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# Dosimetric predictors of treatment-related lymphopenia induced by palliative radiotherapy: predictive ability of dose-volume parameters based on body surface contour

Tetsuo Saito, Ryo Toya, Tomohiko Matsuyama, Akiko Semba, Natsuo Oya

Department of Radiation Oncology, Kumamoto University Hospital, 1-1-1 Honjo, Chuo-ku, Kumamoto-shi, Kumamoto, 860-8556, Japan

Correspondence to: Tetsuo Saito, M.D., Department of Radiation Oncology, Kumamoto University Hospital, 1-1-1, Honjo, Chuo-ku, Kumamoto-shi, Kumamoto, 860-8556, Japan. Phone: +81 96 373 5261; Fax: +81 96 373 5342; E-mail: tsaito@kumamoto-u.ac.jp

**Background.** Radiation-related lymphopenia has been associated with poor patient outcome. Our aim was to identify predictors of lymphopenia after palliative radiotherapy, with a focus on dose-volume parameters.

**Patients and methods.** To retrospectively assess patients with various cancers who had undergone palliative radiotherapy, we delineated three organs at risk: the volume enclosed by the body surface contour (body A), the volume left after excluding air, pleural effusion, ascites, bile, urine, and intestinal content (body B), and the volume of the bone marrow (BM). We then noted the absolute volume of the three organs at risk that had received 5–30 Gy, and assessed the predictive value for post-treatment lymphopenia of grade 3 or higher (LP3+).

**Results.** Of 54 patients, 23 (43%) developed LP3+. Univariate logistic regression analysis showed that body A V5, body A V10, body B V5, body B V10, the number of fractions, and splenic irradiation were significant predictors of LP3+ (p < 0.05). By multivariate analysis, body A V5, body A V10, body B V5, body B V10, and the number of fractions retained significance (p < 0.05). BM dose-volume parameters did not predict lymphopenia.

**Conclusions.** Higher body A and body B dose-volume parameters and a larger number of fractions may be predictors of severe lymphopenia after palliative radiotherapy.

Key words: palliative radiotherapy; radiation-related lymphopenia; dose-volume parameters

## Introduction

The important role of lymphocytes in the immune response to cancer[1] is evidenced by the better survival of lung-, colorectal-, and breast cancer-, and glioblastoma patients whose cancer tissues manifest lymphocyte infiltration.[2-5] Survival tends to be poor in cancer- and lymphoma patients with lymphopenia before undergoing treatment[6-10], and treatment-related lymphopenia is associated with a poor outcome in patients subjected to curative chemoradiotherapy for pancreatic-, lung-, cervical-, and nasopharyngeal cancer and malignant glioma.[11-18]

The irradiation of circulating peripheral blood may elicit radiation-related lymphopenia.[19,20] Although studies to evaluate the effect of irradiation on lymphocytes showed that radiation-related lymphopenia was associated with organ-specific (lung[14] and brain[21]) dose-volume parameters, dosimetric predictors applicable at various treatment sites remained to be identified.

Radiation-related lymphopenia has been studied mainly in patients who had received curative treatment[11-18]; there are few reports on patients subjected to palliative radiotherapy (RT). Because lymphocytes are highly radiosensitive, exposure to even low doses of radiation can lead to a decrease

in the number of peripheral blood lymphocytes.[22,23] Consequently, even low radiation doses delivered by palliative RT can lead to lymphopenia affecting the immune system and the treatment outcome.

Focusing on dose-volume parameters, we attempted to identify predictors of lymphopenia after palliative RT. We used organs at risk based on body surface contour to evaluate their predictive value.

## Patients and methods

### Patients

This retrospective study was approved by the institutional review board of Kumamoto University Hospital (No. 1171). The study was carried out according to the Declaration of Helsinki. Our inclusion criteria were as follows: patients treated with palliative RT between October 2010 and June 2013 at the Kumamoto University Hospital; the availability of laboratory data acquired within 2 weeks prior to the start of RT; and of two or more laboratory data obtained within one month after the start of RT, the latest data recorded at least 2 weeks after the start of RT. The exclusion criteria were hematologic tumor; chemotherapy, molecular targeted therapy, interferon treatment, or radiotherapy delivered from one month before to one month after the start of RT; or grade 2 or higher lymphopenia based on the Common Terminology Criteria for Adverse Events (CTCAE) v 4.0 at the start of RT. Patient-, tumor-, and treatment data were obtained from medical charts.

