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Effects of lymphopenia on survival in proton therapy with chemotherapy for non-small cell lung cancer

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ABSTRACT

Lymphocytes play an important role in the cancer immune system. In the present study, we aimed to evaluate the associations of lymphopenia during proton beam therapy (PBT) and concurrent chemotherapy with clinical outcomes and to determine whether lung or bone is more influential on lymphopenia during PBT. Data from 41 patients with stage III non-small cell lung cancer (NSCLC) who received PBT of 74 GyE with concurrent chemotherapy between 2007 and 2017 were reviewed retrospectively. The correlation between dosimetry parameters obtained from dose–volume histograms of the bone and lung and lymphopenia during PBT were analyzed. Minimum absolute lymphocyte count (ALCmin) and maximum neutrophil/lymphocyte ratio (NLRmax) were used as indicators of lymphopenia. Bone V5–20 and lung V5–50 were significantly correlated with the ALCmin and NLRmax during PBT. Multivariable analysis showed that the NLRmax, but not the ALCmin, was associated with overall survival (OS), progression-free survival (PFS) and distant metastasis-free survival (DMFS). The 3-year rates of OS, PFS and DMFS of patients with a low (≤ 6.3) versus high (> 6.3) NLRmax 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. Lung V20 was significantly associated with DMFS on multivariable analyses (hazard ratio: 1.094, $P = 0.008$), whereas bone V5 had no impact on survival outcomes. We concluded that the NLRmax was a better prognostic indicator than the ALCmin, and the lung dose had more influence than the bone dose on the main survival outcomes in stage III NSCLC patients treated with PBT combined with concurrent chemotherapy.

Keywords: proton beam therapy (PBT); radiation-induced lymphopenia; stage III non-small cell lung cancer (NSCLC); dose–volume histogram (DVH); survival

INTRODUCTION

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

In the field of radiotherapy (RT), many previous studies have demonstrated the important roles of radiation-induced immune responses in the success of cancer treatment, and attention to this subject has increased since the introduction of ICIs [11–13]. Lymphocytes are radiosensitive cells, and RT-induced lymphopenia

results from decreased numbers of circulating lymphocytes and depletion of progenitor cells in the bone marrow and spleen [14–17]. However, despite advances in irradiation techniques, the lung and lymphoid organs such as the bone marrow are exposed to unnecessary radiation doses during thoracic RT. Intensity modulated RT (IMRT) enables more intensive high-dose irradiation of the target compared with three-dimensional conformal RT but increases lung volumes at lower radiation doses [18].

Proton beam therapy (PBT) is recognized for its unique ability to deliver high-dose radiation to the target while reducing unnecessary radiation to healthy tissues, because a spread-out Bragg peak of protons can be created to match the depth and thickness of the target [19–21]. Therefore, compared with photon-based RT, PBT potentially has the advantage of minimizing RT-induced lymphopenia because it was reported that lymphopenia is associated with the lung volumes irradiated at low to medium doses such as lung V5 and V10 [22, 23]. In fact, overall survival (OS) and lymphocyte counts were superior in the PBT group to those in the IMRT group in a matched-pair analysis of CRT for esophageal cancer [24].

On the other hand, no report has investigated the effects of irradiation of the bone marrow on lymphopenia or prognosis after CRT in patients with stage III NSCLC. Furthermore, it is unclear whether lymphopenia predicts survival. Therefore, the purpose of this study was to analyze the clinical outcomes of patients with stage III NSCLC who received definitive PBT with concurrent chemotherapy and to examine the associations of survival rates with lymphopenia indicators and the doses to the lung and bone marrow.

MATERIALS AND METHODS

Patient population

The present study was approved by the institutional review board of our institution (Approval No. R01–309). Data from 41 patients with unresectable locally advanced stage III NSCLC who received definitive PBT at a total dose of 74 GyE in 37 fractions combined with concurrent chemotherapy between November 2007 and December 2017 at our institution were reviewed retrospectively. 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 and IIIB in 12 (29.3%) and 29 (71.7%) patients, respectively. Histopathological examination revealed squamous cell carcinoma in 11, adenocarcinoma in 24 and NSCLC in 6. Thirty-two (78.0%) patients received chemotherapy consisting of cisplatin (CDDP) and vinorelbine (VNR). The remaining nine patients also received platinum-doublet chemotherapy (carboplatin plus S-1 in four patients, CDDP plus S-1 in two, carboplatin plus pemetrexed in one, carboplatin plus paclitaxel in one and carboplatin plus VNR in one). Thirty-seven (90.2%) patients completed two courses of chemotherapy and four (9.8%) patients received one course of chemotherapy concurrently with PBT. No patient received ICIs as consolidation therapy.

