



# Lipocalin-2 as a prognostic biomarker and its association with systemic inflammation in small cell lung cancer

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## Abstract

**Background:** Systemic inflammation is believed to contribute to small cell lung cancer (SCLC) progression, but the underlying relationship remains unclear. Lipocalin-2, a potential biomarker of inflammation, has been implicated in various cancers but its prognostic value in SCLC is underexplored.

**Methods:** We retrospectively analyzed 191 patients with SCLC (72 with limited-stage [LD] and 119 with extensive-stage) treated using platinum-based chemotherapy. Lipocalin-2 expression was evaluated using immunohistochemistry. Optimal cutoff values for lipocalin-2 and neutrophil-to-lymphocyte ratio (NLR) were determined using time-dependent receiver operating characteristic curve analysis. The pectoralis muscle index was used to assess sarcopenia.

**Results:** In LD-SCLC, high lipocalin-2 expression was associated with worse progression-free survival (PFS; median: 7.0 vs. 15.9 months,  $p = 0.015$ ) and overall survival (OS; median: 12.9 vs. 30.3 months,  $p = 0.035$ ) compared with low lipocalin-2 expression. Patients were stratified into three prognostic groups by combining lipocalin-2 with NLR: low lipocalin-2/low NLR, high lipocalin-2/low NLR or low lipocalin-2/high NLR, and high lipocalin-2/high NLR (median PFS: 17.3 vs. 11.0 vs. 6.3 months,  $p = 0.004$ ; median OS: 30.5 vs. 17.3 vs. 8.6 months,  $p = 0.002$ ). Similar trends were observed when combining lipocalin-2 with the pectoralis muscle index. High lipocalin-2 expression was also associated with lower complete response rates (18.9% vs. 34.3%,  $p = 0.035$ ). No significant prognostic implications were found for lipocalin-2 in extensive-stage SCLC.

**Conclusions:** High lipocalin-2 expression is potentially associated with poorer survival in LD-SCLC. Combining lipocalin-2 with other inflammation-related markers could improve prognostic stratification.

## KEYWORDS

biomarkers, inflammation, lipocalin-2, sarcopenia, small cell lung carcinoma

## INTRODUCTION

Small cell lung cancer (SCLC) is a rapidly progressing malignancy with a poor prognosis.<sup>1</sup> Smoking, the primary risk factor for SCLC, introduces carcinogenic compounds in the body and triggers systemic inflammation, which may contribute to cancer progression.<sup>1,2</sup> Recent studies

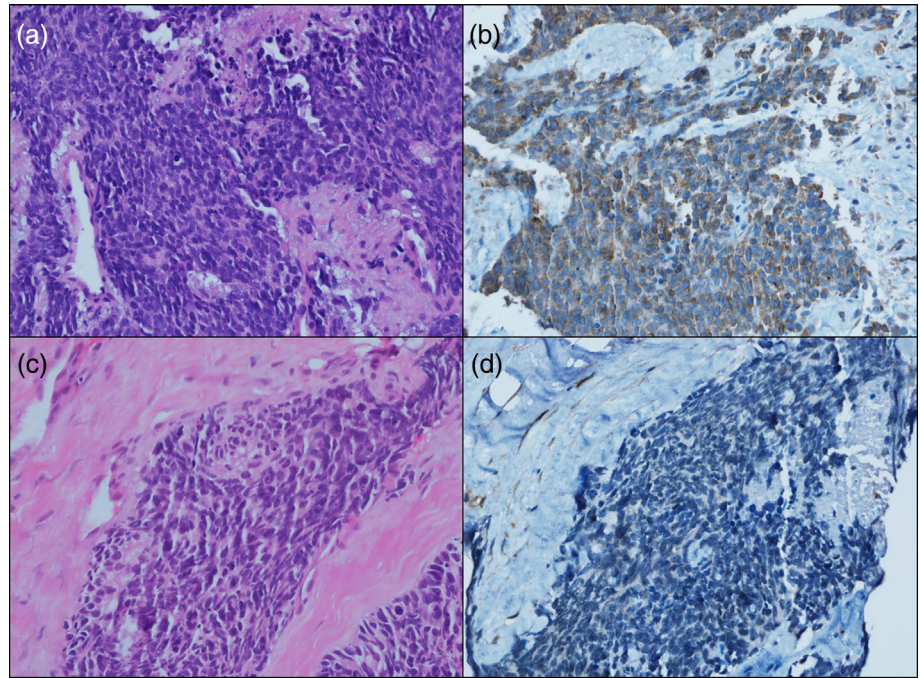
suggested the clinical relevance of inflammatory markers in SCLC, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio, systemic immune-inflammation index, and skeletal muscle index (SMI), which is associated with sarcopenia.<sup>3-6</sup> However, direct experimental evidence supporting the relationship between systemic inflammation and SCLC progression is limited, possibly due to the heterogeneity and complexity of SCLC biology.<sup>7,8</sup>