### Laboratory data

The study endpoints were (1) the absolute lymphocyte count at nadir, defined as the lowest value recorded within one month after the start of RT, (2) the lymphocyte count ratio, obtained by dividing the nadir absolute lymphocyte count by the pre-RT absolute lymphocyte count, and (3) lymphopenia of grade 3 or higher (LP3+, absolute lymphocyte count < 500 x $10^6$/L) determined by CTCAE v 4.0, with the highest grade within one month after the start of RT recorded for analysis.

### Dose-volume parameters

All patients underwent CT simulation in the supine position, and three-dimensional treatment planning. For this study, we delineated organs at risk on planning CT images with commercially

available software (Velocity AI, Velocity Medical System, Atlanta, GA, USA). To evaluate the effect of radiation on peripheral blood lymphocytes, one radiation oncologist delineated the volume of two organs at risk where body A is the volume enclosed by the body surface contour, and body B is the volume left after excluding air, pleural effusion, ascites, bile, urine, and intestinal content (Figure 1). For body A, the body surface contour was obtained first by using threshold-based segmentation and then by manual correction. To obtain body B, we excluded volumes whose irradiation would not contribute to the reduction of lymphocytes; lung tissue was included and the trachea and bronchi were excluded. For bone marrow (BM), all bones were delineated by threshold-based segmentation and manual correction (Figure 1); intervertebral disks and costal-, thyroid-, cricoid-, and tracheal cartilage were excluded. The distal half of the femur and humerus were also excluded from BM because they contain little proliferating bone marrow.[24] The absolute volumes of the three organs at risk receiving 5-, 10-, 20-, and 30 Gy (V5, V10, V20, and V30) were recorded.

### Statistical analysis

Data were summarized by using descriptive statistics (frequency, percentage, median, range). The correlation between the dose-volume parameters and the nadir lymphocyte count was evaluated with the Spearman correlation coefficient. For univariate and multivariate logistic regression analysis, the age, interval from tumor diagnosis, the pre-RT absolute lymphocyte count, total radiation dose, number of fractions, total monitor units, total irradiation time, and all dose-volume parameters were the continuous variables. The categorical variables included the gender, previous RT, previous chemotherapy, concurrent steroid use, bone metastasis, brain metastasis, splenic irradiation, and thymic irradiation. Variables that were significant
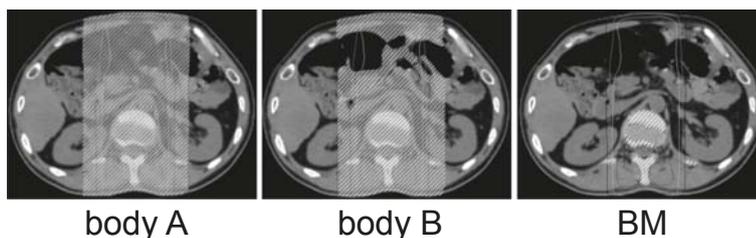


**FIGURE 1.** Delineation of the three organs at risk. Body A is the volume enclosed by the body surface contour. Body B excludes air, pleural effusion, ascites, bile, urine, and the intestinal content. BM = bone marrow.

**TABLE 1.** Patient and treatment characteristics (n = 54)

| Characteristic | No. of patients | % |
|---|---|---|
| **Patient characteristics** | | |
| Male gender | 33 | 61 |
| Age (years) | | |
| Median | 69 | |
| Range | 39-86 | |
| Primary tumor | | |
| Lung | 14 | 26 |
| Gastrointestinal | 9 | 17 |
| Skin | 4 | 7 |
| Liver | 3 | 6 |
| Uterus | 3 | 6 |
| Others[a] | 21 | 39 |
| Previous radiotherapy | 15 | 28 |
| Previous chemotherapy | 26 | 48 |
| Concurrent steroid use | 23 | 43 |
| Bone metastasis | 27 | 50 |
| Brain metastasis | 11 | 20 |
| Interval from tumor diagnosis to radiotherapy (months) | | |
| Median | 13 | |
| Range | 0-168 | |
| Pre-radiotherapy absolute lymphocyte count (x 10⁶/L) | | |
| Median | 1356 | |
| Range | 844-3468 | |
| **Treatment characteristics** | | |
| Total radiation dose (Gy) | | |
| Median | 30 | |
| Range | 16–50 | |
| Number of fractions | | |
| Median | 10 | |
| Range | 4–25 | |
| Fraction size (Gy) | | |
| Median | 3 | |
| Range | 2–5 | |
| Total monitor units for all fractions | | |
| Median | 4433 | |
| Range | 1896–13890 | |
| Total irradiation time for all fractions (minutes) | | |
| Median | 7.5 | |
| Range | 3.2–37.7 | |
| Treatment site | | |
| Head and neck | 14 | 26 |
| Chest | 24 | 44 |
| Abdomen | 10 | 19 |
| Pelvis | 11 | 20 |
| Limb | 1 | 2 |
| Splenic irradiation[b] | 9 | 17 |
| Thymic irradiation[c] | 15 | 28 |

