

# Analysis of the Association Between Low Dose Bone, Lung, and Heart Irradiation and Survival Rates for Patients Who Received High-dose Proton Beam Therapy With Concurrent Chemotherapy for Stage III Non-small Cell Lung Cancer

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## Research

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# Abstract

**Background:** Lymphocytes play an important role in the cancer immune system. We investigated influences of irradiated doses and volumes of the bone on lymphopenia and survivals in chemoradiotherapy for stage III non-small cell lung cancer (NSCLC).

**Methods:** Data from 41 patients with stage III unresectable NSCLC who received definitive proton beam therapy (PBT) of 74 GyE with concurrent chemotherapy between 2007 and 2017 were retrospectively reviewed. The correlation between dosimetry parameters obtained from dose-volume histograms (DVHs) of the bone, lung, and heart and lymphopenia during PBT were analyzed. Maximum and minimum absolute lymphocyte counts (ALCmax and ALCmin) and maximum neutrophil/lymphocyte ratio (NLRmax) were used as indicators of lymphopenia. Clinical factors, dosimetry parameters, and indicators of lymphopenia were also evaluated for the correlation with overall survival (OS), progression-free survival (PFS), and distant metastasis-free survival (DMFS).

**Results:** Significant inverse correlations were observed between bone V5 and ALCmax ( $\rho = -0.377$ ,  $p = 0.015$ ) and ALCmin ( $\rho = -0.441$ ,  $p = 0.003$ ) during the treatment period. Also, significant correlation between bone V5 and NLRmax ( $\rho = 0.398$ ,  $p = 0.010$ ) was observed, but bone V5 exhibited no significant association with OS, PFS, or DMFS. On the other hand, heart V5 (Hazard ratio [HR]: 1.032,  $p = 0.023$ ), ALCmax (HR: 0.999,  $p = 0.049$ ), and NLRmax (HR: 1.035,  $p = 0.007$ ) were significantly associated with OS in univariable analysis. Lung V5 tended to be associated with PFS in univariable analysis (HR: 1.047,  $p = 0.056$ ) and was significantly associated with DMFS (HR: 1.075,  $p = 0.005$ ). In multivariable analysis, heart V5 was not associated with OS, whereas ALCmax was significantly associated with OS (HR: 0.999,  $p = 0.022$ ). Lung V5 was not a factor associated with PFS, but it was significantly associated with DMFS (HR: 1.062,  $p = 0.027$ ).

**Conclusions:** In PBT with chemotherapy for stage III NSCLC, lymphopenia was correlated with irradiation doses to the bone and lung, and lung dose but not bone dose was associated with DMFS.

## Background

The standard treatment for patients with unresectable and locally advanced stage III non-small cell lung cancer (NSCLC) is chemoradiotherapy (CRT) [1, 2]. Recently, the PACIFIC study revealed that durvalumab after CRT for NSCLC improved treatment outcomes of patients with stage III NSCLC [3], and immuno-oncology is becoming more popular in clinical practice. With the advent of immune checkpoint inhibitors (ICI), immuno-oncology is garnering attention in the field of radiation oncology. Furthermore, lymphocytes, especially T-cell lymphocytes, are known to play an important role in the cancer immune system [4, 5]. Some studies have reported that, in the treatment for various cancers, survival rates are associated with lymphopenia and neutrophil lymphocyte ratio (NLR) as representative markers [6–10].

In the field of radiotherapy (RT), many previous studies have attested to the important roles of radiation-induced immune response in the success of cancer treatment and there has been renewed attention to

this subject since the introduction of ICIs [11–13]. It is well known that lymphocytes are radiosensitive cells and that RT-induced lymphopenia results from a decrease of circulating lymphocytes in the lung and heart and depletion of progenitor cells in the bone marrow and spleen [14–17]. However, the lung, heart, and lymphoid organs like bone marrow are exposed to unnecessary radiation doses during thoracic RT. These days, the ability to calculate dose volume histograms (DVHs) of the targets and organs at risk (OARs) enables the examination the effects of irradiation on various OARs [18]. However, despite advances in RT and the adoption, in many facilities, of intensity modulated radiotherapy (IMRT), which enables a more intensive high-dose irradiation of the target than three-dimensional conformal RT, concerns remain over lower doses of radiation to the normal tissues becoming more diffuse [19].

Among the more recent developments in RT, proton beam therapy (PBT) is recognized for its unique ability to deliver high-dose irradiation to the target while reducing unnecessary irradiation of healthy tissues, because a spread-out Bragg peak of protons can be created to match the depth and thickness of the target [20, 21]. Therefore, compared to X-ray RT, PBT can potentially yield better clinical outcomes and is also considered to have the advantage of minimizing RT-induced lymphopenia. Regarding the use of PBT in NSCLC, a randomized trial has been conducted for locally advanced NSCLC [22]. As for the effect of irradiation on the lung, which is regarded as a blood pool, it has been reported that lymphopenia is associated with lung V5, and that lower lymphocyte nadirs during RT were correlated with worse overall survival (OS) [23]. However, no report has investigated the effects of irradiation of the bone marrow on lymphopenia and patient prognosis in RT or PBT combined with chemotherapy for stage III NSCLC. Therefore, the purpose of the current study was to analyze the clinical outcomes of patients with stage III NSCLC who received definitive PBT with concurrent chemotherapy and examine the associations with survival rates and doses to normal tissues including lung, heart, and bone marrow.

