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ONCOLOGY

The vertebral 3′-deoxy-3′-^{18}F-fluorothymidine uptake predicts the hematological toxicity after systemic chemotherapy in patients with lung cancer

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Abstract

Objectives Although hematological toxicities (HT) are the leading adverse events of systemic chemotherapy, the estimation of severe HT is challenging. Recently, 3′-deoxy-3′-[^{18}F]-fluorothymidine (^{18}F-FLT) accumulation with PET has been considered a biomarker of the cell proliferation. This study aims to elucidate whether the vertebral accumulation of ^{18}F-FLT could estimate severe HT during platinum-doublet chemotherapy.

Methods In this Institutional Review Board–approved retrospective study, 50 patients with primary lung cancer underwent ^{18}F-FLT PET scan before platinum-doublet chemotherapy. We evaluated the standardized uptake value, total vertebral proliferation (TVP), and TVP/body surface area (TVP/BSA) of the vertebral body (Th4, Th8, Th12, and L4), and then the associations between those parameters and frequency of severe HT during platinum-doublet chemotherapy were assessed.

Results Severe HT (grade 3/4) was observed in 40.0% of patients during the first cycle. The ROC curve analyses revealed that the TVP/BSA of L4 was the most discriminative parameter among PET parameters for the prediction of severe HT. The multivariate logistic regression analysis revealed the TVP/BSA of L4 (odds ratio [OR], 0.94; $p = 0.0036$) and the frequency of the grade 3/4 hematological toxicity in previous clinical trials (OR, 1.03; $p = 0.023$) were independent predictors. Furthermore, the sensitivity, specificity, and accuracy of the TVP/BSA of L4 cut-off of 68.7 to predict grade 3/4 HT were 80.0%, 86.7%, and 84.0%, respectively. A low TVP/BSA of L4 (< 68.7) as a binary variable was a significant indicator of severe HT (OR, 26.0; $p = 0.000026$).

Conclusions The low ^{18}F-FLT uptake in the lower vertebral body is a predictor of severe HT in patients with lung cancer who receive platinum-doublet chemotherapy.

Trial registration Trial registration: UMIN000027540

Key Points

- *The vertebral ^{18}F-FLT uptake with PET is an independent predictor of the severe hematological toxicity during the first cycle of platinum-doublet chemotherapy.*
- *The ^{18}F-FLT uptake in L4 vertebral body estimated hematological toxicities better than that in the upper vertebra (Th4, Th8, and Th12).*
- *The evaluation of the amount and activity of hematopoietic cells in the bone marrow cavity using ^{18}F-FLT PET imaging could provide predictive data of severe hematological toxicities and help determine an appropriate drug combination or dose intensity in patients with advanced malignant diseases.*

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Keywords Thymidine · Positron emission tomography (PET) · Lung cancer · Chemotherapy · Lumbar vertebrae

Abbreviations

^{18}F-FLT	3′-Deoxy-3′-[^{18}F]-fluorothymidine
AUC	Area under the curve
BMI	Body mass index
BSA	Body surface area
CI	Confidence interval
ECOG	Eastern Cooperative Oncology Group
G-CSF	Granulocyte colony–stimulating factor
HT	Hematological toxicity
L	Lumbar vertebra
MIP	Maximum intensity projection
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NPV	Negative predictive value
OR	Odds ratio
PPV	Positive predictive value
PPV	Proliferative vertebral volume
PS	Performance status
SUV	Standardized uptake value
Th	Thoracic vertebra
TVP	Total vertebral proliferation
VOI	Volume of interest
WBC	White blood cell

Introduction

In several malignant diseases, hematological toxicities are the leading adverse events and the dose-limiting toxicity during systemic chemotherapy using cytotoxic agents. For patients with advanced solid tumor, including non-small cell lung cancer, one of the most crucial goals of systemic chemotherapy is not cure but prolonging the survival time or palliation. Thus, the dose adjustment of cytotoxic agents is imperative to evade severe hematological toxicities and maintain the patients' quality of life. Reportedly, the prevalence of various malignant diseases increases with advancing age of patients [1]. With rapidly increasing aging population worldwide and the fact that the hematopoietic function of the bone marrow decreases in older patients, hematological toxicities of cancer chemotherapy could become a primary concern for healthcare providers in the future.

Although the whole bone marrow cavity comprises hematopoietic cells at birth, these are gradually replaced by fat tissue during life [2], and the hematopoietic function of the bone marrow decreases with age. However, as the chronological age is not a precise indicator of the hematopoietic function of the bone marrow, it is challenging to determine an appropriate drug combination or dose intensity in individual patients. Thus, it is anticipated that noninvasive measures of the hematopoietic function of the bone marrow can predict severe hematological toxicities of cancer chemotherapy.