[a] Others include head and neck (3 patients), breast (3 patients), mediastinal (3 patients), urogenital (8 patients), and soft tissue (4 patients) tumors; [b] Yes, if any part of the spleen was covered by the 5 Gy idodose line; [c] Yes, if any part of the thymus was covered by the 5 Gy idodose line.

in univariate analysis were included in multivariate analysis. The overall survival, calculated from the start of RT, was estimated with the Kaplan-Meier method; differences determined with the log-rank test. Values of $p < 0.05$ were considered statistically significant. All statistical analyses were performed with SPSS software, version 23 (IBM SPSS, Armonk, NY, USA).

# Results

## Patients

We included 54 patients whose solid tumors were treated with palliative RT. The patient and treatment characteristics are summarized in Table 1. As we excluded patients with lymphopenia of grade 2 or higher (absolute lymphocyte count < 800 x 10⁶/L), all included patients had a pre-RT absolute lymphocyte count ≥ 800 x 10⁶/L. The median follow-up period from the start of RT was 4.5 months (range 0–42.0 months).

## Laboratory data

The median pre-RT absolute lymphocyte count was 1356 x 10⁶/L (range 844–3468 x 10⁶/L), and the median nadir post-RT absolute lymphocyte count was 536 x 10⁶/L (range 131–1653 x 10⁶/L). The median lymphocyte count ratio, obtained by dividing the nadir- by the pre-RT absolute lymphocyte count, was 0.410 (range 0.108–0.983). Of the 54 patients, 12 (22%), 19 (35%), 20 (37%), and 3 (6%) patients had post-treatment lymphopenia of grade 1, 2, 3, and 4, respectively; a total of 23 (43%) patients developed LP3+.

## Dose-volume parameters

The median (range) V5, V10, V20, and V30 for body A were 2.880 (0.399–8.976), 2.519 (0.344–6.596), 1.629 (0.000–4.255), and 0.660 (0.000–3.365) x 10³ mL, respectively. These values were 2.704 (0.392–8.143), 2.384 (0.341–5.959), 1.597 (0.000–3.919), and 0.645 (0.000–3.326) x 10³ mL for body B and 0.348 (0.000–0.974), 0.258 (0.000–0.909), 0.174 (0.000–0.737), and 0.076 (0.000–0.724) x 10³ mL for BM.

## Correlation between the dose-volume parameters and the nadir lymphocyte count

There was a negative correlation between body dose-volume parameters (V5, V10, and V30 for

body A and body B) and the nadir lymphocyte count (p < 0.05, Table 2). Higher body A and body B dose-volume parameters were correlated with a lower post-RT lymphocyte count. There was no significant correlation between BM dose-volume parameters and the nadir lymphocyte count (Table 2). We observed a strong correlation between body A V5 and body B V5 (Speaman's rho = 0.992, p < 0.001), between body A V10 and body B V10 (Speaman's rho = 0.992, p < 0.001), between body A V20 and body B V20 (Speaman's rho = 0.997, p < 0.001), and between body A V30 and body B V30 (Speaman's rho = 0.999, p < 0.001).

### Predictors of severe treatment-related lymphopenia

Univariate logistic regression analysis showed that body A V5, body A V10, body B V5, body B V10, the number of fractions, and splenic irradiation were significant predictors of LP3+ (p < 0.05, Table 3). For multivariate analysis, we took into account factors with p < 0.05 by univariate analysis (number of fractions and splenic irradiation) to test the independent significance of dose-volume parameters (Table 4). We found that body A V5, body A V10, body B V5, body B V10, and the number of fractions retained significance (p < 0.05). Higher body A and body B dose-volume parameters and a larger number of fractions were predictive of LP3+.

