the $p < 0.05$ level.

* Likelihood-ratio test comparing model with model 1.

† Likelihood-ratio test comparing model with model 2.

‡ Likelihood-ratio test comparing model with model 4.
The proportion of patients with further chemotherapy between the end of radiotherapy and EoRT lymphocyte count was low for both intrathoracic tumours (37; 6%) and for patients with breast cancer (2; 1%).

Patients with breast cancer had very low body V2 and MHD due to the predominance of tangential field techniques used in the department. We therefore analysed patients with breast cancer separately in the subsequent modelling. In contrast, patients with intrathoracic tumours, particularly patients with oesophageal cancer, received larger MHDs. Raw data on body V2 and MHD are plotted against EoRT lymphocyte count for all cancer diagnoses in Figure 1.

### Modelling of End-of-Radiation-Therapy Lymphocyte Count

Overall, goodness of fit improved by adding any dosimetric variable (MHD or body V2) to the baseline model in patients with intrathoracic tumours. Here, the baseline model predicted EoRT lymphocyte counts from age, gender, number of fractions, concomitant chemotherapy and pre-treatment lymphocyte count. Further modest improvement was

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**Table 2**

Baseline characteristics of 915 patients who had available lymphocyte counts, stratified by diagnosis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Breast</th>
<th>Intrathoracic tumours</th>
<th>NSCLC</th>
<th>SCLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>303</td>
<td>291</td>
<td>265</td>
<td>56</td>
</tr>
<tr>
<td>Female sex – no. patients (%)</td>
<td>300 (99.0)</td>
<td>74 (25.4)</td>
<td>124 (46.8)</td>
<td>25 (44.6)</td>
</tr>
<tr>
<td>Age, years – median (IQR)</td>
<td>59.8 (52.2–68.6)</td>
<td>65.4 (59.7–70.4)</td>
<td>67.1 (60.2–74.1)</td>
<td>66.0 (58.5–71.1)</td>
</tr>
<tr>
<td>Charlson comorbidity index, points – median (IQR)</td>
<td>2 (2–3)</td>
<td>3 (2–3)</td>
<td>2 (2–4)</td>
<td></td>
</tr>
<tr>
<td>Dose per fraction – no. patients (%)</td>
<td>1 (0.3)</td>
<td>49 (16.8)</td>
<td>2 (0.8)</td>
<td>53 (94.6)</td>
</tr>
<tr>
<td>&lt; 2 Gy</td>
<td>188 (62.1)</td>
<td>237 (81.4)</td>
<td>212 (80.0)</td>
<td>2 (3.6)</td>
</tr>
<tr>
<td>&gt; 2 Gy</td>
<td>114 (37.6)</td>
<td>5 (1.7)</td>
<td>51 (19.3)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Physical dose, Gy – median (IQR)</td>
<td>50 (40–50)</td>
<td>50 (50–50)</td>
<td>66 (66–66)</td>
<td>45 (25–45)</td>
</tr>
<tr>
<td>EQD2, Gy – median (IQR)</td>
<td>50 (40–50)</td>
<td>50 (50–50)</td>