3′-Deoxy-3′-[^{18}F]-fluorothymidine (^{18}F-FLT) is a thymidine analog trapped into cells through the phosphorylation by thymidine kinase 1. As the ^{18}F-FLT uptake with PET correlates with the Ki-67 immunohistochemical scoring in several malignant tumors [3–5], ^{18}F-FLT PET has been developed for imaging the cellular proliferation. Besides proliferative tumors, the physiological ^{18}F-FLT uptake has been observed in the liver and bone marrow because of glucuronidation of ^{18}F-FLT in the liver and high proliferative activity of hematopoietic cells in the marrow. Because low ^{18}F-FLT uptake in the bone marrow is associated with dysregulated proliferation of myeloid cells and shows the same distribution pattern as bone marrow scintigraphy in patients with myelofibrosis, ^{18}F-FLT uptake is considered as a reliable marker to assess the hematopoietic activity in the bone marrow [6]. Moreover, some recent studies have reported that a change in the ^{18}F-FLT uptake of the bone marrow before and after chemoradiotherapy signifies a change in local hematopoiesis [7–9]. However, to date, whether the baseline ^{18}F-FLT uptake of the bone marrow estimates the hematological toxicity of cancer chemotherapy remains unclear. This study aims to assess the impact of the pretreatment ^{18}F-FLT uptake in the vertebral bone marrow on the hematological toxicity following the initiation of systemic chemotherapy with platinum doublets for patients with primary lung cancer.

Materials and methods

Study design and patient selection

This study protocol was approved by the Institutional Review Board of our hospital, and the clinical trial was registered at the University Hospital Medical Information Network Clinical Trials Registry (www.umin.ac.jp/ctr/; UMIN000027540). In this study, a retrospective review was performed on patients who underwent an ^{18}F-FLT PET scan for the assessment of newly diagnosed primary lung cancer at the University of Fukui Hospital (Fukui, Japan) from June 2010 to February 2018. All eligible patients of the present study had to have a histological diagnosis of primary lung cancer and receive standard platinum-doublet chemotherapy, including cisplatin (60–80 mg/m^2) or carboplatin (area under the concentration-time curve, 5–6 mg/mL/min). Conversely, the exclusion criteria were blood disorders and prior use of cytotoxic agents or concurrent use of immunosuppressive agents for other condition(s). Of 75 patients who underwent ^{18}F-FLT PET scan, we subsequently excluded 6

because of the final diagnosis of benign lung diseases and 19 because they received treatments other than platinum-doublet chemotherapy (operation, molecular-targeted drugs, chemoradiotherapy, and single-agent chemotherapy). Thus, we enrolled 50 patients with newly diagnosed primary lung cancer in this study (Fig. 1).

All patients underwent a ^{18}F-FLT PET scan using a whole-body scanner (ADVANCE, GE Healthcare) within 2 weeks before initiation of chemotherapy, and a whole-body ^{18}F-FDG PET/CT examination using a PET/CT scanner (Discovery LS or Discovery ST Elite; GE healthcare) and a whole-body CT scan with a multi-detector row CT system (LightSpeed ULTRA or Discovery CT750 HD; GE Healthcare) within 4 weeks before undergoing the ^{18}F-FLT PET scan. In our institution, the whole-body ^{18}F-FDG PET/CT scan was used as a routine technique of staging, and the whole-body PET scanner was used for the clinical research because of a radiation exposure concern.

We recorded the following baseline measures: (a) tumor characteristics comprised the tumor type, cancer stage, and bone metastasis; (b) the clinical variables comprised gender, age, the Eastern Cooperative Oncology Group (ECOG) performance status (PS), diastolic blood pressure, and body mass index (BMI); and (c) the laboratory data comprised the white blood cell (WBC) count, neutrophil count, hemoglobin, and creatinine clearance. Furthermore, we recorded the treatment characteristics.

Chemotherapy was administered until the disease progression, development of an unacceptable toxicity, or patient's wish to withdraw. During the treatment period with the first-line chemotherapy, any hematological and non-hematological adverse events were closely monitored. In addition, chemotherapy-related toxicities were graded by using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.0.

^{18}F-FLT PET image acquisition

In this study, ^{18}F-FLT was synthesized using methods described previously [10]. In addition, ^{18}F-FLT was radiosynthesized in a TRACERlab MX-FDG (GE Healthcare) using an FLT kit (ABX GmbH). No-carrier-added ^{18}F-fluoride was produced via the ^{18}O(p,n)^{18}F reaction from > 98% enriched ^{18}O-water (Cambridge Isotope Laboratories) on an RDS eclipse RD/HP medical cyclotron (Siemens Healthcare). The radiochemical purity of the final product was > 99%. Approximately 185 MBq of ^{18}F-FLT was intravenously administered, and emission scans were obtained at 1 h after injection. We obtained a transmission scan for 10 min using a standard rod source of ^{68}Ge/^{68}Ga for attenuation correction after each emission scan using the bed position similar to the emission scan.