Se-Il Go and Jung Wook Yang contributed equally to this study.

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**FIGURE 1** Representative images of immunohistochemical staining for lipocalin-2. (a, hematoxylin–eosin stain) small cell carcinoma with (b) lipocalin-2 high expression showing cytoplasmic staining and (c, hematoxylin–eosin stain) small cell carcinoma with (d) lipocalin-2 low expression.



Lipocalin-2, which is also known as neutrophil gelatinase-associated lipocalin, is emerging as a potential biomarker in various diseases. It plays essential roles in iron transport, immune response regulation, and cellular homeostasis.<sup>9–11</sup> It is expressed by various cell types, including neutrophils, epithelial cells, and some cancer cells.<sup>12</sup> Beyond its basic biological functions, lipocalin-2 acts as an acute phase protein and is involved in inflammation and injury response processes.<sup>13,14</sup> In addition, lipocalin-2 has been suggested to be related to tumor progression, metastasis, and treatment response in various types of cancer.<sup>15–18</sup> However, the clinical implication of lipocalin-2 in SCLC is unclear, particularly regarding other potential biomarkers for systemic inflammation. This study was performed to investigate the prognostic value of lipocalin-2 and assess its relationship with other inflammation-related markers, such as NLR and SMI, in patients with SCLC.

## METHODS

### Patient selection

We retrospectively reviewed the records of all consecutive patients with histologically confirmed SCLC who were treated at the Gyeongsang National University Hospital between July 2006 and March 2020. Patients were included in the study using the following criteria: (1) platinum-based chemotherapy as the first-line treatment, (2) available archival tumor samples or recent biopsy specimens suitable for immunohistochemistry (IHC) analysis of lipocalin-2, and (3) available clinical information to assess the SMI and NLR, as well as key endpoints, such as progression-free survival (PFS), overall survival (OS), and treatment response. Patients were excluded from the study if they had a concurrent malignancy or history of another cancer

within the previous 5 years, or if they experienced a histological transformation from non-small cell lung cancer to SCLC. The study was conducted in accordance with the principles of the Declaration of Helsinki (1964) and its latest amendments. The Institutional Review Board of the Gyeongsang National University Hospital approved this study (GNUH 2023-12-002). The requirement for written informed consent was waived owing to the retrospective nature of the study.

### IHC analysis for lipocalin-2

IHC was performed on 4- $\mu$ m-thick sections from formalin-fixed paraffin-embedded specimens (biopsy or cellblock). The tissue sections were attached to glass slides, deparaffinized, rehydrated, and incubated in 3% hydrogen peroxide for 10 min to block the endogenous peroxidase activity. The slides were subsequently heated for 20 min in 10 mmol/L sodium citrate buffer (pH 6.0, homemade) in a microwave oven (700 W) and incubated with Ultra V Block (Lab Vision; Thermo Fisher Scientific, Inc.) for 7 min at room temperature (20–25°C) to block background staining. The slides were then incubated with a monoclonal anti-lipocalin-2 antibody (dilution, 1:200; Clone #220310, MAB1757; R&D Systems) for 1 h at room temperature. UltraVision LP Detection System HRP DAB (Thermo Fisher Scientific) was used to visualize antigens, and the sections were counterstained using Mayer's hematoxylin.