## Methods

### Patient population

Data of 41 patients with stage III unresectable locally advanced NSCLC who received definitive PBT at 74 GyE with concurrent chemotherapy between November 2007 and December 2017 at our institution were retrospectively reviewed. Patient characteristics are shown in Table 1. There were 31 men and 10 women, and the median age was 62 years (range = 42–79 years). According to the 7th version of the Union for International Cancer Control TNM classification, the clinical stage was IIIA in 12 patients and IIIB in 29, and histopathological examination revealed squamous cell carcinoma in 11, adenocarcinoma in 24, and NSCLC in 6.

Table 1  
Patient and tumor characteristics

Characteristic	No. of patients
Age (years)	42–79 (median, 62)
Sex	
Male	31 (75.6%)
Female	10 (24.3%)
Performance Status	
0	27 (65.8%)
1	14 (34.1%)
Histology	
Squamous cell carcinoma	11 (26.8%)
Adenocarcinoma	24 (58.5%)
Non-small cell carcinoma, NOS	6 (14.6%)
7th UICC clinical stage	
IIIA	12 (29.3%)
IIIB	29 (70.7%)
Clinical Target volume (cc)	21.5–820.4 (median, 228.9)
Chemotherapy regimen	
Cisplatin and vinorelbine	32 (78.0%)
Others	9 (22.0%)
Follow-up time (months)	6.4–139.0 (median, 41.6)
NOS, not otherwise specified; UICC, Union for International Cancer Control	

## Proton beam therapy

For treatment planning, chest computed tomography (CT) images were taken at 2.5-mm or 5.0-mm intervals with the patients in a body cast in the treatment position (Engineering System Co., Matsumoto, Japan) using a respiratory-gated system during the end-expiratory phase. Passive-scattering PBT plans were constructed, and dose calculations were performed using the pencil beam method for PBT (Proton Treatment Planning Software version 1.7 or 2, Hitachi Inc., Ibaraki, Japan). Proton beams of 155 to 250 MeV were used in the treatment plans. The treatment planning system automatically estimated the

conditions required for beam delivery, which included a ridge filter, range shifter, collimator, and bolus. The beam delivery system created a homogenous dose distribution at the prescription dose using the spread-out Bragg peak.

In general, initial clinical target volume (CTV1) encompassed the primary tumor, the positive lymph nodes, and hilar and mediastinal lymph nodes as prophylactic areas where clinically positive lymph nodes existed. Clinically positive lymph nodes were defined as nodes measuring  $\geq 1$  cm (as visualized on CT) or as positron emission tomography (PET)-positive lymph nodes. Second CTV (CTV2) encompassed the primary tumor and the positive lymph nodes, and the third CTV (CTV3) included only the primary tumor. The planning target volume (PTV) encompassed the CTV with a 7- to 10-mm margin in all directions and an additional 5-mm margin in the caudal direction to compensate for the respiratory motion. After delivering a dose of 40 GyE in 20 fractions to the PTV1, 66 GyE in 33 fractions was delivered to the PTV2, followed by a total boost of 74 GyE in 37 fractions to the PTV3. In general, two to three ports in the optimal direction were used to meet the following dose constraints: the percentage of the lung volume receiving a dose of  $\geq 20$  GyE ( $V_{20}$ )  $\leq 35\%$ , maximum dose to the spinal cord  $< 46$  GyE biologically equivalent dose in 2 GyE per fraction (EQD2), maximum dose to the esophagus  $< 70$  GyE (EQD2), and maximum dose to the bronchus  $< 70$  GyE (EQD2).

## **Dosimetry analysis and evaluation of blood cell counts**

Dosimetry parameters of patients were obtained from available DVHs of the bone, lung, and heart. In the present study, the vertebrae from Th1 to Th10, the bilateral first to seventh ribs, and the whole sternum were contoured on planning chest CT as bone for DVH analysis. The organ contoured as bone included all irradiated bones in every patient.

During PBT with concurrent chemotherapy, complete blood count (CBC) was performed at least once a week. When grade 3 or severe cytopenia according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 occurred, CBC was performed at least twice a week until cytopenia improved to grade  $\leq 2$ . For evaluation of maximum and minimum absolute lymphocyte counts (ALCmax and ALCmin), and maximum NLR (NLRmax), results of CBCs performed from the first to the last day of PBT were used, whereas CBCs within 2 to 3 days after administration of steroids used as antiemetic drugs were excluded.

## **Follow-up and statistical analysis**

The patients were followed up with a physical examination, chest radiography, blood test, CT or PET/CT, and magnetic resonance imaging every 2–3 months during the first year and at 3- to 6-month intervals thereafter. Local progression at the primary site was defined as an increase in tumor size, significant positive accumulation on PET/CT, or histological diagnosis. Regional recurrence was defined as regrowth or new lymphadenopathy in the hilar, mediastinum, or supraclavicular lesion. Distant metastasis was defined as failure at any other site. Adverse events were assessed according to the CTCAE version 4.0.