Proton beam therapy

For treatment planning, chest computed tomography (CT) images were taken at 2.5- or 5.0-mm intervals in 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 155–250 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, the initial clinical target volume (CTV1) encompassed the primary tumor, 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 ≥ 1 cm (as visualized on CT) or as F-18-fluorodeoxyglucose (FDG) positron emission tomography (PET)-positive lymph nodes. The second CTV (CTV2) encompassed the primary tumor and 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 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 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 ≥ 20 GyE (V20) $\leq 35\%$, 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

The dosimetric parameters of the patients were obtained from available dose–volume histograms (DVHs) of the bone and lung. 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. V5–50 values of the bone and lung, which were percentages of the volumes receiving doses of $\geq$ 5–50 GyE, were used for this analysis.

During PBT with concurrent chemotherapy, complete blood count (CBC) analysis was performed at least once a week. In cases of grade ≥ 3 cytopenia, according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, CBCs were obtained at least twice a week until the cytopenia improved to grade ≤ 2. For evaluation of minimum absolute lymphocyte counts (ALCmin) and maximum NLR (NLRmax) [25], CBCs obtained from the first to last day of PBT were used, whereas CBCs obtained within 2–3 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

Table 1. Patient and tumor characteristics

Characteristic	No. of patients
Age (years)	42–79 (median, 62)
Sex	
Male	31 (75.6%)
Female	10 (24.4%)
Performance status	
0	27 (65.9%)
1	14 (34.1%)
Histology	
Squamous cell carcinoma	11 (26.8%)
Adenocarcinoma	24 (58.6%)
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)

Abbreviations: NOS, not otherwise specified; UICC, Union for International Cancer Control.

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 FDG 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), and distant metastasis-free survival (DMFS) 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

The pretreatment absolute lymphocyte count in all patients ranged from 172 to 2862/μL (mean $\pm$ standard deviation [SD] = 1612 $\pm$ 582/μL; median = 1517/μL). The ALCmin during PBT in all patients ranged from 60 to 1089/μL (mean $\pm$ SD = 375 $\pm$ 213/μL; median = 368/μL). A decrease in the lymphocyte count during CRT was observed in all patients, and grades 1, 2, 3 and 4 lymphopenia were detected in 2 (4.8%), 7 (17.1%), 24 (58.6%) and 8 (19.5%) patients, respectively. The pretreatment NLR ranged from 1.00 to 40.0 (mean $\pm$ SD = 3.8 $\pm$ 6.1; median = 2.5). The NLRmax during PBT ranged from 1.76 to 78.4 (mean $\pm$ SD = 10.3 $\pm$ 12.9; median = 5.6). The median time from the start of PBT to the ALCmin and NLRmax were 45 days (range = 13–60 days) and 37 days (range = 6–60 days), respectively.

Survival

At the last follow-up, 33 (80.4%) patients had died: 30 (73.2%) from cancer, 1 (2.4%) from suffocation due to repeated aspiration and 2 (4.8%) from unknown causes without any cancer recurrence. 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. 1).

Lymphopenia and dosimetric parameters of the bone and lung

Bone and lung V5–50 values, which were percentages of the volumes of each organ receiving doses of $\geq$5–50 GyE, and Spearman's rank correlation coefficients for the associations between lymphopenia and bone and lung V5–50 are shown in Table 2. Regarding the bone dose, bone V5–20 values were significantly associated with the ALCmin and NLRmax, and bone V5 ranging from 12.9 to 51.5% (mean $\pm$ SD = 33.4 $\pm$ 10.9%; median = 33.1%) showed the strongest association with the ALCmin (R = $-$0.441, P = 0.004). Lung V5–50 values were also correlated with both the ALCmin and NLRmax, and lung V20 ranging from 6.1 to 39.6% (mean $\pm$ SD = 18.9 $\pm$ 6.3%; median = 19.9%) showed the strongest association with the ALCmin

Table 2. Correlations of bone and lung dosimetric parameters with lymphopenia

Organ	Dosimetric parameter		Minimum ALC		Maximum NLR	
	mean ± SD (%)		R	*P* value	R	*P* value
Bone	V5	33.4 ± 10.9	−0.441	0.004	0.398	0.010
	V10	30.4 ± 11.0	−0.426	0.006	0.401	0.009
	V20	25.4 ± 10.9	−0.388	0.012	0.362	0.019
	V30	17.6 ± 8.4	−0.279	0.077	0.208	0.190
	V40	10.7 ± 5.6	−0.257	0.104	0.129	0.421
	V50	7.0 ± 4.2	−0.255	0.106	0.113	0.479
Lung	V5	25.0 ± 7.6	−0.408	0.008	0.449	0.003
	V10	22.2 ± 7.0	−0.419	0.006	0.446	0.003
	V20	18.9 ± 6.3	−0.443	0.004	0.465	0.002
	V30	15.9 ± 5.9	−0.419	0.006	0.438	0.004
	V40	13.1 ± 5.1	−0.422	0.006	0.420	0.006
	V50	10.5 ± 4.4	−0.391	0.011	0.360	0.020

Abbreviations: ALC, absolute lymphocyte count; NLR, neutrophil/lymphocyte ratio; SD, standard deviation.