Lipocalin-2 was expressed in the cytoplasm. Staining intensity was scored as 0 (negative), 1 (mild), 2 (moderate), or 3 (high). The proportional score of stained tumor cells was scored as a percentage (0%–100%). The H-score was calculated by multiplying the intensity score by the proportional score (0–300; Figure 1).

## Assessments

The pectoralis muscle index (PMI), calculated using the cross-sectional area of the pectoralis major and minor muscles, was used as a surrogate for the SMI in this study.<sup>6</sup> The bilateral pectoralis muscle area was measured by manually outlining the outermost part of the pectoralis muscles at the T4 level with a range of  $-29$  to  $100$  HU, through computed tomography histogram analysis using the “X section” analysis tool (Advantage Window 4.4; GE Healthcare). The mean value of the bilateral pectoralis muscle area was then adjusted for height ( $m^2$ ), resulting in the PMI being expressed in  $cm^2/m^2$ . The sex-specific cutoff values of PMI to indicate sarcopenia and categorize into low and high PMI groups were  $4.4\text{ cm}^2/m^2$  and  $3.1\text{ cm}^2/m^2$  for male and female patients, respectively.<sup>19</sup> The NLR was determined by dividing the total number of neutrophils by the total number of lymphocytes.<sup>20</sup> We collected baseline characteristics, including age at diagnosis, Eastern Cooperative Oncology Group performance status, and tumor stage at diagnosis as either limited-stage (LD) or extensive-stage (ED), from the electronic medical records of the patients. In addition, details on the types of treatments, chemotherapy protocols, number of treatment cycles, intensity of doses, and administration of concurrent chemoradiotherapy and prophylactic cranial irradiation were documented. Follow-up evaluations were performed every 6–12 weeks or when necessary to monitor disease progression, assess treatment response, and identify any adverse event. The assessment of treatment response was based on the Response Evaluation Criteria in Solid Tumors 1.1 criteria. Early treatment discontinuation, which was defined as stopping treatment early for reasons other than cancer progression before completing the scheduled cycles of platinum-based chemotherapy, and treatment-related mortality, which was defined as death within 30 days after the last dose of initial chemotherapy not caused by cancer progression, were also recorded. The relative dose intensity was calculated by dividing the actual dose received per cycle by the standard dose per cycle, presented as a percentage.

## Statistical analysis

Time-dependent receiver operating characteristic curve analysis was conducted to assess the predictive validity of lipocalin-2 and the NLR at various time points, aiming to identify the optimal cutoff values for these biomarkers. This analysis involved plotting the sensitivity (true positive rate) against 1-specificity (false positive rate) across a range of potential cutoff values for each marker. The optimal cutoff was determined by the point on the curve that maximized the Youden index (sensitivity + specificity  $-1$ ), aiming to identify patients with survival times greater than the median OS. The analysis was planned to be conducted separately for patients with LD and ED, because of anticipated differences

in survival patterns and the potential variability in the prognostic value of lipocalin-2 and NLR across stages.

Descriptive statistics, including the chi-square, Fisher's exact, Mann–Whitney U, and Cuzick's trend tests, and the Cochran–Armitage trend test were used to analyze the baseline characteristics, treatment-related factors, and treatment response of the study population. Kaplan–Meier survival analysis with log-rank tests was conducted to evaluate PFS and OS. Variables with  $p < 0.1$  in univariate analyses were subsequently included in the multivariate Cox proportional hazards models to evaluate the prognostic significance of covariates. Two-sided  $p < 0.05$  was considered statistically significant for all tests. All statistical analyses were conducted using STATA version 16.1 (StataCorp LLC).

