Effect of liver toxicity on clinical outcome of patients with non-small-cell lung cancer treated with pemetrexed

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Abstract. Liver toxicity (LT) is a common side effect of pemetrexed (PEM); however, the effect of LT on clinical outcome has not been investigated in patients with non-small-cell lung cancer (NSCLC) treated with PEM. Between June, 2009 and June, 2012, a total of 95 chemo-naive NSCLC patients received a PEM-containing regimen in our hospital. We reviewed the medical records of those 95 patients and evaluated the incidence of LT. Furthermore, we investigated the association between LT and clinical outcome. In this analysis, LT was defined as any grade of aspartate aminotransferase or alanine aminotransferase elevation. A total of 67 patients (70.5%) developed LT, which occurred mostly during the first treatment cycle. Among these, 10 patients (10.5%) required a delay in treatment or a dose reduction from the subsequent cycle and PEM discontinuation was required in 1 patient. The response rate (RR) was 43.3 and 21.4% in patients with and in those without LT, respectively (P=0.0387). The median progression-free survival (PFS) and overall survival (OS) were 6.3 and 24.2 months in patients with LT and 2.9 and 18.3 months in patients without LT, respectively (P<0.0001 for PFS and P=0.2426 for OS). The multivariate analysis demonstrated that LT exerted a significant positive effect on PFS (hazard ratio = 0.341; P<0.0001). In conclusion, LT was frequently observed in NSCLC patients treated with PEM; however, it was generally easily manageable. The improvement in RR and PFS observed in patients with LT suggested that LT may be a useful predictor of a favorable outcome in this patient population.

Introduction

Lung cancer is the leading cause of cancer-related mortality in numerous industrialized countries, with an incidence that is increasing worldwide (1). Platinum-based combination chemotherapy has been shown to improve the survival and quality of life of patients with advanced non-small-cell lung cancer (NSCLC), which accounts for ~80% of all lung cancers; however, its prognosis remains poor.

Pemetrexed (PEM) is a novel pyrrolo[2,3-d]pyrimidine-based antifolate. It is transported into the cells via the reduced folate carrier. Upon cell entry, PEM is polyglutamylated to the activate pentaglutamate in a reaction catalyzed by folylpolyglutamate synthase. PEM inhibits multiple enzymes involved in pyrimidine and purine synthesis, including thymidylate synthase, dihydrofolate reductase and glycinamide ribonucleotide formyltransferase (2,3). Available data suggest that PEM is exclusively more effective in non-squamous NSCLC compared to other non-platinum agents (4,5).

Elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels has been commonly reported in previous clinical studies of PEM (6-9). In a randomized study of two different doses of PEM, the incidence of ≥grade 2 AST and ALT elevation was 29.8 and 34.2%, respectively, in patients who received the standard dose (500 mg/m^2) of PEM (8). Despite the high incidence of AST and ALT elevation, however, its effect on clinical outcome has not been investigated. If carcinoma cells exhibit characteristics similar to those of host cells, the therapeutic effect may be predictable from the reaction of hepatocytes to PEM.

In this study, we defined as liver toxicity (LT) any grade of AST or ALT elevation from baseline and investigated the association between LT and clinical outcome in patients with non-squamous NSCLC who were treated with a PEM-containing regimen.

Materials and methods

Patients. Between June, 2009 and June, 2012, 95 consecutive patients with advanced NSCLC were treated with PEM or PEM plus a platinum agent as first-line chemotherapy at the Department of Respiratory Medicine, Kyoto University Hospital. Patients who had received PEM-based chemotherapy as perioperative treatment were excluded from the analysis. Staging was performed according to the 7th edition of TNM classification (10). This study was approved by the institutional review board of Kyoto University and informed consent was obtained from all patients prior to enrollment in this study.

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Evaluation of response and survival. Tumor response was assessed by the response evaluation criteria in solid tumors (RECIST, version 1.1) (11) every 2 cycles during chemotherapy and then based on clinical practice. Survival data were obtained through active follow-up based on the verification of the patients' vital status up to March 1, 2012. Progression-free survival (PFS) was defined as the time from the first administration of PEM to disease progression or death from any cause.