The follow-up interval was defined from the first day of PBT to the date of death or the last follow-up. The OS, progression-free survival (PFS), distant metastasis-free survival (DMFS), local progression-free (LPF), and regional control (RC) rates were calculated from the first day of PBT to the date of that event or the last follow-up using the Kaplan–Meier method. Significant differences between survival curves were assessed using the generalized Wilcoxon test and Cox proportional hazard model. A  $p$  value < 0.05 was considered significant. SAS version 9.4 (SAS Institute, Cary, NC, USA) was used for the statistical analyses.

## Results

### Lymphopenia during treatment

ALCmax and ALCmin during PBT in all patients ranged from 544 to 2,824/ $\mu$ L (mean  $\pm$  standard deviation [SD] = 1,321  $\pm$  527/ $\mu$ L; median = 1,274/ $\mu$ L) and from 60 to 1,089/ $\mu$ L (mean  $\pm$  SD = 368  $\pm$  213/ $\mu$ L; median = 368/ $\mu$ L), respectively. NLRmax ranged from 1.76 to 78.4 (mean  $\pm$  SD = 10.3  $\pm$  12.9; median = 5.6). A decrease in lymphocyte count in relation to CRT for grades 0, 1, 2, 3, and 4 was observed in 0, 2 (4.8%), 7 (17.1%), 24 (58.6%), and 8 (19.5%) patients, respectively.

### Survival and locoregional control

At the last follow-up, 33 (80.4%) patients were deceased: 30 (73.1%) from cancer, 1 (2.4%) from suffocation due to repeated aspiration, and 2 (4.8%) from unknown causes. The median follow-up time from the first day of PBT was 41.6 (range = 6.4–139.0) months for all patients and 63.8 (range = 37.0–139.0) months for the surviving patients. The 3-year OS, PFS, and DMFS rates were 60.9% (95% confidence interval [CI] = 46.0–75.9%), 14.6% (95% CI = 3.8–25.5%), and 21.9% (95% CI = 9.3–34.6%), respectively (Fig. 1a). The 3-year LPF and RC rates were 54.4% (95% CI = 37.7–71.0%) and 42.7% (95% CI = 26.0–59.4%), respectively (Fig. 1b).

### Dose-volume analysis of the bone, lung, and heart and lymphopenia

Spearman's rank correlation coefficients of the percentages of the bone, lung, and heart receiving doses of  $\geq 5$  to 50 GyE and lymphopenia are shown in Table 2. Low doses to the bone (V5 to V20) were significantly associated with ALCmax, ALCmin, and NLRmax. Bone V5 showed the highest association with ALCmax and ALCmin. Lung doses (V5 to V50) also correlated with ALCmax, ALCmin, and NLRmax. Because the correlation coefficients of lung parameters were similar, lung V5 was used as an evaluation value for lung dose based on the radiosensitivity of lymphocytes, in accordance with previous reports [23, 24]. Heart doses did not correlate with ALCmax, ALCmin, and NLRmax. Heart V5 was used as the evaluation value in accordance with a previous report [19]. Scatter plots of the bone, lung, and heart V5 versus these three indicators of lymphopenia are shown in Fig. 2.

Table 2  
Spearman's rank correlation coefficients between dosimetry parameters and lymphopenia

Organ	DVH parameter	maximum ALC		minimum ALC		maximum NLR	
		R	<i>p</i> value	R	<i>p</i> value	R	<i>p</i> value
Bone	V5	-0.377	0.015	-0.441	0.003	0.398	0.010
	V10	-0.378	0.015	-0.426	0.005	0.401	0.009
	V20	-0.323	0.039	-0.388	0.012	0.362	0.019
	V30	-0.253	0.109	-0.279	0.077	0.208	0.190
	V40	-0.128	0.425	-0.257	0.104	0.129	0.421
	V50	-0.122	0.446	-0.255	0.106	0.113	0.479
Lung	V5	-0.360	0.020	-0.408	0.008	0.449	0.003
	V10	-0.333	0.033	-0.418	0.006	0.446	0.003
	V20	-0.347	0.026	-0.442	0.004	0.465	0.002
	V30	-0.328	0.036	-0.419	0.006	0.438	0.004
	V40	-0.318	0.042	-0.421	0.006	0.420	0.006
	V50	-0.296	0.059	-0.391	0.011	0.360	0.020
Heart	V5	-0.161	0.314	-0.303	0.053	0.267	0.091
	V10	-0.151	0.345	-0.269	0.088	0.226	0.155
	V20	-0.183	0.252	-0.269	0.088	0.223	0.159
	V30	-0.190	0.231	-0.295	0.060	0.251	0.112
	V40	-0.145	0.363	-0.269	0.088	0.246	0.120
	V50	-0.186	0.242	-0.266	0.092	0.241	0.128

DVH, dose-volume histogram; ALC, absolute lymphocyte count; NLR, neutrophil/lymphocyte ratio

## Effects of lymphopenia on survival

When the cutoff value of ALCmin was set at 200/ $\mu$ L, which was categorized as CTCAE grade 4, the 3-year OS, PFS, and DMFS rates of the high (> 200/ $\mu$ L) and low ( $\leq$  200/ $\mu$ L) ALCmin groups were 66.7% vs. 37.5% ( $p = 0.195$ ), 18.2% vs. 12.5% ( $p = 0.041$ ), and 27.3% vs. 12.5% ( $p = 0.006$ ), respectively (Fig. 3). Table 3 shows the patient characteristics of the high and low ALCmin groups. There were more women than men in the high ALCmin groups, but sex (male vs. female) was not a factor associated with OS ( $p = 0.962$ ), PFS ( $p = 0.855$ ), and DMFS ( $p = 0.507$ ) in this study population.