## RESULTS

### Determination of lipocalin-2 cutoff value in patients with LD and ED

In this study involving 191 patients (72 with LD and 119 with ED), we established a cutoff value of H-score 50 for lipocalin-2 in patients with LD cancer. This threshold, which was used to differentiate patients into low and high lipocalin-2 groups, demonstrated a sensitivity of 69.4% and a specificity of 66.7% for predicting survival beyond 18.9 months (the median OS for patients with LD cancer in the study cohort; Figure S1a). For patients with ED cancer, no significant cutoff value for lipocalin-2 was found to predict survival beyond 11.5 months (the median OS for patients with ED cancer) as indicated by the low area under the curve value and the receiver operating characteristic curve's proximity to the 45-degree diagonal line (Figure S1b). Consequently, further analysis focused on the LD cohort.

### Baseline and treatment characteristics

Table 1 describes the baseline characteristics of the LD cohort, categorized by lipocalin-2 expression levels. The high lipocalin-2 group exhibited a higher frequency of poor performance status and elevated lactate dehydrogenase levels. Patients with low PMI tended to be more common in the high lipocalin-2 group, although this difference did not reach statistical significance. Otherwise, no significant differences were found in baseline characteristics between the low and high lipocalin-2 groups. Regarding treatment characteristics (Table S1), the etoposide plus carboplatin regimen was administered more frequently to patients in the high lipocalin-2 group than to those in the low lipocalin-2 group. However, this imbalance was likely due to chance rather than a direct relationship with lipocalin-2 expression, as the choice between cisplatin and carboplatin in the chemotherapy regimen was based on the physician's assessment of the patient's clinical factors, such as renal function, age, and performance status, and was made independently of the lipocalin-2

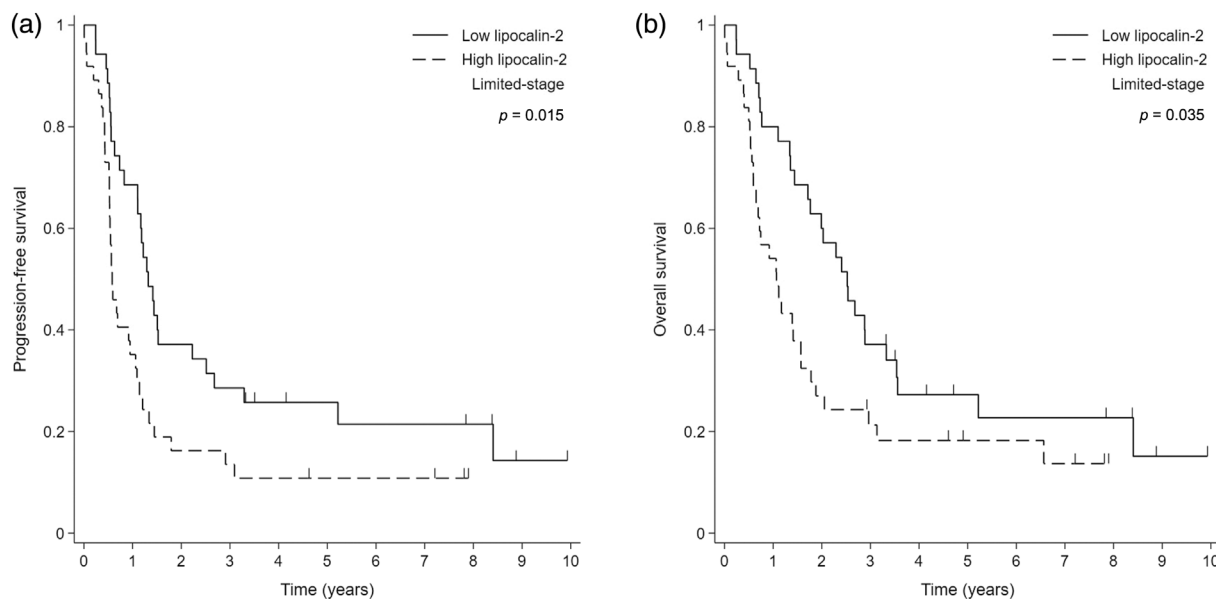
**TABLE 1** Baseline characteristics of patients with limited stage.