### Relationship between radiation-related lymphopenia and overall survival

The median survival for all patients was 6.3 months (95% confidence interval: 4.1–8.5 months). The overall survival based on the grade of radiation-related lymphopenia is shown in Figure 2. There was no statistically significant difference in the overall survival of patients with LP3+ and the other grades of lymphopenia (p = 0.79).

## Discussion

We found that body A V5, body A V10, body B V5, body B V10, and the number of fractions were significant predictors of severe radiation-related lymphopenia. Higher body A and body B dose-volume parameters and a larger number of fractions were predictive of LP3+. In contrast, irradiation to lymphoid organs such as the bone marrow, spleen, and thymus were not predictive of radiation-related lymphopenia.

**TABLE 2.** Spearman correlation coefficients between the dose-volume parameters and the nadir lymphocyte count

| | Nadir lymphocyte count | | | |
|---|---|---|---|---|
| | Absolute lymphocyte count at nadir | | Lymphocyte count ratio[a] | |
| Variable | Spearman's rho | p-value | Spearman's rho | p-value |
| Body A V5 | -0.265 | 0.053 | -0.350 | 0.010 |
| Body A V10 | -0.283 | 0.038 | -0.367 | 0.006 |
| Body A V20 | -0.231 | 0.093 | -0.255 | 0.063 |
| Body A V30 | -0.305 | 0.025 | -0.281 | 0.039 |
| Body B V5 | -0.274 | 0.045 | -0.347 | 0.010 |
| Body B V10 | -0.280 | 0.040 | -0.352 | 0.009 |
| Body B V20 | -0.236 | 0.086 | -0.260 | 0.057 |
| Body B V30 | -0.302 | 0.027 | -0.280 | 0.041 |
| BM V5 | -0.152 | 0.27 | -0.210 | 0.13 |
| BM V10 | -0.161 | 0.25 | -0.207 | 0.13 |
| BM V20 | -0.135 | 0.33 | -0.161 | 0.25 |
| BM V30 | -0.168 | 0.23 | -0.185 | 0.18 |

BM = bone marrow; [a] The lymphocyte count ratio was calculated by dividing the nadir absolute lymphocyte count by the pre-radiotherapy absolute lymphocyte count.
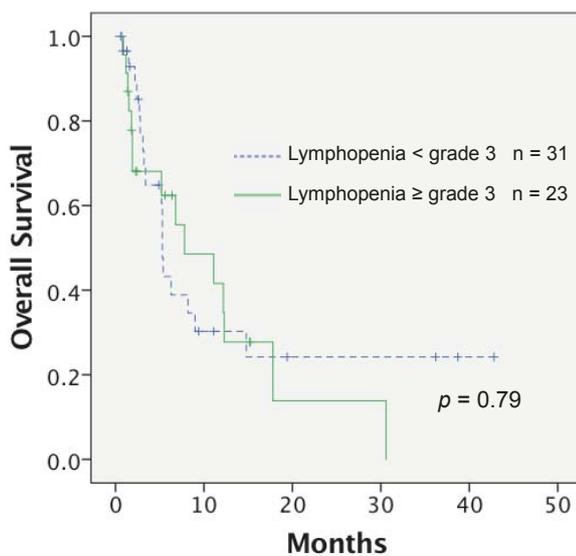


Median survival
Lymphopenia < grade 3    5.3 months (95% CI: 5.1–5.5 months)
Lymphopenia ≥ grade 3    7.8 months (95% CI: 0.5–15.1 months)

**FIGURE 2.** Overall survival according to the grade of radiation-related lymphopenia.

CI = confidence interval.

TABLE 3. Univariate logistic regression analysis for lymphopenia of grade 3 or higher