^{18}F-FLT PET image analysis

The semiquantitative analysis of the ^{18}F-FLT uptake was based on the volume of interest (VOI) analysis by one experienced radiologist and one experienced oncologist, which produced the standardized uptake value (SUV) [local radioactivity concentration/(injected dose/body weight)]. Notably, the interpreting physicians were blinded to patients' clinical history and data. The CT and PET images were co-registered using specific software (Advantage Workstation; GE Healthcare). With this software, ^{18}F-FLT PET images were visualized and conformed into three-dimensional sections. VOIs were placed on each vertebral body (thoracic vertebra (Th) 4, Th8, Th12, and lumbar vertebra (L) 4). When a bone metastasis was detected in the Th4, Th8, Th12, and L4 vertebral bodies on CT or ^{18}F-FDG PET images, VOI was placed on an adjacent lower vertebral body. Besides, the vertebral contour was delineated to include voxels presenting SUV > 40% of SUV_{max} (Fig. 2). We defined the extracted

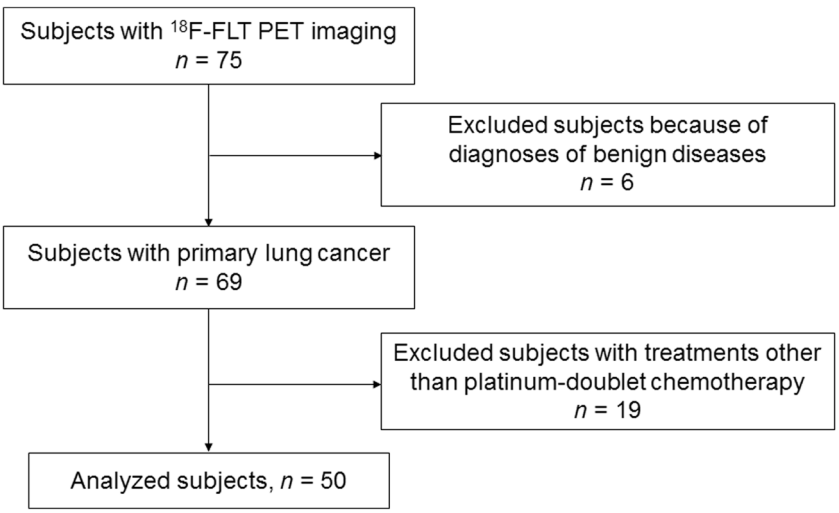

Fig. 1 A flow diagram of the study

vertebral volume as the proliferative vertebral volume (PVV) and calculated the total vertebral proliferation (TVP) as the product of the averaged SUV (SUV_{mean}) and PVV ($TVP = PVV \times SUV_{mean}$). Furthermore, the TVP/body surface area (BSA, m^2) was calculated by dividing the TVP by the BSA to revise the PET parameter with the body build.

Statistical analysis

In this study, we visualized the receiver operating characteristic (ROC) curves and calculated the corresponding area under the curve (AUC) to determine the most discriminative ^{18}F-FLT PET parameter and the optimal threshold of the parameter. In addition, we used bivariate logistic regression analysis adjusted with the highest frequency of grade 3/4 hematological toxicity from published clinical trials that enrolled at least 100 patients to each regimen [11–20] based on recent guidelines [21–23] to consider the risk of hematological toxicities of each chemotherapy regimen. The multivariate logistic regression analysis was used to determine independent factors affecting the severe hematological toxicity. Furthermore, variables selected by a univariate test ($p < 0.05$) were evaluated using the multivariate logistic regression analysis. All statistical analyses were performed using IBM SPSS Statistics 22.0 (IBM), and we considered $p < 0.05$ as statistically significant.