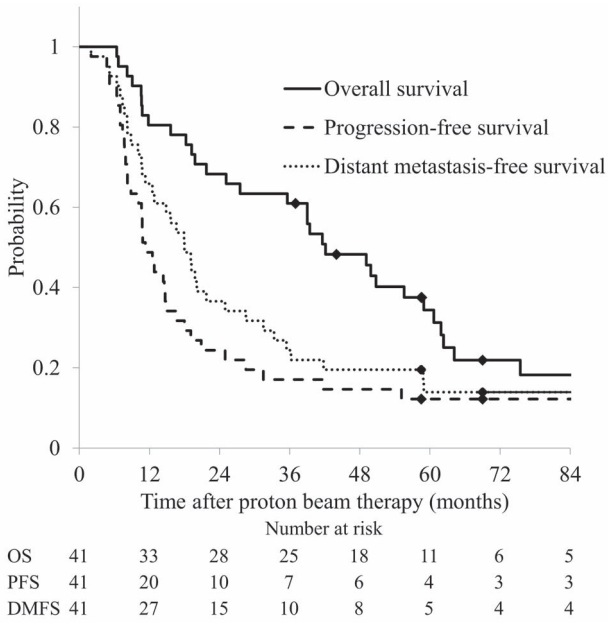

Fig. 1. Kaplan–Meier estimates of OS (straight line), PFS (dashed line), and DMFS (dotted line) survival curves for all patients.

(R = −0.443, $P = 0.004$). Scatter plots of the bone V5 or lung V20 versus these two indicators of lymphopenia are shown in Fig. 2.

Effects of lymphopenia on survival

The 3-year OS, PFS and DMFS rates of the patients with grade ≤ 3 versus grade 4 lymphopenia 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). When the patients were grouped according to a cutoff NLRmax of 6.3, as determined by receiver operating characteristic analysis, the 3-year OS, PFS and DMFS rates of the low versus high 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 (Fig. 4).

Table 3 shows the patient characteristics according to the lymphopenia grade and NLRmax. There were significant differences in sex ($P = 0.019$) and bone V5 ($P = 0.017$) between the patients with grade 4 lymphopenia and those with grade ≤ 3 lymphopenia, but no significant differences in other factors, including clinical stage and CTV, were observed between the groups.

Prognostic factors

The results of univariable analyses of 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, the NLRmax (hazard ratio [HR]: 1.035, $P = 0.008$) was significantly associated with OS. Lung V20 (HR: 1.084, $P = 0.010$), age (HR: 0.178, $P = 0.024$), lymphopenia grade (HR: 2.629, $P = 0.019$) and NLRmax (HR: 1.030, $P = 0.004$) were significantly associated with DMFS.

Table 5 shows the results of the multivariable analysis of survival using the stepwise selection method (inclusion and exclusion criteria were $P = 0.2$). The NLRmax was significantly associated with OS (HR: 1.035, $P = 0.008$), PFS (HR: 1.032, $P = 0.015$) and DMFS (HR: 1.034, $P < 0.001$). Other than the NLRmax, lung V20 (HR: 1.094, $P = 0.008$) and age (HR: 0.265, $P = 0.001$) were independent predictive factors for DMFS.

DISCUSSION

We hypothesized that in patients with stage III NSCLC undergoing PBT, irradiation of bone tissues might cause lymphopenia due to depletion of progenitor cells, which in turn can reduce antitumor immunity and influence survival. However, our findings revealed no significant impact of the bone dose on any of the survival types evaluated, whereas lung V20 was identified as an independent predictor of DMFS in