Table 3  
Patient and tumor characteristics by minimum absolute lymphocyte count

Characteristics		ALC $\leq$ 200/ $\mu$ L (n = 8)	ALC $>$ 200/ $\mu$ L (n = 33)	<i>p</i> value
Age (years)		47–73 (median 66)	42–79 (median 62)	0.499
Sex	Male	3	28	0.019
	Female	5	5	
Performance Status	0	4	22	0.639
	1	4	11	
Histology	SCC	3	8	0.386
	AC	5	19	
	NSCLC, NOS	0	6	
7th UICC clinical stage	IIIA	2	10	0.999
	IIIB	6	23	
Clinical Target volume (cc)		104.6–446.0 (median 233.3)	21.5–820.4 (median 193.9)	0.348
Chemotherapy regimen	CDDP + VNR	7	25	0.807
	Others	1	8	
ALC, absolute lymphocyte count; SCC, squamous cell carcinoma; AC, adenocarcinoma; NSCLC, non-small cell carcinoma; NOS, not otherwise specified; UICC, Union for International Cancer Control; CDDP, cisplatin; VNR, vinorelbine				

In NLRmax, when the cutoff value was set at 6.33, which was determined using the result of the receiver operating characteristic (ROC) curve, the 3-year OS, PFS, and DMFS rates of the low ( $\leq$  6.33) and high ( $>$  6.33) NLRmax groups were 73.9% vs. 44.4% ( $p = 0.042$ ), 26.1% vs. 5.6% ( $p = 0.022$ ), and 39.1% vs. 5.6% ( $p < 0.001$ ), respectively.

## Prognostic factor

The results of univariable analysis for potential prognostic factors associated with OS, PFS, and DMFS are shown in Table 4. Bone V5 did not show a significant association with OS, PFS, or DMFS. Conversely, heart V5 (hazard ratio [HR]: 1.032,  $p = 0.023$ ), ALCmax (HR: 0.999,  $p = 0.049$ ), and NLRmax (HR: 1.035,  $p = 0.007$ ) were significantly associated with OS. Heart V5 was significantly associated with PFS (HR: 1.024,  $p = 0.035$ ), whereas lung V5 (HR: 1.047,  $p = 0.056$ ) and NLRmax (HR: 1.020,  $p = 0.057$ ) were marginally significant factors associated with PFS. Lung V5 (HR 1.075,  $p = 0.005$ ), heart V5 (HR: 1.035,  $p = 0.005$ ),

CTV (HR: 1.002,  $p = 0.036$ ), age (HR: 0.443,  $p = 0.024$ ), ALCmax (HR: 0.999,  $p = 0.025$ ), and NLRmax (HR: 1.030,  $p = 0.005$ ) were significantly associated with DMFS.

Table 4  
Univariable analysis of prognostic factors for survivals

Factors	Overall survival			Progression-free survival			Distant metastasis-free survival		
	HR	95% CI	<i>p</i> value	HR	95% CI	<i>p</i> value	HR	95% CI	<i>p</i> value
Bone V5 (%)	1.010	0.98–1.05	0.561	1.010	0.98–1.04	0.553	1.021	0.99–1.06	0.242
Lung V5 (%)	1.029	0.99–1.08	0.233	1.047	1.00–1.10	0.056	1.075	1.02–1.13	0.005
Heart V5 (%)	1.032	1.00–1.06	0.023	1.210	1.00–1.05	0.035	1.035	1.01–1.06	0.005
CTV volume (cc)	1.002	1.00–1.00	0.109	1.000	0.99–1.00	0.703	1.002	1.00–1.01	0.036
Age (median)	0.699	0.35–1.41	0.317	0.630	0.32–1.23	0.175	0.443	0.22–0.90	0.024
Sex (male or female)	1.143	0.50–2.64	0.754	1.210	0.55–2.66	0.635	0.921	0.42–2.03	0.838
PS (0 or 1)	1.469	0.72–2.99	0.289	1.248	0.63–2.47	0.525	1.045	0.53–2.07	0.900
Histology (AC or others)	0.939	0.47–1.90	0.861	1.041	0.53–2.04	0.906	1.005	0.51–1.98	0.989
Maximum ALC (/ $\mu$ L)	0.999	0.99–1.00	0.049	0.999	0.99–1.00	0.092	0.999	0.99–1.00	0.025
Minimum ALC (/ $\mu$ L)	1.000	0.99–1.00	0.775	0.999	0.99–1.00	0.407	0.999	0.99–1.00	0.172
Maximum NLR	1.035	1.01–1.06	0.007	1.020	1.00–1.04	0.057	1.030	1.01–1.05	0.005

HR, hazard ratio; CI, confidence interval; CTV, clinical target volume; PS, performance status; AC, adenocarcinoma; ALC, absolute lymphocyte count; NLR, neutrophil/lymphocyte ratio