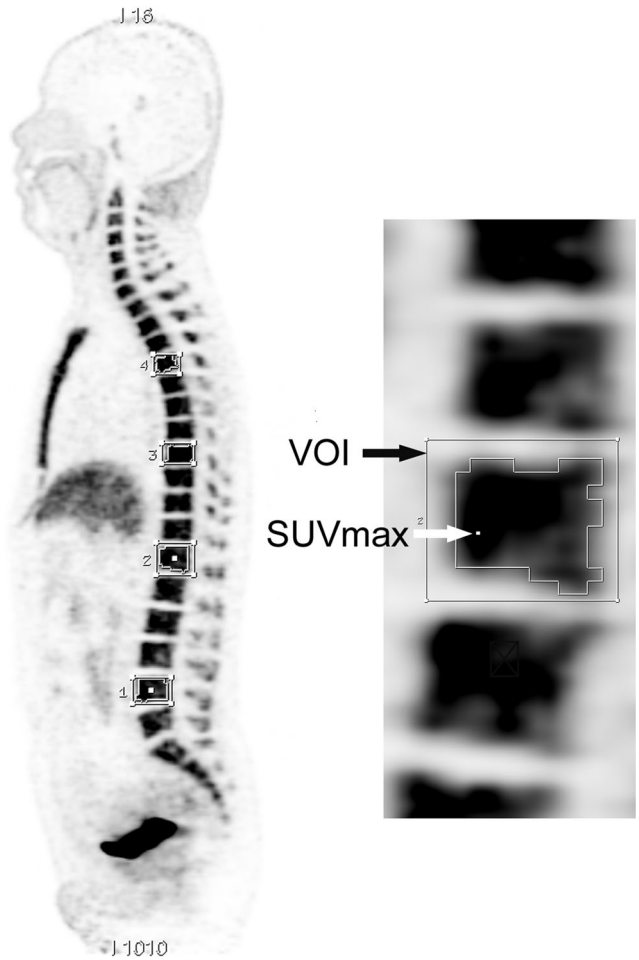

Fig. 2 Representative positron emission tomography (PET) images and the region of interest analysis. Three-dimensional volumes of interest (VOIs) were placed on each vertebral body (thoracic vertebra (Th) 4, Th8, Th12, and lumbar vertebra (L) 4). The vertebral contour was delineated to include voxels presenting SUV > 40% of the SUV_{max}

Results

Patients' characteristics

In this study, 50 patients (median age, 67.5 [range, 41–81] years) with newly diagnosed primary lung cancer underwent a pretreatment ^{18}F-FLT PET scan. Table 1 summarizes patients' characteristics. While 38 patients had non-small cell carcinoma, 12 had small cell carcinoma. We included 8 patients with stage IIA–IIIA in this study because they were judged as a contraindication of surgery or radiotherapy by the attending physician owing to the low pulmonary function. Furthermore, 6 patients had the ECOG-PS of 2.

Table 1 Baseline characteristics

	All subjects ($n = 50$)
Gender, male/female	42/8
Median age (range), years	67.5 (41–81)
ECOG-PS, *n*	
0/1/2	24/20/6
Median diastolic blood pressure (range), mmHg	67.5 (48–94)
Median white blood cell (range), /μL	6850 (2800–14,900)
Median neutrophil count (range), /μL	4273 (1008–12,485)
Median hemoglobin (range), g/dL	12.6 (9.1–16.1)
Median platelet count (range), $\times 10^4$/μL	24.9 (11.0–62.6)
Median creatinine clearance (range), mL/min	72.9 (36.1–131.1)
Histology, *n*	
Adenocarcinoma	21
Squamous cell carcinoma	14
Large cell carcinoma	1
Not otherwise specified	2
Small cell carcinoma	12
Stage, *n*	
IIA	1
IIIA	7
IIIB	9
IV	33
Bone metastasis, *n*	15

ECOG-PS, Eastern Cooperative Oncology Group performance status

Patients were treated with various treatment regimens (Table S1), at the discretion of each attending physician. While 15 patients received cisplatin-based regimens, 35 received carboplatin-based regimens. The incidence of the hematological toxicity of each regimen was represented by the rate of the most frequent grade 3/4 hematological toxicity of a regimen using data provided by previous clinical trials [11–20].

Chemotherapy-related adverse events

During the first cycle of chemotherapy, all patients did not receive a prophylactic use of granulocyte colony–stimulating factors (G-CSF). Table 2 summarizes hematological toxicities for the first-line systemic chemotherapy. During the first cycle, the most common grade 3/4 hematological toxicity was neutropenia (17 patients, 34%); other grade 3/4 hematological toxicities were leukopenia (14 patients, 28%), thrombocytopenia (6 patients, 12%), anemia (4 patients, 8%), and febrile neutropenia (6 patients, 12%). During all cycles, the most common grade 3/4 hematological toxicity was neutropenia (29 patients, 58%); other grade 3/4 hematological toxicities were leukopenia (17 patients, 34%), anemia (9 patients, 18%), thrombocytopenia (7 patients, 14%), and febrile neutropenia (6 patient, 12%). In this study cohort, we did not observe any treatment-related deaths.

^{18}F-FLT PET imaging biomarkers

We measured the SUV_{max}, SUV_{mean}, TVP, and TVP/BSA of Th4, Th8, Th12, and L4 vertebral bodies to assess the proliferative activity of the bone marrow. Among the 50 patients, 15 had any bone metastases and 9 had vertebral metastasis. Because 4 patients had vertebral metastasis in Th4 (2 patients) and Th12 (2 patients), VOI was placed on the Th5 and L1 vertebral bodies instead of Th4 and Th12. In addition, we analyzed the AUCs of these PET parameters to predict grade 3/4 hematological toxicities during the first cycle of chemotherapy; Table 3 summarizes these PET imaging parameters. The most discriminative parameter for the prediction of grade 3/4 hematological toxicities during the first cycle was the TVP/BSA of L4 (AUC, 0.86). The ROC curve analysis suggested a cut-off value of 68.7 to be the appropriate cut-off point (Fig. 3). Thus, we used 68.7 as the TVP/BSA of L4 cut-off value for further analysis.