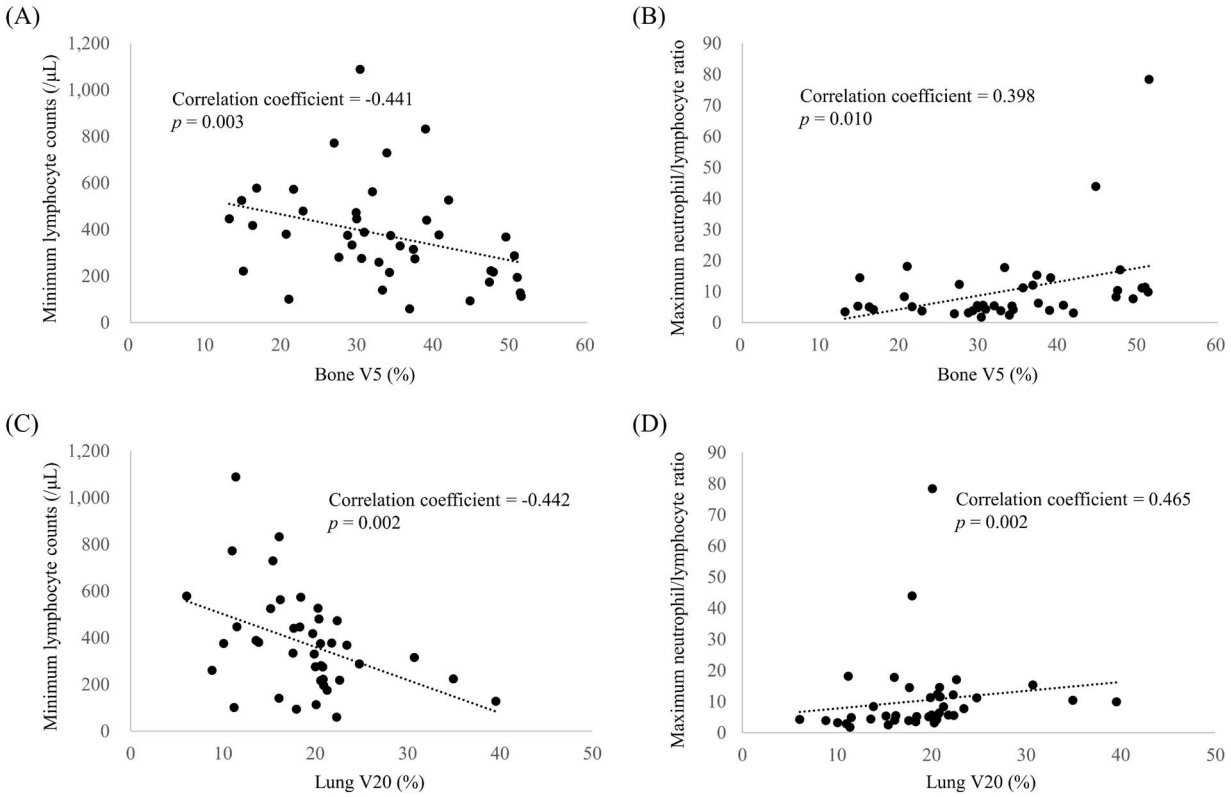

Fig. 2. Scatter plots of bone V5 or lung V20 versus the lymphocyte count. Spearman's rank correlation coefficients for the correlations of bone V5 with the (A) minimum lymphocyte count during PBT and (B) maximum neutrophil/lymphocyte ratio, and of lung V20 with the (C) minimum lymphocyte count during PBT and (D) maximum neutrophil/lymphocyte ratio.

the multivariable analysis. Furthermore, bone V5 and lung V20 were correlated with both the ALCmin and NLRmax, and the NLRmax, but not ALCmin, was associated with survival rates.

Although the impact of chemotherapy on hematologic toxicity is well established, lymphocytes are known to be highly sensitive to radiation and RT could have great effect on lymphopenia. Campian *et al.* reported that patients with stage III NSCLC treated with neoadjuvant chemotherapy did not develop lymphopenia until they began treatment with RT [11]. In addition, Abravan *et al.* reported that a significant reduction was observed in lymphocyte counts during RT compared with baseline, irrespective of chemotherapy delivery in patients with NSCLC [26]. Therefore, radiation-related factors such as gross tumor volume and lung volumes at lower radiation doses could be significantly correlated with lymphopenia during CRT in NSCLC patients, as reported by Tang *et al.* [22].

Lymphopenia induced by CRT using protons is potentially less severe than that induced by CRT using photons because protons can reduce the doses and volumes of the healthy organs such as the bone and lung. Xie *et al.* analyzed 178 NSCLC patients treated with photon-based CRT and reported median pretreatment ALC and ALCmin during CRT were 1630/μL and 260/μL, respectively [23], and Kanzaki *et al.* reported median pretreatment NLR and NLRmax during CRT were 3.1 and 14.7, respectively, in 111 patients with stage III NSCLC treated with photon-based CRT [27]. In the present study, the median ALCmin and NLRmax during PBT with 74 GyE administered concurrently with chemotherapy were 375/μL and 5.6, respectively. Although the patient and treatment characteristics and baseline value of lymphocytes were different among those studies, lymphopenia related to CRT using protons, irrespective of higher dose irradiation at a total dose of 74 GyE, seems to be less severe compared with photon-based CRT.