Evaluation and definition of LT. The following biochemical parameters were evaluated: AST, ALT, alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), total bilirubin (TBil) and renal function (estimated creatinine clearance using the modified Cockcroft-Gault formula). Each toxicity was graded according to the National Cancer Institute Common Toxicity Criteria, version 4.0. The worst grade was identified and toxicities were recorded following deterioration by one or more grades compared to baseline. In this study, LT was defined as any grade of AST or ALT elevation from baseline.

Statistical analysis. Statistical comparisons were performed using the Student's t-test when data were normally distributed and non-parametric analysis using the Wilcoxon signed-rank test otherwise. The significance of the association between individual clinical factors was evaluated using the χ^2 or Fisher's exact tests, as appropriate. A multivariate regression analysis was conducted using the Cox proportional hazards model. The survival rate was calculated by the Kaplan-Meier method and the statistical significance of the differences was evaluated using the log-rank test. $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed using JMP 10.0.0 software (SAS Institute, Cary, NC, USA).

Results

Patient characteristics. A total of 95 patients with NSCLC were included in this analysis. The clinical characteristics of the patients are summarized in Table I. The patients comprised 51 men and 44 women, with a median age of 68 years. The performance status (PS) was generally good, with ~92% of the patients exhibiting a PS of 0 or 1. In total, 55 patients (57.9%) were former or current smokers and 27 patients (28.4%) had a history of habitual alcohol consumption. There were few patients with positive hepatitis virus B (HBV) surface antigen, positive hepatitis virus C (HCV) antibody, or fatty liver. The majority of patients had adenocarcinoma histology and were diagnosed with stage IV disease. Liver metastasis was recorded in 10 patients.

Frequency and severity of LT. Compared with the high incidence of AST (63.2%) or ALT elevation (62.1%), the incidence of cholestatic enzyme elevation was relatively low (ALP, 16.8%; γ-GTP, 37.9%; and TBil, 6.3%). As shown in Fig. 1, 47 of the 67 patients who developed LT (70.1%) did so during the first cycle of chemotherapy. All cases of grade 1 LT improved spontaneously and there was no effect on subsequent PEM administration. By contrast, of the 16 patients with ≥grade 2 LT, 10 patients required a treatment delay or a dose reduction from the subsequent cycle and PEM discontinuation was required in 1 patient (Table II).

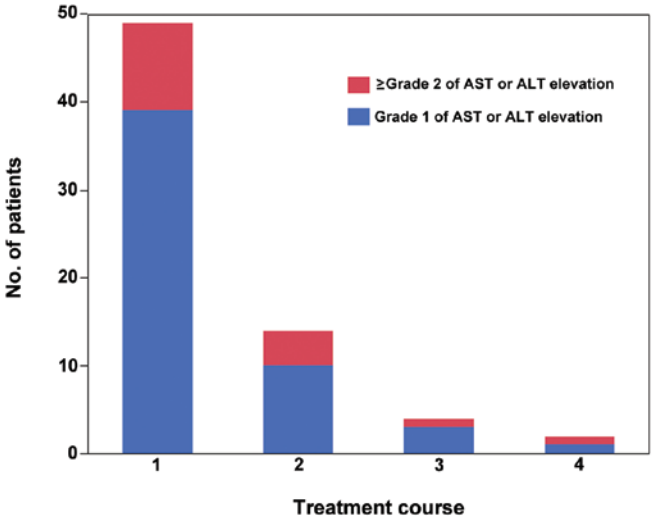

Figure 1. Frequency of liver toxicity of different grades during the treatment course. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Risk factors for AST and ALT elevation. We investigated the association between LT and clinical factors, such as gender, age, body mass index (BMI), PS, disease stage, liver metastasis, alcohol consumption, hepatic comorbidity, treatment regimen, estimated creatinine clearance, dose intensity of PEM and baseline liver function, that may affect the pharmacokinetics of PEM (Table I). There was no significant difference between the LT and non-LT groups, with the exception of liver metastasis and the dose intensity of PEM: the incidence of liver metastasis and the dose intensity were higher in the non-LT group (P=0.0332 and 0.0478, respectively).

Efficacy. There were no recorded cases with complete response (CR), 35 with partial response (PR), 45 with stable disease (SD) and 15 with progressive disease (PD). The response rate (RR) and disease control rate (DCR) were 36.8 and 84.2%, respectively.