Table 2 The incidence of drug-related hematological toxicities

	CTCAE grade, *n* (%)		
	All	3	4
First cycle			
White blood cell decreased	27 (54)	12 (24)	2 (4)
Neutrophil count decreased	35 (70)	11 (22)	6 (12)
Anemia	30 (60)	4 (8)	0 (0)
Platelet count decreased	34 (68)	5 (10)	1 (2)
Febrile neutropenia	–	6 (12)	–
Any grade 3/4 hematological toxicity		20 (40)	
All cycles			
White blood cell decreased	37 (74)	14 (28)	3 (6)
Neutrophil count decreased	45 (90)	22 (44)	7 (14)
Anemia	42 (84)	8 (16)	1 (2)
Platelet count decreased	38 (76)	5 (10)	2 (4)
Febrile neutropenia	–	6 (12)	–
Any grade 3/4 hematological toxicity		31 (62)	

CTCAE, Common Terminology Criteria for Adverse Events

Predictive value of the vertebral ^{18}F-FLT uptake and clinical biomarkers for severe hematological toxicities

Table 4 presents the results of logistic regression analyses to estimate indicators for the grade 3/4 hematological toxicity during the first cycle. Univariate analyses revealed that the lower initial WBC count (100/μL count unit; odds ratio [OR], 0.97; 95% confidence interval [CI], 0.94–0.99, $p = 0.042$), lower

Table 3 The vertebral ^{18}F-FLT accumulation and AUC to predict grade 3/4 hematological toxicities during the first cycle

Parameters	Mean ± SD	AUC (95% CI)
SUV_{max}		
Th4	10.7 ± 9.4	0.59 (0.43–0.76)
Th8	10.0 ± 2.7	0.73 (0.58–0.88)
Th12	9.3 ± 2.5	0.71 (0.56–0.87)
L4	9.0 ± 2.0	0.70 (0.54–0.85)
SUV_{mean}		
Th4	6.4 ± 5.1	0.62 (0.45–0.79)
Th8	5.9 ± 1.6	0.73 (0.58–0.88)
Th12	5.2 ± 1.4	0.71 (0.56–0.86)
L4	5.2 ± 1.2	0.71 (0.56–0.86)
TVP		
Th4	45.1 ± 11.8	0.61 (0.44–0.78)
Th8	72.6 ± 19.9	0.72 (0.58–0.87)
Th12	91.3 ± 27.6	0.66 (0.50–0.82)
L4	125.0 ± 40.1	0.81 (0.69–0.94)
TVP/BSA		
Th4	27.6 ± 5.8	0.63 (0.47–0.80)
Th8	44.4 ± 10.7	0.77 (0.63–0.90)
Th12	56.1 ± 16.5	0.67 (0.51–0.82)
L4	76.5 ± 23.3	0.86 (0.74–0.97)

SUV, standardized uptake value; *Th*, thoracic vertebrae; *L*, lumbar vertebrae; *TVP*, total vertebral proliferation; *BSA*, body surface area; *AUC*, area under receiver operating characteristics curves; *CI*, confidence interval

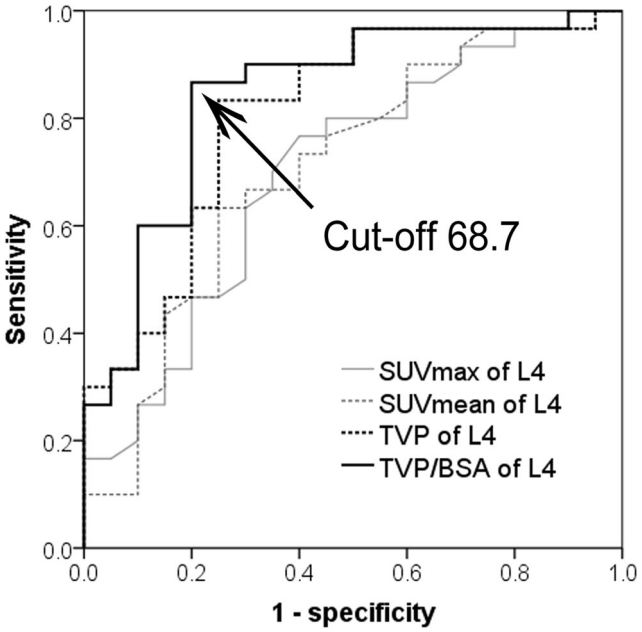

Fig. 3 The result of the ROC curve analysis of the SUV_{max}, SUV_{mean}, TVP, and TVP/BSA of L4 to predict the severe hematological toxicity during the first cycle of platinum-doublet chemotherapy. The optimal cut-off value of the TVP/BSA of L4 was estimated to be 68.7

TVP/BSA of L4 as a continuous variable (OR, 0.93; 95% CI, 0.88–0.97, $p = 0.00080$), and frequency of the grade 3/4 hematological toxicity of each regimen in previous clinical trials (OR, 1.04; 95% CI, 1.01–1.06, $p = 0.0016$) were the indicators of grade 3/4 hematological toxicities during the first cycle, whereas other biomarkers, including age, ECOG-PS, diastolic blood pressure, creatinine clearance, bone metastasis, and vertebral metastasis, were insignificant.