<td>66 (66–66)</td>
<td>43 (43–43)</td>
</tr>
<tr>
<td>Duration of radiation therapy, days – median (IQR)</td>
<td>33 (22–36)</td>
<td>33 (33–36)</td>
<td>45 (42–48)</td>
<td>22 (22–23)</td>
</tr>
<tr>
<td>Volume of the body irradiated with 2 Gy – l, median (IQR)</td>
<td>5.1 (3.4–6.6)</td>
<td>11.9 (9.3–15.4)</td>
<td>8.0 (5.1–10.4)</td>
<td>8.1 (6.2–10.4)</td>
</tr>
<tr>
<td>Mean heart dose – Gy, median (IQR)</td>
<td>1.2 (0.7–1.9)</td>
<td>17.3 (7.7–25.0)</td>
<td>4.8 (1.1–10.8)</td>
<td>7.6 (3.0–12.9)</td>
</tr>
<tr>
<td>Concomitant chemotherapy* – no. patients (%)</td>
<td>7 (2.3)</td>
<td>278 (95.5)</td>
<td>174 (65.7)</td>
<td>49 (87.5)</td>
</tr>
<tr>
<td>None</td>
<td>296 (97.7)</td>
<td>13 (4.5)</td>
<td>91 (34.3)</td>
<td>7 (12.5)</td>
</tr>
<tr>
<td>Platinum compounds</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>14 (5.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Platinum compounds and vinca alkaloids</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>149 (56.2)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Platinum compounds and topoisomerase 2 inhibitors</td>
<td>0 (0)</td>
<td>1 (0.3)</td>
<td>1 (0.4)</td>
<td>45 (80.4)</td>
</tr>
<tr>
<td>Platinum compounds and antimitabolites</td>
<td>0 (0)</td>
<td>199 (63.4)</td>
<td>1 (0.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Antimitabolites</td>
<td>1 (0.3)</td>
<td>59 (20.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (1.3)</td>
<td>17 (5.8)</td>
<td>9 (3.4)</td>
<td>3 (5.4)</td>
</tr>
<tr>
<td>Pre-treatment lymphocyte count, cells/μl x 10³ – median (IQR)</td>
<td>1.4 (1.0–2.0)</td>
<td>1.8 (1.4–2.3)</td>
<td>1.8 (1.4–2.5)</td>
<td>1.9 (1.4–2.5)</td>
</tr>
<tr>
<td>No. patients (%) – lymphocyte count within 1 month before radiation start</td>
<td>195 (64.4)</td>
<td>186 (63.9)</td>
<td>175 (66.0)</td>
<td>42 (75.0)</td>
</tr>
<tr>
<td>Time from radiation therapy start to pre-treatment lymphocyte count (days) – median (IQR)</td>
<td>20 (13–57)</td>
<td>14 (5–42)</td>
<td>20 (3–39)</td>
<td>20 (7–31)</td>
</tr>
<tr>
<td>End-of-radiation-therapy lymphocyte count, cells/μl x 10³ – median (IQR)</td>
<td>1.2 (0.8–2.5)</td>
<td>0.5 (0.3–0.7)</td>
<td>0.8 (0.5–1.2)</td>
<td>0.7 (0.5–1.1)</td>
</tr>
<tr>
<td>No. patients (%) – lymphocyte count within a month of radiation end</td>
<td>115 (38.0)</td>
<td>220 (75.6)</td>
<td>140 (52.8)</td>
<td>49 (87.5)</td>
</tr>
<tr>
<td>Time from end-of-radiation-therapy lymphocyte count to end of radiation therapy (days) – median (IQR)</td>
<td>56 (12–114)</td>
<td>1 (−3–27)</td>
<td>22 (0–76)</td>
<td>5 (0–21)</td>
</tr>
</tbody>
</table>