| Variable | OR | 95% CI | p-value |
|---|---|---|---|
| **Patient characteristics** | | | |
| Male vs. female | 1.39 | 0.46–4.22 | 0.55 |
| Age (per 1 year increase) | 0.99 | 0.94–1.03 | 0.53 |
| Previous radiotherapy (yes vs. no) | 1.02 | 0.30–3.47 | 0.98 |
| Previous chemotherapy (yes vs. no) | 0.53 | 0.18–1.58 | 0.26 |
| Concurrent steroid use (yes vs. no) | 1.07 | 0.36–3.17 | 0.91 |
| Bone metastasis (yes vs. no) | 0.86 | 0.29–2.53 | 0.78 |
| Brain metastasis (yes vs. no) | 0.72 | 0.18–2.84 | 0.64 |
| Interval from tumor diagnosis to radiotherapy (per 1 month increase) | 1.00 | 0.99–1.02 | 0.81 |
| Pre-radiotherapy absolute lymphocyte count (per increase of $1 \times 10^6$/l) | 0.99 | 0.99–1.00 | 0.23 |
| **Treatment characteristics** | | | |
| Total radiation dose (per 1-Gy increase) | 1.06 | 0.99–1.14 | 0.11 |
| Number of fractions (per 1-fraction increase) | 1.18 | 1.01–1.38 | 0.036 |
| Total monitor units over the entire treatment course (per increase of 100 monitor units) | 1.01 | 0.99–1.03 | 0.29 |
| Total irradiation time over the entire treatment course (per 1-minute increase) | 0.99 | 0.92–1.08 | 0.91 |
| Splenic irradiation (yes vs. no) [a] | 6.34 | 1.18–34.24 | 0.032 |
| Thymic irradiation (yes vs. no) [b] | 0.58 | 0.17–2.03 | 0.39 |
| Body A V5 (per $1 \times 10^3$ mL increase) | 1.55 | 1.06–2.26 | 0.025 |
| Body A V10 (per $1 \times 10^3$ mL increase) | 1.60 | 1.04–2.45 | 0.032 |
| Body A V20 (per $1 \times 10^3$ mL increase) | 1.60 | 0.96–2.68 | 0.074 |
| Body A V30 (per $1 \times 10^3$ mL increase) | 1.87 | 0.95–3.68 | 0.069 |
| Body B V5 (per $1 \times 10^3$ mL increase) | 1.58 | 1.05–2.38 | 0.027 |
| Body B V10 (per $1 \times 10^3$ mL increase) | 1.63 | 1.04–2.56 | 0.035 |
| Body B V20 (per $1 \times 10^3$ mL increase) | 1.59 | 0.94–2.72 | 0.087 |
| Body B V30 (per $1 \times 10^3$ mL increase) | 1.84 | 0.93–3.67 | 0.082 |
| BM V5 (per $1 \times 10^3$ mL increase) | 3.62 | 0.37–35.36 | 0.27 |
| BM V10 (per $1 \times 10^3$ mL increase) | 2.88 | 0.27–31.10 | 0.38 |
| BM V20 (per $1 \times 10^3$ mL increase) | 1.78 | 0.15–20.92 | 0.65 |
| BM V30 (per $1 \times 10^3$ mL increase) | 4.73 | 0.19–115.78 | 0.34 |

BM = bone marrow; OR = odds ratio; CI = confidence interval; [a] Yes, if any part of the spleen was covered by the 5 Gy idodose line; [b] Yes, if any part of the thymus was covered by the 5 Gy idodose line.

Others[19,20] suggested that the irradiation of circulating peripheral blood may lead to the development of radiation-related lymphopenia. This hypothesis is supported by findings that lymphopenia was observed after the delivery of RT to various body parts that did, or did not, include lymphoid organs.[19,25] The irradiation of extracorporeal blood can lead to long-lasting lymphopenia.[26] Tang et al.[14] found that in lung cancer patients, higher lung V5

to V10 values were associated with a lower lymphocyte nadir, and Huang et al.[21] reported that in patients with high-grade glioma, higher brain volume receiving 25 Gy was a significant predictor of acute severe lymphopenia during RT and concurrent temozolomide. We document that the body dose-volume parameters we applied are useful predictors of lymphopenia in patients exposed to RT at different sites including the head and neck, the chest, abdomen, and the pelvis.

Our univariate and multivariate logistic regression analysis showed that irradiation to the bone marrow, spleen, and thymus was not a consistently significant predictor. Lymphoid organs such as the thymus, bone marrow, and spleen are central components of the mammalian immune system; lymphocytes are developed in these organs.[27] While splenic irradiation was a significant predictor of LP3+ by univariate logistic regression analysis, it lost its significance upon multivariate analysis. Because the 95% confidence interval was wide in our multivariate analysis (Table 4), the predictive value of splenic irradiation should be examined in large patient populations. We detected no significant association between lymphopenia and bone marrow irradiation although Sini et al.[28] reported that the exposure of bone marrow to radiation played a significant role. They found that higher BM V40 was associated with higher risk of acute Grade3 or late Grade2 lymphopenia in prostate cancer patients treated with whole-pelvis RT. Because information on the role of lymphoid organs in radiation-related lymphopenia is limited, additional studies are warranted.