The chemotherapy regimen–specific frequency of the grade 3/4 hematological toxicity in previous clinical trials with the adjusted bivariate logistic regression analysis revealed that the TVP/BSA of L4 correlated with the toxicity (OR, 0.94; 95% CI, 0.89–0.98, $p = 0.0036$). In addition, the initial WBC count and neutrophil count were associated with the toxicity. Furthermore, the multivariate analysis model, including the TVP/BSA of L4, WBC count, and frequency of the grade 3/4 hematological toxicity in previous clinical trials, demonstrated that the TVP/BSA of L4 (OR, 0.94; 95% CI, 0.89–0.98, $p = 0.0036$) and frequency of grade 3/4 hematological toxicity in previous clinical trials (OR, 1.03; 95% CI, 1.00–1.06, $p = 0.023$) were independent predictors of grade 3/4 hematological toxicity. As the neutrophil count exhibited a robust significant correlation coefficient ($r = 0.89$; $p < 0.0001$, Spearman's rank correlation) with the WBC count, we excluded the neutrophil count from the multivariate analysis.

Table 5 presents the predictive value of the TVP/BSA of L4 for grade 3/4 hematological toxicities during the first cycle and all cycles. Upon adopting the cut-off value of 68.7, the sensitivity, specificity, and accuracy to predict severe hematological toxicities during the first cycle were 80.0%, 86.7%, and 84.0%, respectively. In addition, a univariate logistic regression analysis revealed that the low TVP/BSA of L4 (< 68.7) as a binary variable was a significant indicator of grade 3/4 hematological toxicities (OR, 26.0; 95% CI, 5.69–118.81; $p = 0.000026$). Conversely, the accuracy was weak (66.0%) when it was applied to the prediction for grade 3/4 hematological toxicities throughout chemotherapy. Figure 4 shows the representative ^{18}F-FLT PET images used for the prediction of severe hematological toxicities in the present primary lung cancer cases.

Table 4 The logistic regression analysis of factors on the diagnostic sensitivity for the hematological toxicity during the first cycle platinum-based chemotherapy

	Univariate analysis			Bivariate analysis		
	OR	95% CI	*p*	OR	95% CI	*p*
Age (years)	1.08	0.99–1.17	0.066	1.05	0.96–1.16	0.30
ECOG-PS	1.04	0.46–2.36	0.93	0.85	0.33–2.19	0.74
WBC count, 100/μL count unit	0.97	0.94–0.99	0.042*	0.96	0.93–1.00	0.032*
Neutrophil count, 100/μL count unit	0.96	0.93–1.00	0.063	0.95	0.91–1.00	0.048*
Hemoglobin (g/dL)	0.87	0.62–1.22	0.41	1.03	0.70–1.52	0.88
Diastolic blood pressure (mmHg)	0.99	0.94–1.05	0.79	0.98	0.91–1.04	0.50
Creatinine clearance (mL/min)	1.00	0.98–1.03	0.82	1.00	0.97–1.03	0.94
Bone metastasis	1.00	0.29–3.44	1.00	1.67	0.38–7.30	0.50
Vertebral metastasis	2.74	0.51–14.8	0.24	1.57	0.23–10.2	0.67
TVP/BSA of L4	0.93	0.88–0.97	0.00080*	0.94	0.89–0.98	0.0036*
Frequency of grade 3/4 hematological toxicity in previous clinical trials (%)	1.04	1.01–1.06	0.0016*	Adjustment variable		

ECOG-PS, Eastern Cooperative Oncology Group performance status; *TVP*, total vertebral proliferation; *BSA*, body surface area; *L*, lumbar vertebrae; *OR*, odds ratio

*$p < 0.05$

Table 5 The predictive value of the TVP/BSA of L4 (cut-off, 68.7) for the grade 3/4 hematological toxicity after platinum-based chemotherapy

	Sensitivity, *n* (%)	Specificity, *n* (%)	PPV, *n* (%)	NPV, *n* (%)	Accuracy, *n* (%)
Any grade 3/4 HT during first cycle	16/20 (80.0)	26/30 (86.7)	16/20 (80.0)	26/30 (86.7)	42/50 (84.0)
Any grade 3/4 HT during all cycles	17/31 (54.8)	16/19 (84.2)	17/20 (85.0)	16/30 (53.3)	33/50 (66.0)

HT, hematological toxicity; *PPV*, positive predictive value; *NPV*, negative predictive value

Discussion

In this study, we examined 50 patients with primary lung cancer to assess the predictive factor for grade 3/4 hematological toxicities after standard platinum-doublet chemotherapy. The results revealed that the low TVP/BSA of L4 vertebral body and frequency of the grade 3/4 hematological toxicity in previous clinical trials were the prominent predictors of severe hematologic toxicities during the first cycle; however, established indicators, including age, ECOG-PS, creatinine clearance, and diastolic blood pressure, were not prominent predictors. To the best of our knowledge, this is the first study to analyze the correlation between the pretreatment ^{18}F-FLT uptake of the bone marrow and the degree of hematological toxicities, revealing that the manifestation frequency of severe hematological toxicities was considerably higher in patients with the low ^{18}F-FLT uptake (TVP/BSA, < 68.7) compared with those with high ^{18}F-FLT uptake (≧ 68.7).