The association between clinical characteristics, including LT, and response to PEM, is shown in Table III. Younger age (<70 years), good PS (<2) and a doublet regimen were significant positive predictive factors for disease control (PR+SD) in the univariate analysis. The incidence of LT was significantly higher among non-PD patients compared to that in PD patients (P=0.0352). The RR and DCR were 43.3 and 89.6%, respectively, in patients with LT and 21.4 and 71.4%, respectively, in patients without LT (RR, P=0.0387; DCR, P=0.0352).

The median PFS of all patients was 5.6 months (95% CI: 4.2-6.5 months). Patients with LT achieved a significantly longer PFS compared to those without LT (6.3 vs. 2.9 months, P<0.0001; Fig. 2A). Similarly, the 16 patients with grade 2 or worse LT achieved a significantly longer PFS compared to their counterparts (6.3 and 4.0 months, respectively; P=0.0105; Fig. 2B). Furthermore, the median survival time and 1-year survival rate from the beginning of the treatment were 24.2 months and 79.6%, respectively, in patients with grade 2 or worse LT vs. 18.3 months and 74.3%, respectively, in their counterparts. There was no difference in overall survival (OS) (P=0.2426 vs. P=0.3109; Fig. 2C and D).

Table I. Patient characteristics (n=95).

Variables	Patient no. (n=95)	%	Patient no. without LT (n=28)	Patient no. with LT[a] (n=67)	P-value
Gender					
Male	51	53.7	17	34	0.3728
Female	44	46.3	11	33	
Age, years [median (range)]	68 (35-85)	-	68 (41-85)	68 (35-82)	0.6184
BMI, kg/m^2 (mean ± standard deviation)	-	-	21.0±2.1	22.2±3.0	0.0540
ECOG PS					
0-1	87	91.6	24	63	0.2290
2	8	8.4	4	4	
Smoking history					
Never	40	42.1	10	30	0.4123
Former + current	55	57.9	18	37	
History of alcohol consumption					
Yes	27	28.4	8	19	0.9832
No	68	71.6	20	48	
HBV or HCV or alcoholic hepatitis					
Yes	4	4.2	1	4	1.000
No	91	95.8	27	63	
Disease stage at the beginning of the treatment					
IIIA	2	2.1	0	2	0.1789
IIIB	5	5.3	3	2	
IV	88	92.6	25	63	
Liver metastasis					
Yes	10	10.5	6	4	0.0332[b]
No	85	89.5	22	63	
Histology					
Adenocarcinoma	93	97.9	27	66	0.5048
NOS/other	2	2.1	1	1	
Treatment regimen					
CBDCA+PEM	59	62.1	16	43	0.8139
CDDP+PEM	3	3.2	1	2	
PEM	33	34.7	11	22	
Treatment course [median (range)]	4 (1-11)	-	4 (1-6)	5 (1-11)	-
Maintenance					
Yes	21	22.1	5	16	0.5125
No	74	77.9	23	51	
PEM dose intensity, mg/m^2/week (mean ± standard deviation)	153.2±15.6	-	158.1±10.1	151.2±17.1	0.0478[b]
Baseline liver enzyme elevation[a]					
Yes	13	13.7	5	8	0.5163
No	82	86.3	23	59	
eCCr (mean ± standard deviation)	78.9±30.9	-	74.2±23.9	80.9±33.4	0.3293

[a]Grade 1 or higher aspartate aminotransferase (AST) or alanine aminotransferase (ALT) elevation. [b]Student's t-test or χ^2 test. LT, liver toxicity; BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; PS, performance status; HBV, hepatitis B virus; HCV, hepatitis C virus; NOS, not otherwise specified; CBDCA, carboplatin; PEM, pemetrexed; CDDP, cisplatin; eCCr, creatinine clearance assessed with the Cockcroft Gault formula.

Table II. Characteristics of 16 patients with grade 2 or higher AST or ALT elevation following chemotherapy with PEM.