In the present study, we examined the lymphopenia indicators ALCmin and NLRmax as prognostic factors, but the ALCmin was associated with only DMFS in the univariable analysis. On the other hand, the patients with a low NLRmax had significantly better OS, PFS and DMFS and the NLRmax was significantly associated with OS, PFS and DMFS in not only the univariable but also multivariable analyses. Thus, the NLRmax seems to be more important for predicting survival after PBT combined with chemotherapy for stage III NSCLC patients, and various reports have analyzed lymphopenia as a prognostic factor after surgery, systemic therapy and RT. Although studies evaluating the relationships between clinical outcomes and both ALC and NLR simultaneously are limited [6–9], the study by Xia *et al.* supports our findings [28]. In their retrospective study analyzing the relationships

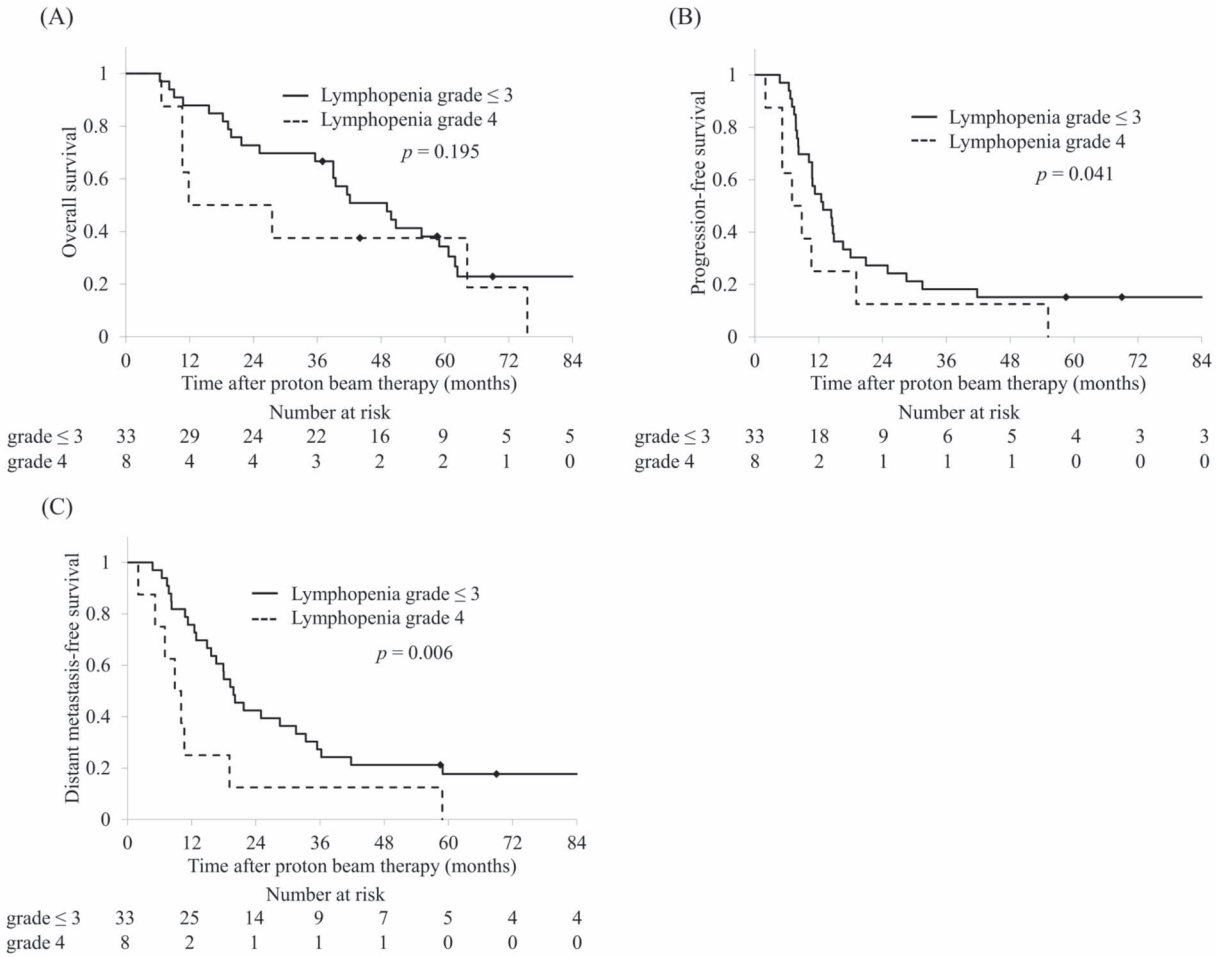

Fig. 3. Survival according to grade ≤ 3 versus grade 4 lymphopenia. (A) OS, (B) PFS, and (C) DMFS.

of survival with the ALC, NLR and platelet/lymphocyte ratio in 224 NSCLC patients receiving definitive RT, the NLR during RT was an independent prognostic factor for OS (HR: 1.049, $P = 0.001$) and PFS (HR: 1.040, $P = 0.004$) on multivariable analysis, whereas the ALCmin was not associated with PFS or OS.