Table 5 shows the results of the multivariable analysis for survival by stepwise selection method (inclusion and exclusion criteria were set as  $p = 0.2$ ). Heart V5 was not significantly associated with OS (HR: 1.028,  $p = 0.104$ ), whereas age (HR: 0.400,  $p = 0.035$ ), sex (HR: 3.915,  $p = 0.018$ ), and ALCmax (HR: 0.999,  $p = 0.022$ ) were significantly associated with OS. For PFS, CTV volume (HR: 0.995,  $p = 0.008$ ), age (HR: 0.237,  $p = 0.001$ ), sex (HR: 11.09,  $p = 0.001$ ), histology (HR: 2.723,  $p = 0.033$ ), ALCmax (HR: 0.999,  $p =$

0.012), and NLRmax (HR: 1.065,  $p = 0.001$ ) were significant. For DMFS, lung V5 was an independent predictive factor (HR: 1.062,  $p = 0.027$ ). Age (HR: 0.251,  $p = 0.001$ ), sex (HR: 3.215,  $p = 0.030$ ), ALCmax (HR: 0.999,  $p = 0.008$ ), and NLRmax (HR: 1.041,  $p = 0.003$ ) were also significantly associated with DMFS.

Table 5  
Multivariable analysis of prognostic factors for survivals

Factors	Overall survival			Progression-free survival			Distant metastasis-free survival		
	HR	95% CI	$p$ value	HR	95% CI	$p$ value	HR	95% CI	$p$ value
Lung V5 (%)	-			1.048	0.99–1.10	0.092	1.062	1.00–1.11	0.027
Heart V5 (%)	1.028	0.99–1.06	0.104	-			-		
CTV volume (cc)	-			0.995	0.99–1.00	0.008	-		
Age	0.400	0.17–0.93	0.035	0.237	0.10–0.55	0.001	0.251	0.11–0.56	0.001
Sex (male or female)	3.915	1.26–12.1	0.018	11.09	2.87–42.8	0.001	3.215	1.12–9.23	0.030
Histology (AC or others)	-			2.723	1.08–6.83	0.033	-		
Maximum ALC (/ $\mu$ L)	0.999	0.99–1.00	0.022	0.999	0.99–1.00	0.012	0.999	0.99–1.00	0.008
Maximum NLR	1.030	0.99–1.06	0.094	1.065	1.02–1.10	0.001	1.041	1.01–1.06	0.003

HR, hazard ratio; CI, confidence interval; CTV, clinical target volume; PS, performance status; AC, adenocarcinoma; ALC, absolute lymphocyte count; NLR, neutrophil/lymphocyte ratio

## Discussion

We conducted this study hypothesizing that, in patients with stage III NSCLC undergoing PBT, irradiation of bone tissues might cause lymphopenia owing to depletion of progenitor cells, which would, in turn, reduce antitumor immunity and thereby influence survival. Our findings revealed that bone V5 and lung V5 correlated with lymphopenia and that lymphopenia was significantly associated with survival rates. In this study, however, there was no significant impact of bone V5 on survival rates, but lung V5 affected to DMFS on the multivariate analysis.

In RT with concurrent chemotherapy for NSCLC, it has been pointed out that low-dose irradiation of the thoracic vertebral body is associated with grade  $\geq 3$  leukopenia, which can result in poor survival and control rates owing to incomplete chemotherapy or treatment that could not be performed as planned

[25]. Some studies have also reported that doses to the bone were related to the survival rate in CRT for other primary tumors or with radiation alone [26, 27]. In the present study, the lymphocyte count tended to decrease with increasing bone irradiation doses, but lung V5 rather than bone V5 strongly correlated with lymphopenia. Conceivably, lung V5 irradiation doses increased relative to the bone V5 irradiation dose, and it is possible that lymphopenia, caused by the increase in doses to the bone, may be a spurious correlation (Fig. 4). However, there was no significant difference in the survival rate related to irradiation doses to the bone. In the present study, the thoracic vertebrae (Th1 to Th10), the sternum, and the first to seventh ribs were contoured as bones. Hayman et al. reported that the relative contribution of the thoracic vertebra, sternum, and ribs/clavicle to the active proliferating bone marrow was approximately 20%, 3%, and 9%, respectively [28]. Thus, one reason that bone irradiation may not have a large effect on the survival rate could be that myelosuppression is mainly caused by the concurrent use of chemotherapy.

In the current study, we examined not only ALC but also NLR as lymphocyte-related factors, as there are various reports describing how these factors relate to the prognosis of surgery and systemic treatment [9, 10, 29]. It has been reported that inflammatory cytokines are involved in cancer progression and associated with chemotherapy [30, 31]. Furthermore, high NLR levels are inflammatory markers that are one of the poor prognostic factors for programmed cell death receptor-1 (PD-1) inhibitor treatment in patients with lung cancer [30, 31]. Likewise, high NLR was a predictive factor for lower PFS and DMFS on multivariable analysis in the present study. Therefore, because the standard treatment for locally advanced lung cancer is CRT and immune checkpoint inhibitor therapy, it is important to reduce lymphopenia during RT.