Case no.	Gender	Age (years)	PS	Alcohol history	Hepatic comorbidity	Liver metastasis	Regimen	Best response	Cycle at event	AST peak value (IU/l)	ALT peak value (IU/l)	Treatment interruption due to liver dysfunction
1	F	61	0	No	No	No	CBDCA+PEM	SD	2	101	150	Yes[a]
2	F	69	0	No	HBV	No	CBDCA+PEM	PR	1	73	155	Yes[b]
3	F	60	0	No	HCV	Yes	CBDCA+PEM	PR	1	100	102	Yes[b]
4	F	43	1	No	No	No	CBDCA+PEM	SD	2	75	94	Yes[b]
5	M	63	0	No	No	No	CBDCA+PEM	PR	1	85	160	No
6	M	64	0	No	No	No	CBDCA+PEM	PR	4	73	155	Yes[a]
7	F	51	0	No	No	No	CBDCA+PEM	SD	1	141	247	Yes[a]
8	F	74	0	No	No	No	CBDCA+PEM	SD	4	125	122	Yes[b]
9	F	74	1	No	No	No	CBDCA+PEM	PD	1	152	83	Yes[b]
10	M	62	0	Yes	Fatty liver	Yes	CBDCA+PEM	PR	1	174	344	Yes[a]
11	M	35	0	No	No	No	CBDCA+PEM	SD	1	107	234	Yes[a]
12	F	39	0	Yes	No	No	CBDCA+PEM	PR	4	95	97	No
13	M	75	1	Yes	No	No	PEM	PR	1	94	130	No
14	M	70	1	Yes	No	No	PEM	SD	1	113	190	Yes[c]
15	F	82	0	No	No	No	PEM	SD	4	129	82	No
16	F	75	1	Yes	No	No	PEM	PR	3	223	160	No

[a]Required dose reduction and delay in subsequent chemotherapy cycle. [b]Required dose reduction in subsequent chemotherapy cycle. [c]Discontinuation of chemotherapy. F, female; M, male; PS, performance status; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; CBDCA, carboplatin; PEM, pemetrexed; SD, stable disease; PR, partial response; PD, progressive disease.

Table III. Association between clinical characteristics and LT (n=95).

Variables	No. of patients with controlled disease (CR+PR+SD) (n=80)	No. of patients with progressive disease (PD) (n=15)	P-value
Gender			
Male	42	9	0.5916
Female	38	6	
Age, years [median (range)]	67 (35-85)	75 (64-84)	
<70	52	3	0.0011[a]
≥70	28	12	
ECOG PS			
0-1	76	11	0.0199[a]
2	4	4	
Smoking history			
Never	36	4	0.1773
Former + current	44	11	
Histology			
Adenocarcinoma	78	15	1.000
Other	2	0	
EGFR mutation status			
Mutation-positive	22	4	1.000
Wild-type or unknown	58	11	
Disease stage			
III	6	2	0.6080
IV	74	13	
Treatment regimen			
Platinum+PEM	58	4	0.0008[a]
PEM	22	11	
PEM dose intensity, mg/m^2/week (mean ± standard deviation)	152.1±16.5	160.0±7.6	0.1863
Presence of LT			
Yes	60	7	0.0352[a]
No	20	8	

[a]Fisher's exact test or χ^2 test. LT, liver toxicity; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ECOG, Eastern Cooperative Oncology Group; PS, performance status; EGFR, epidermal growth factor receptor; PEM, pemetrexed.

Subsequently, we conducted a Cox regression analysis to determine the correlation between PFS and clinical factors such as age (<70 vs. ≥70 years), gender (female vs. male), PS (0-1 vs. 2), epidermal growth factor receptor (EGFR) mutation status (mutant vs. wild-type/unknown), disease stage (III vs. IV), first-line therapy regimen (platinum plus PEM vs. PEM) and the presence of LT (present vs. absent). Among these factors, the presence of LT [hazard ratio (HR)=0.389, 95% CI: 0.244-0.633 and P=0.0002] exerted a significant positive effect on PFS based on the univariate analysis. The multivariate analysis revealed that platinum plus PEM therapy (HR=0.438, 95% CI: 0.210-0.823 and P=0.0119) and the presence of LT (HR=0.341, 95% CI: 0.206-0.574 and P<0.0001) exerted a significant positive effect on PFS (Table IV).

Discussion

The incidence of LT in this study was 70.5%. This is almost identical to that reported by previous clinical trials (6,8,9). Although approximately two-thirds of the patients who developed ≥grade 2 LT required a treatment delay or a dose reduction from the subsequent cycle, PEM was successfully continued, except in 1 patient. These results suggest that LT is common among patients treated with PEM, although it is generally easily manageable. The most interesting result in this study was that patients who developed LT achieved a significantly higher RR and PFS compared to those without LT.