A potential reason why the NLR had a stronger prognostic impact compared with the ALC in the present study is that the NLR takes into account the influence of the suppressed cytolytic activity of lymphocytes by neutrophils. The NLR, which is the ratio of the peripheral circulating neutrophil count to lymphocyte count, is an inflammatory marker that reflects the imbalance between immune surveillance and tumor progression [29–31]. A high NLR indicates an abnormal host-immune surveillance status, which might favor tumor proliferation, invasion and metastasis [32]. A meta-analysis revealed that a high NLR before cancer treatment initiation was a predictor of poor prognosis after lung cancer treatment [33]. In addition, the incidence of severe lymphopenia was lower in our PBT study than in the photon studies, and it might have masked the effects of the ALCmin on the outcomes in the present study.

In photon-based CRT, doses to the bone or lung are reportedly related to hematologic toxicities including lymphopenia [22, 34, 35]. Similarly, both bone V5–20 and lung V5–50 values were associated with lymphopenia in our PBT study, but the latter had a stronger impact than the former on lymphopenia induction. This suggests that circulating lymphocytes in the lungs have a greater effect on lymphopenia during CRT compared with proliferating lymphocytes in the bone marrow, because bone marrow may be suppressed not only by RT but also by systemic chemotherapy. Tang *et al.* analyzed the correlation between lung V5–70 values (in 5-Gy increments) and lymphopenia in 515 NSCLC patients treated with CRT and reported that lung V5–55 values were associated with lymphopenia [22]. Likewise, lung V5–50 values were associated with lymphopenia in the present study. Although lung V5 showed the strongest association in their study ($P < 0.001$), the correlations between the lung V5–40 values and lymphopenia were similar in strength (R = −0.40 to −0.44, $P = 0.004$ to 0.008) in our study (Table 2). Because an acceptable dose distribution can be obtained using a few proton beams (usually two or three ports), the difference between V5 and V20 is smaller in PBT than in