Radiation pneumonitis (RP) is a potentially life-threatening adverse event in chest RT, and lung V20 is frequently used as an index of RP [32–35]. The National Comprehensive Cancer Network Guidelines have described lung V5 as an RP risk factor; however, owing to the increased use of IMRT and the results of the RTOG 0617 study, the description of lung V5 has been removed from the guidelines regarding dose constraints in lung cancer treatment [35, 36]. In the present study, lung V5 strongly correlated with lymphopenia and have a significant impact on DMFS. This suggests that while lung V5 does not often reduce the survival rate due to RP, it may inhibit a patient's anticancer immunity in association with lymphopenia. In the PACIFIC study, it is suggested that distant metastases might be reduced by maintaining anti-tumor immunity, in which lymphocytes play an important role [3]. Thus, careful attention should be paid not only to lung V20 but also to lung V5 in the treatment planning for NSCLC.

Nowadays, irradiation doses to the heart are known to be important in NSCLC patients treated with CRT. In the RTOG 0617 study, heart V5 was identified as a prognostic factor for OS [19]. In the present study, heart V5 was significantly associated with OS in univariable analysis. Unlike IMRT, PBT has the advantage of concentrating high doses of irradiation on the CTV while avoiding low doses to the lung and heart [20]. Therefore, in the treatment of lung cancer, PBT is considered to be more useful than IMRT because it can lower lung V20 and heart V5 exposure while suppressing the increase of lung V5. In chest irradiation with concurrent chemotherapy for esophageal cancer, PBT has advantages over IMRT in terms of lymphopenia and survival rate, and a prospective study is being conducted [12, 37, 38]. In lung cancer,

a randomized control trial comparing passive-scattering PBT and IMRT for locally advanced NSCLC did not prove the superiority of PBT because, despite PBT, DVHs of the lung and heart remained extremely high [39]. We speculate that in this randomized control trial, technical deficiencies in the delivery of the PBT probably affected the results because RP and local failure rates at 12 months for patients enrolled before versus after the trial midpoint were 31.0% and 13.1%, respectively ( $p = 0.027$ ). Therefore, the results of the NRG 1308 trial, which compares PBT and IMRT and is focused on low doses to at-risk organs and lymphopenia, are highly anticipated to shed light on this important issue [22].

The major limitations of the current study are its retrospective nature, small number of participants, clinical heterogeneity, and long period of patient accrual. The number of the patients with severe lymphopenia ( $ALC_{min} \leq 200/\mu L$ ) was also small, although there was a significant difference in survivals between the patients with and without severe lymphopenia. However, the PBT protocol, such as the definition of the CTV, prescription dose and fractionation, beam arrangement, treatment machine, and methods of respiratory-motion management, has not changed over the study period. Spot-scanning irradiation techniques have become widespread but passive-scattering PBT plans have been still carried at our facility. Furthermore, chemotherapy regimens were not identical although all patients received concurrent platinum-doublet chemotherapy. Large multicenter prospective studies, such as RTOG 1308, are required to address the abovementioned limitations and resolve the question of whether PBT could significantly improve OS in patients with stage III unresectable locally advanced NSCLC.

## Conclusions

This analysis showed that lymphopenia was associated with a lower irradiation dose to the lung as well as bone in CRT using proton beams for patients with stage III unresectable locally advanced NSCLC. Furthermore, patients with severe lymphopenia during the course of CRT had poor survival rates. Although lung doses were associated with DMFS, bone doses were not associated with both OS and DMFS.

Taken together, our findings indicate lung doses are more important than bone doses in CRT for stage III NSCLC and add weight to the argument that PBT has advantages over photon therapy because it is not only capable of delivering high-dose irradiation to lesions but also highly effective for reducing doses to surrounding healthy organs.

## Abbreviations

ALC: Absolute lymphocyte count

CBC: Complete blood count

CI: Confidence interval

CRT: Chemoradiotherapy

CT: Computed tomography

CTCAE: Common Terminology Criteria for Adverse Events

CTV: Clinical target volume

DMFS: Distant metastasis-free survival

DVH: Dose-volume histogram

EQD2: Biologically equivalent dose in 2 GyE per fraction

HR: Hazard ratio

ICI: Immune checkpoint inhibitors

IMRT: Intensity modulated radiotherapy

LPF: Local progression-free

NLR: Neutrophil lymphocyte ratio

NSCLC: Non-small cell lung cancer

OARs: Organs at risk

OS: Overall survival

PBT: Proton beam therapy

PET: Positron emission tomography

PFS: Progression-free survival

PTV: Planning target volume

RC: Regional control

ROC: Receiver operating characteristic

RP: Radiation pneumonitis

RT: Radiotherapy

RTOG: Radiation Therapy Oncology Group

SD: Standard deviation

## Declarations

### *Ethics approval and consent participate*

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This retrospective study was approved by the Institutional Review Board at University of Tsukuba Hospital (Approval No. R01-309) which waived the need for re-informed consent.

### *Consent for publication*

Not applicable.

### *Availability of data and materials*

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### *Competing interests*

The authors declare no competing interests.

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### *Authors' contributions*

Conceptualization, HI and MN; methodology, HI, KM, and MN; investigation, KO, YM, KB, and MN; resources, KO, KN, TS, and TO; data collection, KO, YM, KB, and MN; writing (original draft preparation), MN; writing (review and editing), HI, IS, and KM; supervision, HS; and funding acquisition, HS. All authors have read and agreed to the published version of the manuscript.