EQT2, equivalent dose to 2 Gy; IQR, interquartile range; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

* Two (0.7%) patients with breast cancer and two (0.7%) with oesophageal cancer did not have details on the type of concomitant chemotherapy used.
Fig 1. Association between the volume of the body exposed to at least 2 Gy (body V2) and the end-of-radiation-therapy (EoRT) lymphocyte count (left panels); and between the mean heart dose (MHD) and the EoRT lymphocyte count (right panels), according to cancer diagnosis.
obtained when the MHD was added to the model with body V2. $R^2$ values ranged from 0.18 to 0.35 depending on cancer diagnosis (Table 1). Models with an interaction term between MHD and body V2 did not show any improvement compared with models with both parameters as covariables, except for patients with SCLC. Models behaved differently for patients with breast cancer and showed no improvement when adding any of the dosimetric variables. Sensitivity analyses for patients with intrathoracic tumours and patients with breast cancer did not substantially change our results (data not shown).

Multivariable linear regression fitting formulas for each of the models are displayed in the Supplementary Material. A dynamic nomogram was created in RShiny for general accessibility and to potentially evaluate clinical credibility [15].

For patients with NSCLC (Figure 2), the predicted EoRT lymphocyte count decreased when patients received both a high MHD and a high body V2. There was also a reduction in the predicted EoRT lymphocyte count for patients who received concomitant chemotherapy. Patients who received radiation schemes of fewer than 15 fractions, mostly hypofractionated/stereotactic regimens, had a higher predicted EoRT lymphocyte count than those receiving 15 or more fractions. Further understanding of model predictions can be obtained by working with the shiny app at https://dcccrt.shinyapps.io/Terrones-Campos_Lymphocyte_Dose_Model.

In a subanalysis of 181 patients with NSCLC where the EDRIC was calculated, we also found that it negatively associated with the EoRT lymphocyte count (see Supplementary Figure S4).

**End-of-Radiation-Therapy Lymphopenia and Risk of Death and Infection**

We found that patients with intrathoracic tumours and EoRT lymphopenia grade ≥3 had a higher risk of death, infection of any definition and a definite infection in the first 3 months after the start of radiotherapy, compared with patients with intrathoracic tumours without EoRT lymphopenia. We observed a high risk of death for patients with NSCLC, a high risk of infection of any definition for patients with oesophageal cancer and NSCLC, and a high risk of a definite infection for patients with NSCLC in the first 6 months after the start of radiotherapy if EoRT lymphopenia grade ≥3. We only found a higher risk of cancer treatment failure for patients with SCLC when comparing patients with EoRT lymphopenia grade ≥3 with patients without lymphopenia (Figure 3). No significant differences were found when comparing patients with EoRT lymphopenia grade 1–2 with patients without EoRT lymphopenia (data not shown).

**Discussion**

This study shows that among patients with oesophageal cancer, NSCLC and SCLC, the MHD further contributes to

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**Fig 2.** Example of RShiny app functionality for the non-small cell lung cancer (NSCLC) model, varying concomitant chemotherapy, the mean heart dose (MHD), the volume of the body exposed to at least 2 Gy (body V2) and the number of fractions. The graph shows the predicted end-of-radiation-therapy (EoRT) lymphocyte count for patients with a low MHD and a low body V2 (cases 1, 2, 5, 6), high MHD and high body V2 (cases 3, 4, 7, 8), with (cases 2, 4, 6, 8) and without concomitant chemotherapy (cases 1, 3, 5, 7), receiving <15 (cases 5–8) and ≥15 fractions (cases 1–4), if patients are males, 60–69 years old with a pretreatment lymphocyte count of 2.0 × 10³ cells/μl. The table describes variable values and predictions for specific cases displayed in the plot. Vertical lines represent thresholds for grade 3 lymphopenia (red solid line) and grade 1 lymphopenia (red dotted line). More cases can be run at https://dcccrt.shinyapps.io/Terrones-Campos_Lymphocyte_Dose_Model.
Fig 3. Hazard ratios for death, cancer treatment failure, infection of any definition and definite infections, for all intrathoracic tumours and stratified by cancer diagnosis. Adjusted for age, gender and Charlson comorbidity index. The red and orange colours indicate significant P-values of $<0.01$ and $<0.05$, respectively. LymEoRT, end-of-radiation-therapy lymphocyte count.
explain the variation in the EoRT lymphocyte count. We also confirmed an association between EoRT lymphopenia grade ≥3 and a high risk of death, infection of any category and definite infections in the 6 months following the start of radiotherapy.

A large case-control study of 901 patients with lung cancer and 305 patients with oesophageal cancer reported that thoracic vertebrae volume exposed to 20 Gy or more, mean lung dose and MHD were associated with radiation-induced lymphopenia and subsequently that lymphopenia grade 3 or more was associated with decreased survival [16]. This study used deformable registration to a single reference patient to assess dose volume data, where we, in contrast, used individual heart contour delineation and dose extraction on the unperturbed treatment planning scans. This was made feasible by using an interactive deep learning model to speed up the process and allow accurate heart delineations in about 2 min per heart [7]. Thus, the two studies show different avenues towards high-volume outcome modelling but with the main findings in agreement.