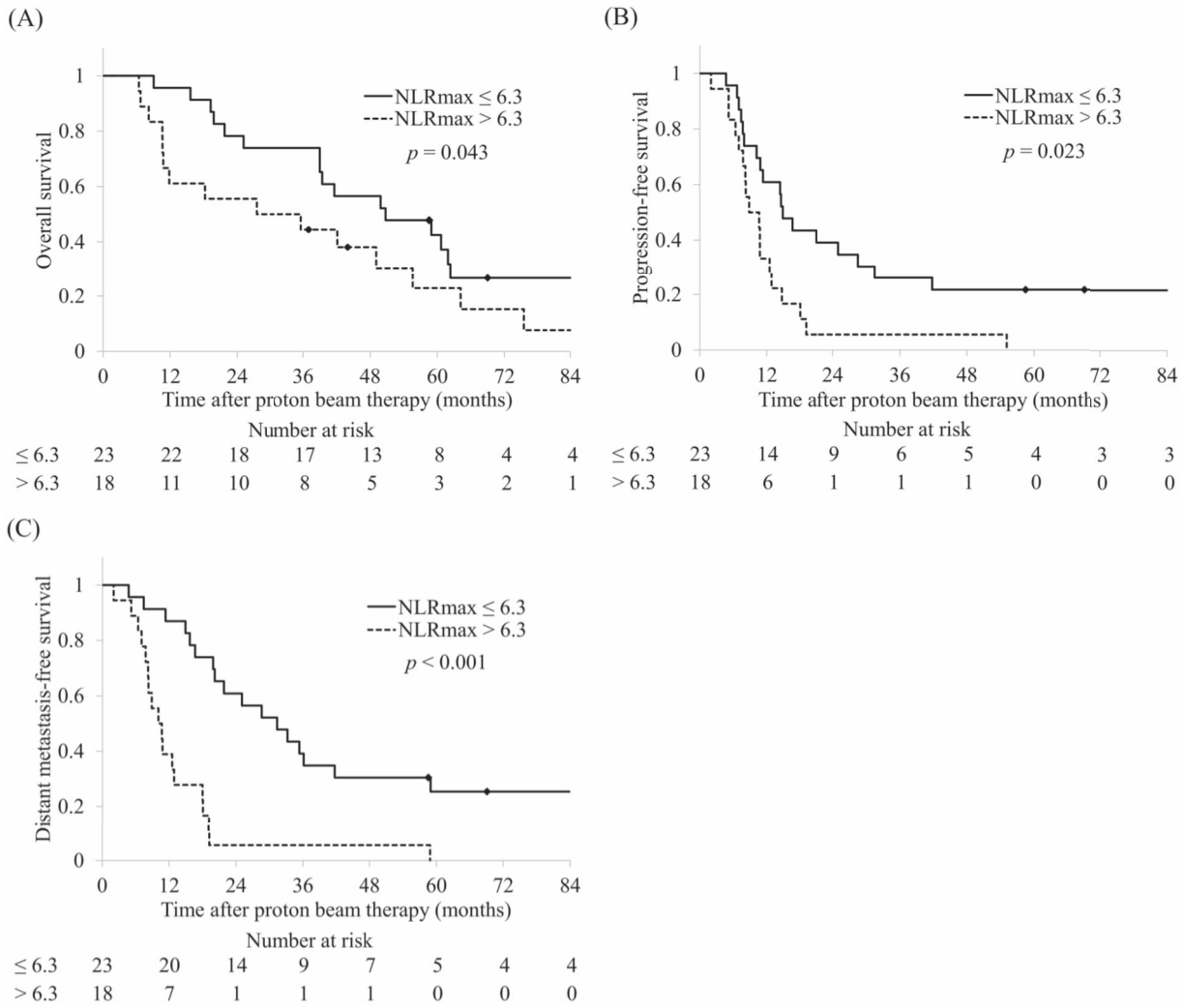

Fig. 4. Survival according to a maximum neutrophil/lymphocyte ratio ≤ 6.3 (straight line) versus >6.3 (dashed line). (A) OS, (B) PFS, and (C) DMFS.

photon-based RT [20, 21, 36]. As a result, V20 might be a predictor of lymphopenia in PBT.

In addition to the association between the lung dose and lymphopenia, lung V20 was associated with DMFS in multivariable analyses. This suggests that minimizing changes to lymphocytes and the immune system during PBT by reducing the irradiated lung volume and dose may decrease the risk of tumor metastasis. In the PACIFIC study, the rate of distant metastasis was reduced by maintaining anti-tumor immunity with the anti-programmed cell death ligand 1 antibody durvalumab [3]. Recent studies have shown that severe lymphopenia during CRT is associated with poor PFS in patients with NSCLC receiving adjuvant durvalumab [37, 38]. Additionally, lung V20 is an important predictive factor for radiation pneumonitis (RP), which is a potentially life-threatening adverse event caused by thoracic RT [39–42], and severe RP leads to discontinued administration of durvalumab after CRT. Thus, lung V20 should be regarded as an important factor for both lymphopenia and RP in treating NSCLC with CRT and immunotherapy.

The major limitations of the present study are its retrospective nature, small number of participants, clinical heterogeneity and long period of patient accrual. However, the PBT protocol, such as the CTV definitions, prescription dose and fractionation, beam arrangement, treatment machine and methods of respiratory-motion management, did not change over the study period. Bone V5–50 values were relative not to the bones of the whole body but to the bones of the chest, although it would be better to use relative V5–50 values to the bones of the whole body in order to evaluate radiation-induced lymphopenia. Because CT images of the whole body for treatment planning were not taken in patients with lung cancer, the same bones of the chest were contoured as the bone in each patient and bone V5–50 values relative to the bones of the chest were used for the analysis. Furthermore, chemotherapy regimens were not identical, although all patients received concurrent platinum-doublet chemotherapy, and no significant differences in the severity of lymphopenia between CDDP plus VNR and the other platinum-doublet chemotherapy regiments, such

Table 3. Patient and tumor characteristics according to lymphopenia grade and maximum NLR