The number of fractions was a significant predictor of severe radiation-related lymphopenia. This finding agrees with earlier observations.[20,29] MacLennan et al.[29] analyzed the consequences of prophylactic cranial irradiation in children with leukemia. In their prospective study, the total radiation dose was constant (24 Gy) and the number of fractions was determined by the participating centers. They found that the level of radiation-related lymphopenia induced by that total dose depended on the number of fractions into which it was divided. The mean lymphocyte count of patients examined 3 months after receiving this dose in 5-, 12-, and 20 fractions was 1.84-, 1.12-, and $0.64 \times 10^9$/L, respectively. Yovino et al.[20] analyzed a model that calculated the radiation dose received by circulating lymphocytes; they found that as the number of fractions increased, the percentage of blood receiving ≥ 0.5 Gy increased rapidly. We also found that the number of fractions was a significant predictor

of radiation-related lymphopenia, however, in our study the total radiation dose was not a significant predictor. Because lymphocytes are highly radio-sensitive[22,23], their number killed by one fraction may not be strongly associated with the dose per fraction. A larger dose per fraction might be relatively less effective in killing lymphocytes than a small dose.

We observed a strong correlation between body A and body B dose-volume parameters when the volume was equal (*e.g.* body A and body B exposed to 5 Gy). It is easier to obtain the volume of body A than body B because body A is based on the body surface contour that can be acquired by auto-segmentation using commercially available software tools. Body A dose-volume parameters may be a convenient tool for predicting radiation-related lymphopenia.

Our data showed that there was no significant difference in the overall survival of patients with LP3+ and other grades of lymphopenia. Although lymphopenia related to curative chemoradiotherapy has been shown to be associated with poor patient outcomes[11-18], its prognostic value for palliative RT remains to be determined.

Our study has some limitations. The study population was small and some useful predictors of lymphopenia may have gone undetected. Also, as our study was retrospective, laboratory data were acquired at different points after the start of RT. Consequently, the true nadir lymphocyte count may not have been evaluated in some patients.

In summary, we identified body A and body B dose-volume parameters were useful new predictors of radiation-related lymphopenia. These body dose-volume parameters were acquired by delineating the body surface and they may be convenient for predicting radiation-related lymphopenia. As the parameters are not organ-specific, they are applicable at various treatment sites. Although their predictive value for radiation-related lymphopenia should be examined in groups of patients with different diseases, our findings may help to elucidate the mechanisms underlying the elicitation of radiation-related lymphopenia in patients treated with palliative RT.

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**TABLE 4.** Multivariate logistic regression analysis for lymphopenia of grade 3 or higher

| Variable | OR | 95% CI | p-value |
|---|---|---|---|
| Body A V5 (per 1 x 10³ mL increase) | 1.58 | 1.03–2.42 | 0.036 |
| Number of fractions (per 1-fraction increase) | 1.26 | 1.03–1.53 | 0.027 |
| Splenic irradiation (yes vs. no) ᵃ | 5.12 | 0.74–35.28 | 0.098 |
| Body A V10 (per 1 x 10³ mL increase) | 1.68 | 1.04–2.70 | 0.034 |
| Number of fractions (per 1-fraction increase) | 1.26 | 1.03–1.55 | 0.026 |
| Splenic irradiation (yes vs. no) ᵃ | 5.25 | 0.77–35.76 | 0.090 |
| Body B V5 (per 1 x 10³ mL increase) | 1.63 | 1.03–2.57 | 0.038 |
| Number of fractions (per 1-fraction increase) | 1.25 | 1.03–1.53 | 0.028 |
| Splenic irradiation (yes vs. no) ᵃ | 5.46 | 0.79–37.53 | 0.085 |
| Body B V10 (per 1 x 10³ mL increase) | 1.72 | 1.04–2.87 | 0.036 |
| Number of fractions (per 1-fraction increase) | 1.26 | 1.03–1.55 | 0.027 |
| Splenic irradiation (yes vs. no) ᵃ | 5.59 | 0.82–37.99 | 0.078 |

CI = confidence interval; OR = odds ratio; ᵃ Yes, if any part of the spleen was covered by the 5 Gy idodose line; Factors with $p < 0.05$ by univariate analysis (number of fractions and splenic irradiation) were taken into account to test the independent significance of the dose-volume parameters.

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