PEM is primarily eliminated in the urine, with 70-90% of the dose recovered as the unchanged parent drug within the first 24 h. It was hypothesized that PEM undergoes

Table IV. Cox proportional hazards model analysis of factors affecting progression-free survival (n=95).

A, Univariate analysis

Factors	OR	95% CI	P-value
Gender (female vs. male)	1.109	0.727-1.685	0.6289
Age (<70 vs. ≥70 years)	0.768	0.504-1.178	0.2237
ECOG PS (0-1 vs. ≥2)	0.800	0.396-1.916	0.5860
EGFR mutation status (positive vs. wild-type/unknown)	1.235	0.762-1.942	0.3815
Stage (III vs. IV or postoperative recurrence)	0.678	0.261-1.449	0.3402
Chemotherapy regimen (platinum+PEM vs. PEM alone)	0.631	0.407-0.998	0.0492[a]
LT (present vs. absent)	0.389	0.244-0.633	0.0002[a]

B, Multivariate analysis

Factors	OR	95% CI	P-value
Gender (female vs. male)	1.295	0.824-2.029	0.2601
Age (<70 vs. ≥70 years)	1.189	0.675-2.145	0.5543
ECOG PS (0-1 vs. ≥2)	2.200	0.839-6.343	0.1109
EGFR mutation status (positive vs. wild-type/unknown)	1.228	0.725-2.027	0.4366
stage (III vs. IV or postoperative recurrence)	0.508	0.188-1.149	0.1087
Chemotherapy regimen (platinum+PEM vs. PEM alone)	0.416	0.210-0.823	0.0119[a]
LT (present vs. absent)	0.341	0.206-0.574	<0.0001[a]

[a]Fisher's exact test or χ^2 test. ECOG, Eastern Cooperative Oncology Group; PS, performance status; EGFR, epidermal growth factor receptor; PEM, pemetrexed; LT, liver toxicity; OR, odds ratio; CI, confidence interval.