Characteristics		Lymphopenia		*P* value	Maximum NLR		*P* value
		grade 4 ($n = 8$)	grade ≤ 3 (n = 33)		≤ 6.3 ($n = 23$)	>6.3 ($n = 18$)	
Age (years)		47–73 (median 66)	42–79 (median 62)	0.499	48–79 (median 62)	42–73 (median 62)	0.990
Sex	Male	3	28	0.019	20	11	0.122
	Female	5	5		3	7	
Performance status	0	4	22	0.639	16	10	0.550
	1	4	11		7	8	
Histology	SCC	3	8	0.386	7	4	0.642
	AC	5	19		12	12	
	NSCLC NOS	0	6		4	2	
7th UICC clinical stage	IIIA	2	10	0.999	8	4	0.595
	IIIB	6	23		15	14	
Clinical target volume (cc)		104.6–446.0 (median 233.3)	21.5–820.4 (median 193.9)	0.348	21.5–437.1 (median 158.1)	102.0–820.4 (median 268.5)	0.028
Bone V5 (mean $\pm$ SD, %)		42.0 ± 10.2	31.3 ± 10.0	0.017	28.9 ± 7.9	39.1 ± 11.5	0.004
Lung V20 (mean $\pm$ SD, %)		21.2 ± 7.7	18.4 ± 5.8	0.383	16.4 ± 4.5	22.2 ± 6.8	0.004
Chemotherapy regimen	CDDP+VNR	7	25	0.807	16	16	0.269
	Other	1	8		7	2	

Abbreviations: NLR, neutrophil/lymphocyte ratio; 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.

Table 4. Univariable analysis of prognostic factors according to different survival types

Factors	OS			PFS			DMFS		
	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value
Bone V5 (%)	1.011	0.98–1.05	0.558	1.010	0.98–1.04	0.563	1.022	0.99–1.06	0.223
Lung V20 (%)	1.023	0.96–1.08	0.452	1.051	0.99–1.11	0.091	1.084	1.02–1.15	0.010
CTV ($\leq$ or $>$ 228.9 cc)	1.373	0.67–2.82	0.387	1.059	0.55–2.05	0.865	1.865	0.93–3.75	0.080
Age ($\leq$ or $>$ 62 years)	0.700	0.35–1.41	0.319	0.632	0.32–1.23	0.178	0.444	0.22–0.90	0.024
Sex (female or male)	1.139	0.49–2.63	0.760	1.217	0.55–2.68	0.625	0.925	0.42–2.04	0.848
PS (0 or 1)	1.473	0.72–3.00	0.286	1.247	0.63–2.47	0.527	1.059	0.54–2.09	0.870
Histology (other or AC)	0.941	0.47–1.90	0.861	1.045	0.53–2.05	0.900	1.011	0.51–1.99	0.974
Lymphopenia (grade 4 or ≤ 3)	1.595	0.68–3.72	0.280	1.910	0.87–4.21	0.108	2.629	1.18–5.88	0.019
NLRmax	1.035	1.01–1.06	0.008	1.020	0.99–1.04	0.056	1.030	1.01–1.05	0.004

Abbreviations: OS, overall survival; PFS, progression-free survival; DMFS, distant metastasis-free survival; HR, hazard ratio; CI, confidence interval; CTV, clinical target volume; PS, performance status; AC, adenocarcinoma; NLRmax, maximum neutrophil/lymphocyte ratio.

as carboplatin plus S-1, were observed. Large multicenter prospective studies, such as RTOG 1308, are required to address the abovementioned limitations.

In conclusion, this analysis showed that lymphopenia was associated with lower radiation doses to the lung, as well as bone, in CRT using proton beams for patients with unresectable locally advanced stage III NSCLC. Furthermore, patients with severe lymphopenia during the CRT course had poor survival rates, and the NLRmax was a useful indicator of lymphopenia. Although the lung doses were associated with DMFS, the bone doses were not associated with OS, PFS, or DMFS. Taken together, our findings indicate that the lung doses are more important than the bone doses in CRT for stage III NSCLC and add weight to the argument that PBT has advantages over IMRT because it not only delivers high-dose radiation to lesions but also effectively reduces the doses to surrounding healthy organs.

Table 5. Multivariable analysis of prognostic factors according to different survival types

Factors	OS			PFS			DMFS		
	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95%CI	*P* value
Lung V20 (%)	-			1.055	0.99–1.12	0.096	1094	1.02–1.17	0.008
Age ($\leq$ or $>$ 62 year)	-			0.492	0.24–1.01	0.054	0.265	0.12–0.60	0.001
Sex (female or male)	-			2.042	0.81–5.15	0.131	1.999	0.77–5.22	0.153
Lymphopenia (grade 4 or $\leq$ 3)	-			-			2.293	0.83–6.35	0.110
NLRmax	1.035	1.01–1.06	0.008	1.032	1.01–1.06	0.015	1.034	1.00–1.07	<0.001

Abbreviations: OS, overall survival; PFS, progression-free survival; DMFS, distant metastasis-free survival; HR, hazard ratio; CI, confidence interval; CTV, clinical target volume; PS, performance status; AC, adenocarcinoma; NLRmax, maximum neutrophil/lymphocyte ratio.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest regarding this manuscript.

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PRESENTATION AT A CONFERENCE

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