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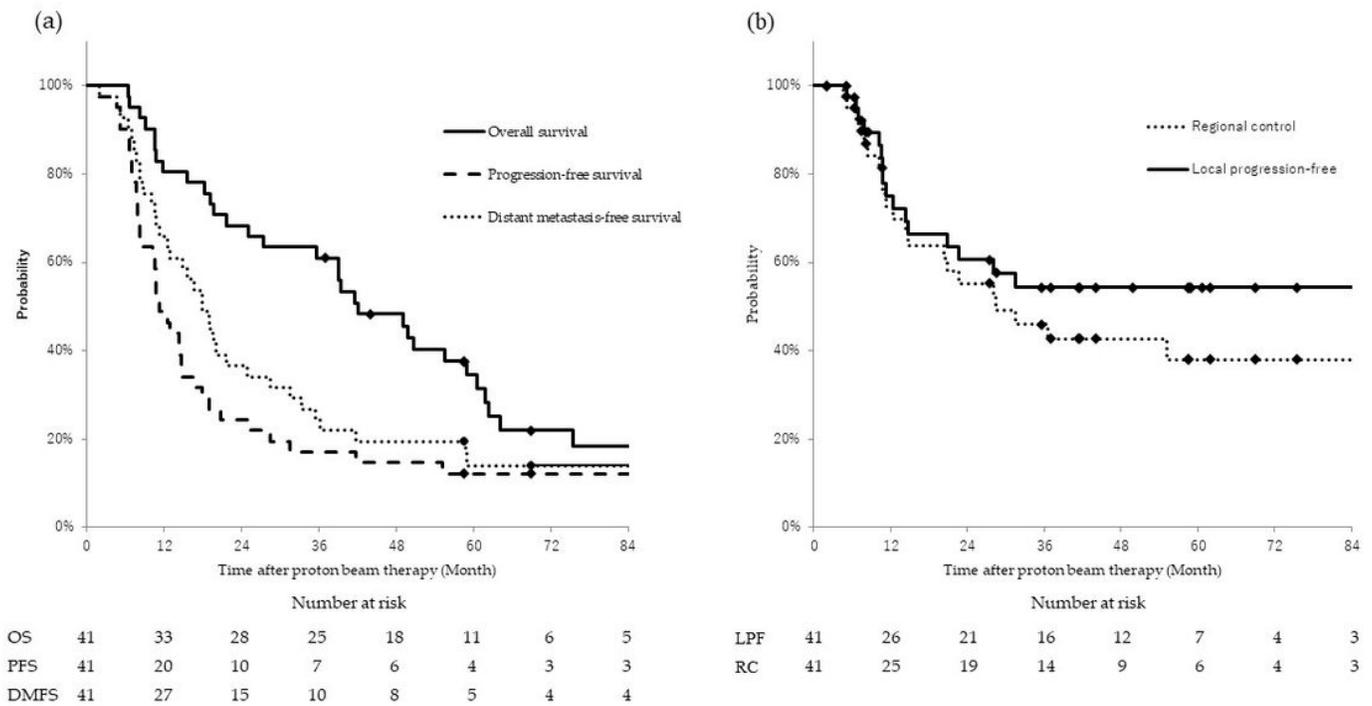
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# Figures



**Figure 1**

Kaplan-Meier estimate of survival, local-progression, and regional control rates. (a) Overall, progression-free, and distant metastasis-free survival curves for the patients in this study. Straight, dashed, and dotted lines indicate overall, progression-free, and distant metastasis-free survival, respectively. (b) Local progression-free and regional control rates. Straight and dotted lines indicate local progression-free and regional control rates, respectively.