Compared with previous studies showing that irradiation of cardiovascular structures leads to increased risk of cardiovascular disease in patients with breast cancer [17] and lymphoma [18], our study suggests that among patients with lung and oesophageal cancer, the cardiac dose is linked to decreased survival not only through cardiac toxicity but also through immunosuppression [19–21]. Patients with oesophageal cancer frequently become immunosuppressed due to chemoradiotherapy combinations, developing low percentages of CD3, CD4 and a decreased CD4/CD8 ratio after radiotherapy. The irradiated volume [22] and the MHD [23] are associated with myelosuppression among these patients.

Radiation-induced lymphopenia is linked to a high risk of bacterial infections despite no neutropenia [13], which is the basis for prophylactic antibiotic prescription [24]. Details on the specific T lymphocyte levels that inform the risk of opportunistic infections [25] are often lacking among patients with solid tumours. Prospective studies should assess lymphocyte subsets to establish recommendations on how to prevent infections that could influence morbidity and mortality.

We only found an association between the EoRT lymphocyte count and the risk of cancer treatment failure for patients with SCLC. Although the results from patients with SCLC need to be assessed with caution due to the small sample size, at least 50% of patients with SCLC remain lymphopenic 3 months after chemoradiotherapy [26]. The duration of lymphopenia may have a different impact on clinical outcomes [27]. In the present study, we only looked at the EoRT lymphocyte count and time windows for lymphocyte count collection were wide. In contrast, a recent article among patients with NSCLC treated with concurrent chemoradiation found that radiation-induced lymphopenia nearly equivalent to grade 4 (<230 cells/µl) was associated with a poor cancer prognosis when compared with grade 0–3 as a group [38]. Also relevant, the EDRIC, which associates with lymphopenia after radiotherapy, associated with poor progression-free survival (PFS) and local progression-free survival (LPFS) in patients with oesophageal and NSCLC [12,39]. Further investigations should include a closer follow-up of the lymphocyte count among patients with lymphopenia after radiotherapy.

Patients with breast cancer did not reveal a significant association between the dosimetric predictors and EoRT lymphocyte counts as could perhaps be expected as the tangential field arrangement leads to minimal exposure of the blood volume in the heart. This is in line with a prior study among patients with breast cancer where irradiation volume was found to be more important than irradiation dose in determining radiation-induced lymphopenia. The irradiation dose was only relevant in promoting lymphopenia when the volume was controlled. The former study concluded, and we agree, that it was important to keep the irradiation volume small as long as target coverage allows it [28].

We were not able to assess lung or thoracic vertebral dosimetric data in our study, which in thoracic tumours may further contribute to the development of lymphopenia as suggested by Abravan et al. [16]. The lack of standardisation of the nomenclature of irradiated structures, plus the inconsistent organ contouring because it is considered to be irrelevant, add to our inability to include these variables in our current analyses. This was our motivation to use an automated tool to segment the whole heart and obtain heart dosimetry. Nevertheless, in a subset of patients with NSCLC for whom we had the mean lung dose available, we were able to calculate the EDRIC, which was further associated with a low EoRT lymphocyte count. Thoracic vertebral bodies are an important proportion of active bone marrow among adults [29] and their irradiation has also been linked to radiation-induced haematological toxicity [16,30,31]. In order to further elucidate how to best administer radiotherapy, it would be of value to identify all relevant structures and include these organ systems in a detailed dose-response model to provide QUANTEC style guidelines for dose volume effects for immune suppression [32]. We suspect that large patient cohorts, as in the current study and the study by Abravan et al. [16], will be needed to provide sufficiently reliable models.

The strengths of our study include the large number of real-world patients with complete clinical, laboratory and dosimetric data. We were able to obtain heart dosimetry by applying a novel artificial intelligence algorithm, which allowed us to have contouring consistency by automating the process. Also, we determined the impact of EoRT lymphopenia on the risk of infection denoting a strong connection with post-radiotherapy immunosuppression.