The ^{18}F-FLT uptake with PET is considered a biomarker of the tumor proliferative state because it is directly associated with the rate of DNA synthesis [3]. In addition, several studies have demonstrated that the ^{18}F-FLT uptake reflects the proliferative state of the bone marrow exposed to radiotherapy or chemotherapy. For instance, it is well documented that irradiated bone marrow steeply decreased the ^{18}F-FLT uptake [7, 8, 24]. Furthermore, Leimgruber et al reported that chemotherapy also decreased the ^{18}F-FLT uptake outside the radiotherapy field, and the change in the ^{18}F-FLT uptake of the vertebral body before and after chemoradiotherapy preceded the neutrophil count reductions after the treatment initiation [7]. A recent in vivo study reported that changes by chemotherapy in the ^{18}F-FLT uptake of hematopoietic organs, including the bone marrow and spleen, implied changes in the proliferative state assessed by the Ki-67 immunohistochemical score and flow cytometry using BrdUrd [9]. These studies suggest that ^{18}F-FLT PET imaging could be a noninvasive tool to assess

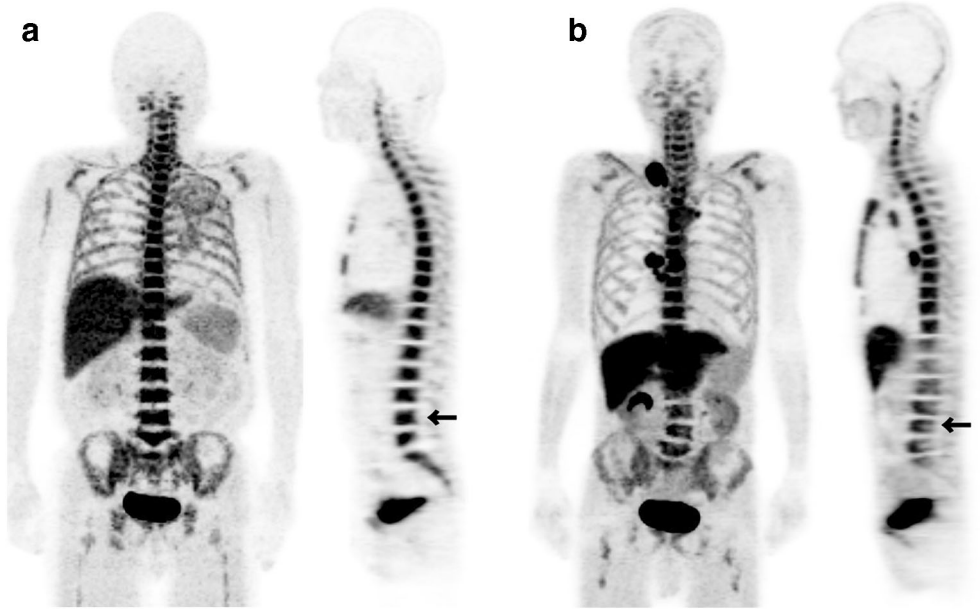

Fig. 4 Pretreatment ^{18}F-FLT PET images from patients with primary lung cancer in this study. **a** The maximum intensity projection (MIP, left) and sagittal image (right) of a 76-year-old male patient with stage IV lung adenocarcinoma who experienced only grade 1 neutropenia during the first cycle of systemic chemotherapy with carboplatin and pemetrexed. He had a high ^{18}F-FLT uptake in the lumbar vertebra (arrow, TVP/BSA of L4, 105.8). **b** The MIP (left) and sagittal image (right) of a 67-year-old male patient with stage IIIB lung squamous cell carcinoma who experienced grade 3 leukocytopenia, neutropenia, and thrombocytopenia and grade 1 anemia during the first cycle of systemic chemotherapy with carboplatin and gemcitabine. He exhibited a low ^{18}F-FLT uptake in the lumbar vertebra (arrow, TVP/BSA of L4, 62.1)

the proliferative state of hematopoietic cells in the bone marrow.

Interestingly, this study demonstrated that the ^{18}F-FLT uptake in L4 vertebral body estimated hematological toxicities better than that in the upper vertebra (Th4, Th8, and Th12). In addition, the SUV_{mean} of L4 vertebral body was lower than that of the upper vertebrae. The red marrow, which is a hematopoietically active marrow, is primarily concentrated in the axial skeleton, including the vertebrae, skull, and pelvis, in adults. Contrarily, the yellow marrow that comprises fat cells is primarily concentrated in the distal parts of the skeleton [2]. The conversion of red to yellow marrow typically progresses from the peripheral to the central skeleton with aging, becoming depleted of hematopoietic cells [25]. Likewise, the conversion of the marrow in the vertebrae might progress from the lower to the upper vertebrae. Thus, the ^{18}F-FLT uptake of the lower vertebrae could be a more sensitive biomarker of hematological toxicities.