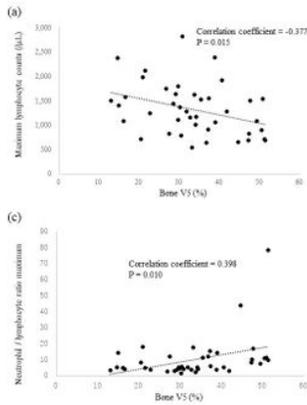


Figure 2A

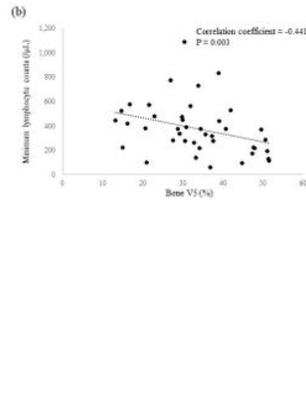


Figure 2B

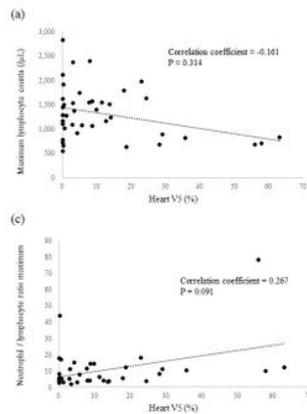
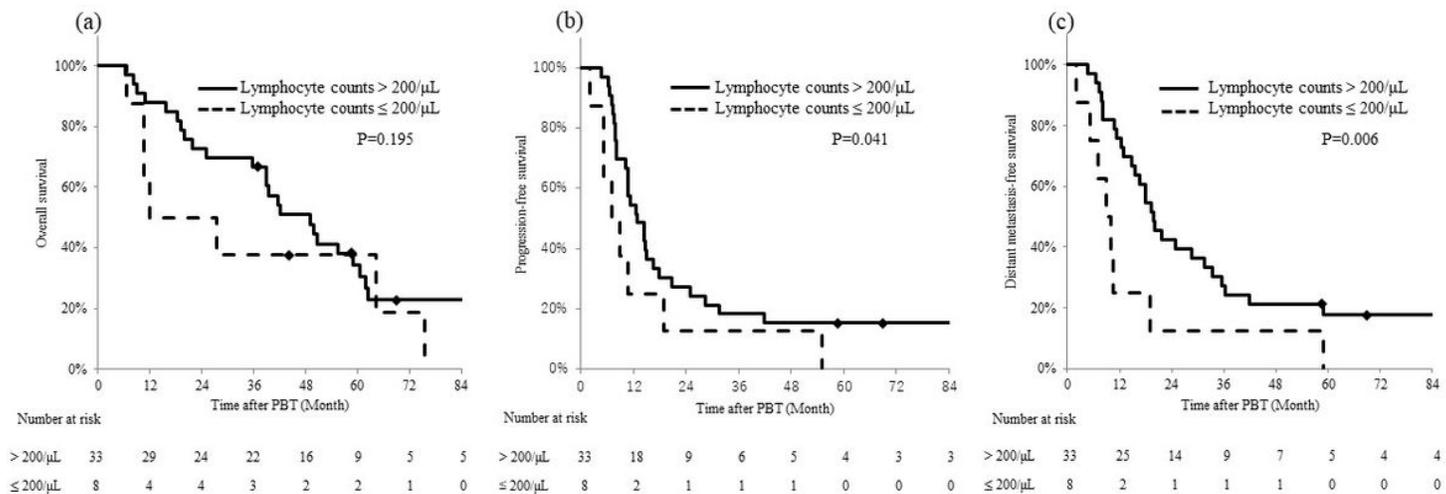


Figure 2C

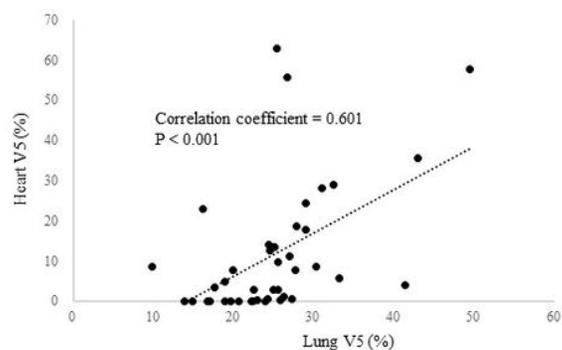
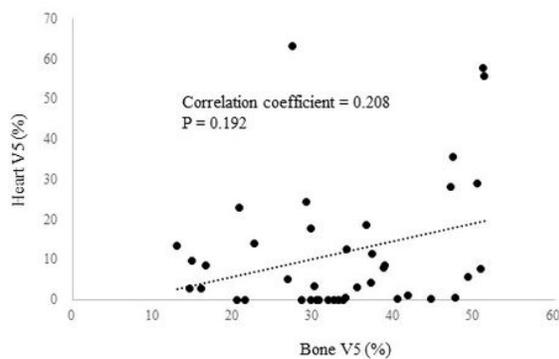
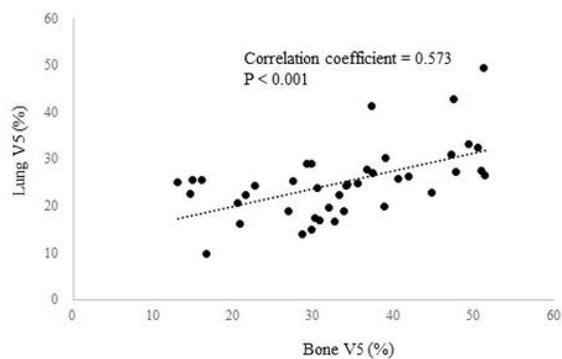
## Figure 2

2A. Scatter plots of bone V5 versus lymphocyte counts. The results of Spearman's rank test were shown. (a) Maximum lymphocyte counts during the period of proton beam therapy. (b) Minimum lymphocyte counts. (c) Maximum neutrophil/lymphocyte ratio. (d) Maximum platelet/lymphocyte ratio. 2B. Scatter plots of lung V5 versus lymphocyte counts. The results of Spearman's rank test were shown. (a) Maximum lymphocyte counts during the period of proton beam therapy. (b) Minimum lymphocyte counts. (c) Maximum neutrophil/lymphocyte ratio. (d) Maximum platelet/lymphocyte ratio. 2C. Scatter plots of heart V5 versus lymphocyte counts. The results of Spearman's rank test were shown. (a) Maximum lymphocyte counts during the period of proton beam therapy. (b) Minimum lymphocyte counts. (c) Maximum neutrophil/lymphocyte ratio. (d) Maximum platelet/lymphocyte ratio.



**Figure 3**

Survivals according to minimum absolute lymphocyte count > 200/ $\mu\text{L}$  (straight line) vs.  $\leq 200/\mu\text{L}$  (dashed line). (a) overall survival. (b) progression-free survival. (c) distant metastasis-free survival.



## Figure 4

Scatter plots of bone V5 versus lung V5 versus heart V5. (a) Correlation of bone V5 to lung V5. (b) Correlation of bone V5 to Heart V5. (c) Correlation of Lung V5 to Heart V5.