In terms of weaknesses, there are the conventional caveats of retrospective cohort studies [33]. We did not have access to tumour volume, stage of the disease nor performance status data. Among patients with lung cancer, gross tumour volume has been associated with lymphocyte nadir [34]. Similarly, among patients with glioblastoma, a reduced planned tumour volume because of a limited margin irradiation is associated with a higher post-radiotherapy lymphocyte count, compared with a conventional margin radiotherapy [35]. This poses the question of whether the tumour volume or in fact the irradiated tumour volume is
more relevant. Unfortunately, limitations in the lack of standardisation of structure nomenclature as mentioned above did not allow us to obtain these measurements. Blood tests were performed out of clinical relevance and selection bias can therefore be present because sicker patients may have had more blood counts collected. However, the dose-response analysis was carried out on patients with the relevant blood counts available (so-called internal controls) and it seems unlikely that the dependence on cardiac dose should be specific for the population with both counts available. The time frame for blood count collection was broad, and some patients received chemotherapy between blood count measurements and the radiation therapy course. However, excluding these patients in sensitivity analyses for patients with intrathoracic tumours and patients with breast cancer did not substantially change our results. Confounding by indication is a risk in terms of the observed dependence on fractionation and chemotherapy, in particular in lung cancer where stereotactic radiotherapy is given to selected stages and patient populations. Ideally, a prospective study should confirm our results in a randomised manner. One could hope that these simple blood samples would be available for some of the large fractionation studies in the literature. Where we did observe a dependence on both survival and infections on EoRT lymphopenia, we cannot claim a causal association between the infections and decreased survival.

The evolution of radiotherapy techniques during the 2010s has significantly changed radiation exposure to non-target tissues and reduced radiation to the heart [36]. We did not evaluate the effect of different radiotherapy techniques on cardiac dose and lymphocyte depletion, but opted instead to analyse the exposure data (MHD and body V2) as the relevant covariates. These exposure data are expected to be the final drivers of toxicity and will also be appropriately handled when new priorities or a learning curve causes different dose distributions to be achieved with the same nominal radiotherapy technique. We expect the dosimetric predictors to be a relevant measure to compare across techniques in the treatment planning process unless hidden confounders of other exposures influencing lymphopenia are present. We believe a large-scale analysis, as presented here, is the most promising way forward to elucidate such correlations if present.

**Conclusion**

The MHD further contributes to explain radiation-related depletion of circulating lymphocytes. Radiation-induced lymphopenia is associated with decreased survival and increased risk of infections among patients with intrathoracic tumours. Modifications of how we deliver radiotherapy may reduce radiation-induced immunosuppression and help us obtain better outcomes, which may be particularly important in the setting of adjuvant immunotherapy in first-line treatments. But as treatment modifications are not always possible without compromising cancer treatment, close monitoring and awareness due to increased death and infection risk are advised among patients who develop lymphopenia after radiotherapy. Future studies should investigate the burden of such infections in this population for more specific recommendations.

Further implementation of automated segmentation of immune organs to minimise radiation exposure could help improve immune cell protection, which is relevant in the context of immunotherapy treatment. Continued investigation of which organs at risk are relevant for this purpose is important but would require large sample sizes for hypothesis generation and test.

**Data Availability**

Research data are stored in the Centre of Excellence for Personalised Medicine of Infectious Complications in Immune Deficiency (PERSIMUNE) data lake and will be shared upon request to the corresponding author.

**Conflicts of Interest**

There are no conflicts of interest related to this study. Righospitalet has research and teaching contracts with Varian Medical Systems and research contracts with Viewray Inc., unrelated to this work. All authors have declared no conflicts of interest.

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**Author Contributions**

CT-C and IRV are the guarantors of integrity of the entire study. CT-C, IRV and LS were responsible for study concepts and design. CT-C carried out the literature research. CT-C, BL, NF, AGS and JP were responsible for experimental studies/data analysis. CT-C and BL carried out the statistical analysis. CT-C prepared the manuscript. CT-C, BL, IRV, LS, MH and JL edited the manuscript.

**Declaration of Interests**

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jens Lundgren reports personal relationships which may be considered as potential competing interests. Righospitalet has research and teaching contracts with Varian Medical Systems and research contracts with Viewray Inc., unrelated to this work.

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Appendix A. Supplementary data

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References