Because many cytotoxic agents target tissue with high proliferation rate, hematopoietic cells with high proliferative state are affected more than those with low proliferative state. However, patients with high proliferative bone marrow according to ^{18}F-FLT PET images were found to be less affected compared with those with low proliferative bone marrow. Thus, when patients are in the pre-chemotherapy state, the ^{18}F-FLT uptake in the bone marrow might signify the amount of hematopoietic cells rather than their proliferative activity. Furthermore, among our ^{18}F-FLT PET parameters, the TVP and TVP/BSA values exhibited a better correlation with the severe hematological toxicity compared with the SUV_{mean}. Because TVP considers the volume of the bone marrow, it can present the number of hematopoietic cells more precisely than SUV_{mean}. Since the volume of the bone marrow cavity is affected by patients' body constitution, we adopted a parameter that was corrected by the BSA. As in almost all cytotoxic anticancer agents, except for carboplatin, the dosage amount was adjusted by the BSA in each patient, and the TVP/BSA could be a more accurate parameter compared with the TVP.

In this study, previously reported biomarkers, including age, ECOG-PS, diastolic blood pressure, and creatinine clearance, were insignificant predictors for the prediction of hematological toxicities. As the ^{18}F-FLT uptake of the bone marrow was a robust biomarker of hematological toxicities in this study, it will be a clinically relevant noninvasive measure for the decision of dose and type of chemotherapy regimen in patients with advanced malignant diseases, especially in elderly patients. For example, according to the recent guideline of myeloid growth factors [26], when using a chemotherapy regimen with intermediate risk for febrile neutropenia (10–20%), the prophylactic use of G-CSF is recommended per patients' risk factors. If ^{18}F-FLT PET imaging is available, the prophylactic use of G-CSF should be considered in patients with the low TVP/BSA of L4. In addition, according to the guideline of older adult oncology [27], if the patients' PS is favorable, a treatment with platinum-doublet chemotherapy is suggested irrespective of the chronological age in patients with advanced non-small cell lung cancer. However, even if the patients' PS is favorable, platinum-doublet chemotherapy might have to be avoided in patients with the low TVP/BSA of L4.

In this study, the low initial WBC and neutrophil counts were significant predictors according to univariate and bivariate analyses, although they were not independent predictors. According to several studies regarding the predictors of hematological toxicities, whether the initial WBC or neutrophil count is associated with the severity of hematological toxicities remains controversial [28–36]. Several reports have not shown any association between WBC or neutrophil count and hematological toxicities [28–32], whereas some have shown this association [33–35]. Although WBC and neutrophil count measurements are simple and low cost, WBC and neutrophil counts are easily altered due to inflammation or cytokines in cancer; thus, they cannot be considered as consistent, reliable predictors of hematological toxicities.

^{18}F-FLT accumulation in the vertebral bone marrow predicted the severe hematological toxicities only in the first cycle of chemotherapy, and the predictive value for the hematological toxicities throughout the remaining chemotherapy was weak. Patients in this study received various cycles (1–6 cycles) of chemotherapy depending on the different disease progression or hematological and non-hematological toxicity due to the therapy; therefore, predicting the toxicities throughout chemotherapy using any biomarker, including ^{18}F-FLT PET imaging, is difficult before the initiation of chemotherapy. This is consistent with a previous study reporting the use of a risk prediction model to predict febrile neutropenia in the first cycle and throughout chemotherapy [33]. Although it is unfeasible, repeated ^{18}F-FLT PET imaging might more precisely predict the toxicities throughout chemotherapy.

This study has several limitations. First, our sample size was small. Second, the patients received various chemotherapy regimens. Thus, we analyzed the bivariate logistic regression analysis adjusted with the frequency of the grade 3/4 hematological toxicity in previous clinical trials. Despite the adjustment, the TVP/BSA of L4 was a significant biomarker of the severe hematological toxicity. Hence, further studies in larger populations with a specific treatment regimen are warranted to validate our findings.

In conclusion, the low TVP/BSA of the L4 vertebral body is an independent biomarker of severe hematological toxicities during the first cycle of platinum-doublet chemotherapy in patients with primary lung cancer. Besides the evaluation of physiological risk factors, the noninvasive evaluation of the amount and activity of hematopoietic cells in the bone marrow cavity using ^{18}F-FLT PET imaging could provide predictive information of severe hematological toxicities and help determine an appropriate drug combination or dose intensity in individual patients.

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Compliance with ethical standards

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Ethical approval Institutional Review Board approval was obtained.

Methodology
- Retrospective
- Diagnostic study or prognostic study
- Performed at one institution

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