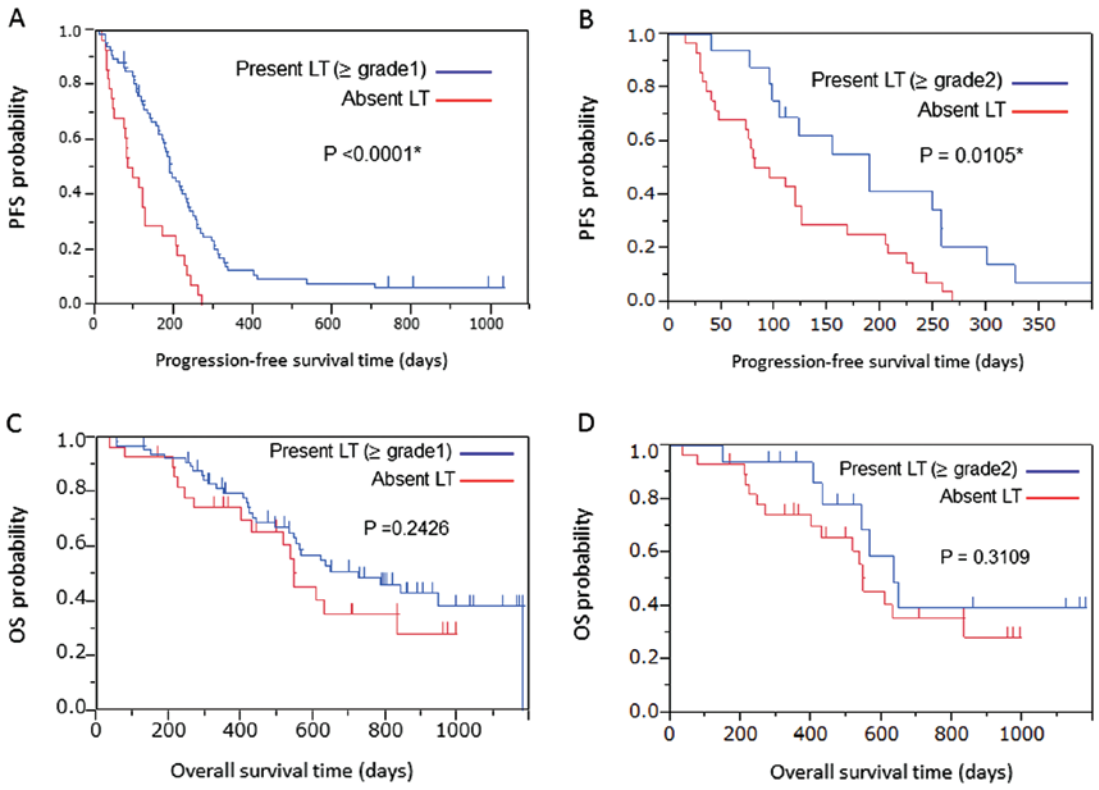

Figure 2. Kaplan-Meier curve for progression-free survival (PFS). (A) The PFS in the ≥grade 1 liver toxicity (LT) group was significantly longer compared to that in the absent LT group (log-rank test, P<0.0001). (B) The PFS in the ≥grade 2 LT group was significantly longer compared to that in the absent LT group (log-rank test, P=0.0105). (C) There was no difference in the overall survival (OS) curves between the ≥grade 1 LT group and the absent LT group (log-rank test, P=0.2426). (D) There was no difference in the OS curves between the ≥grade 2 LT group and the absent LT group (log-rank test, P=0.3109).

limited hepatic metabolism and the precise mechanism of PEM-induced liver injury has not been fully elucidated. The potential determinants of PEM activity include: the folate receptor carrier, which is the predominant route by which folates and several anti-folates gain entry into the cells; γ-glutamyl hydrolase, which removes glutamyl residues from polyglutamylated substrates, decreasing intracellular activity and retention of PEM; thymidylate synthase, which is the main target of this drug; and 5,10-methylenetetrahydrofolate reductase (MTHFR), which has a major impact on the regulation of the folic acid pathway (3,12). These enzymes exist in liver cells as well as in tumor cells; therefore, PEM may also affect liver cells, leading to the development of LT.

The response of cancer cells to chemotherapy depends on a sufficient amount of active drug reaching the target and on whether that target is sensitive to the effects of the drug. These factors may also apply to normal cells, such as liver cells. The availability of the active drug to tumor or normal cells is affected by the pharmacokinetics of the drug, which may produce a similar effect in tumor and normal cells. However, no correlation between the levels of AST or ALT and PEM pharmacokinetic parameters has been reported thus far (13). In the present study, we were unable to verify a correlation between LT and the factors that affect the pharmacokinetics of PEM (such as, dose intensity and estimated creatinine clearance) (14).

The sensitivity of tumor and normal cells to chemotherapeutic agents may also be affected by genetic predisposition, which may similarly affect the two cell types. According to a previous randomized phase II study comparing PEM with PEM plus carboplatin (CBDCA), patients harbouring the C677T homozygous mutation of MTHFR, a key enzyme involved in folate metabolism, exhibited a prolonged PFS compared to patients with wild-type or heterozygous MTHFR mutations (15). This type of mutation has been shown to be associated with efficacy as well as toxicity in patients receiving methotrexate (16). In addition, Taniguchi *et al* (17) reported that the presence of MTHFR 677T was associated with an increased risk of ALT elevation in patients with rheumatoid arthritis treated with antirheumatic drugs. Those observations suggested that the polymorphism of genes that encode enzymes involved in folate metabolism, transportation and activation/inactivation may be associated with clinical outcome and LT in patients with advanced NSCLC receiving PEM-based therapy.

The present study had several limitations. Firstly, there was no definite rule for the evaluation of LT and the follow-up interval was based on clinical practice, due to its retrospective nature. Secondly, a false correlation between LT and patient outcome may have occured, since a higher incidence of LT was expected with the increasing number of chemotherapy cycles and patients surviving longer have a greater chance of receiving additional cycles of chemotherapy; however, these effects appear to be limited, as LT was mostly observed during the first 2 cycles in this analysis. To address this issue, we applied a novel strategy, restricting the primary analysis to patients who remained alive 30 days after the initiation of chemotherapy and the results did not change (data not shown).

To the best of our knowledge, this is the first study suggesting an association between LT and clinical outcome in NSCLC patients treated with PEM. Further studies are required to confirm this hypothesis.

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