Characteristics	Low lipocalin-2 (n = 35)	High lipocalin-2 (n = 37)	p-value
Median age, years (IQR)	66 (59–70)	70 (62–75)	0.120
Sex			0.377
Male	31 (88.6)	30 (81.1)	
Female	4 (11.4)	7 (18.9)	
ECOG PS			0.042
0–1	32 (91.4)	27 (73.0)	
2–3	3 (8.6)	10 (27.0)	
Smoking			>0.998
Never-smoker	2 (5.7)	2 (5.4)	
Current/former smoker	33 (94.3)	35 (94.6)	
Lactate dehydrogenase <sup>a</sup>			0.031
Normal	13 (65.0)	11 (34.4)	
Elevated	7 (35.0)	21 (65.6)	
PMI			0.084
High	32 (91.4)	28 (75.7)	
Low	3 (8.6)	9 (24.3)	
Mean (SD), cm <sup>2</sup> /m <sup>2</sup>	5.8 (1.3)	5.2 (1.3)	0.084
NLR			0.404
Low	25 (71.4)	23 (62.2)	
High	10 (28.6)	14 (37.8)	
Mean (SD)	2.6 (1.5)	3.0 (2.4)	0.714

Note: The variables are presented as number (%) or median (IQR).

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; IQR, interquartile range; NLR, neutrophil-lymphocyte ratio; PMI, pectoralis muscle index; SD, standard deviation.

<sup>a</sup>LDH levels were measured in 52 patients.



**FIGURE 2** Kaplan–Meier survival curves according to lipocalin-2 expression in patients with limited-stage small cell lung cancer. (a) Progression-free survival (PFS) and (b) overall survival (OS).

expression levels, which were not available at the time of treatment selection. No other significant differences were observed between the groups regarding relative dose intensity,

the use of concurrent chemoradiotherapy and prophylactic cranial irradiation, early discontinuation of treatment, or treatment-related mortality.

TABLE 2 Cox regression for overall survival of patients with limited stage.

	Univariate			Multivariate (1)			Multivariate (2)			Multivariate (3)		
	HR	95% CI	<i>p</i> -value	HR	95% CI	<i>p</i> -value	HR	95% CI	<i>p</i> -value	HR	95% CI	<i>p</i> -value
Age	1.062	1.028–1.096	< 0.001	1.047	1.004–1.092	0.034	1.051	1.008–1.097	0.021	1.048	1.007–1.091	0.021
Sex												
Male	Ref.											
Female	0.582	0.273–1.242	0.162									
ECOG PS												
0–1	Ref.			Ref.			Ref.			Ref.		
2–3	4.587	2.291–9.185	< 0.001	3.280	1.560–6.895	0.002	2.904	1.409–5.983	0.004	2.482	1.176–5.236	0.017
Chemotherapy regimen												
Etoposide/carboplatin	Ref.			Ref.			Ref.			Ref.		
Etoposide/cisplatin	0.464	0.270–0.799	0.006	0.853	0.414–1.760	0.667	1.148	0.572–2.304	0.697	0.966	0.503–1.853	0.917
Dose reduction since the first cycle												
No	Ref.			Ref.			Ref.			Ref.		
Yes	2.201	1.175–4.120	0.014	1.022	0.495–2.112	0.952	0.972	0.468–2.017	0.939	1.152	0.561–2.367	0.699
Lipocalin-2												
Low	Ref.			Ref.								
High	1.747	1.034–2.954	0.037	1.193	0.630–2.258	0.588						
Lipocalin-2 + PMI	2.369	1.513–3.709	< 0.001				2.013	1.210–3.351	0.007			
Lipocalin-2 + NLR	1.894	1.297–2.765	0.001							1.708	1.081–2.701	0.022

Note: Since only one factor among lipocalin-2, lipocalin-2 + PMI, and lipocalin-2 + NLR should be selected as a variable in each analysis, used gray shading to indicate 'blank'. Abbreviations: CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; NLR, neutrophil-lymphocyte ratio; PMI, pectoralis muscle index.

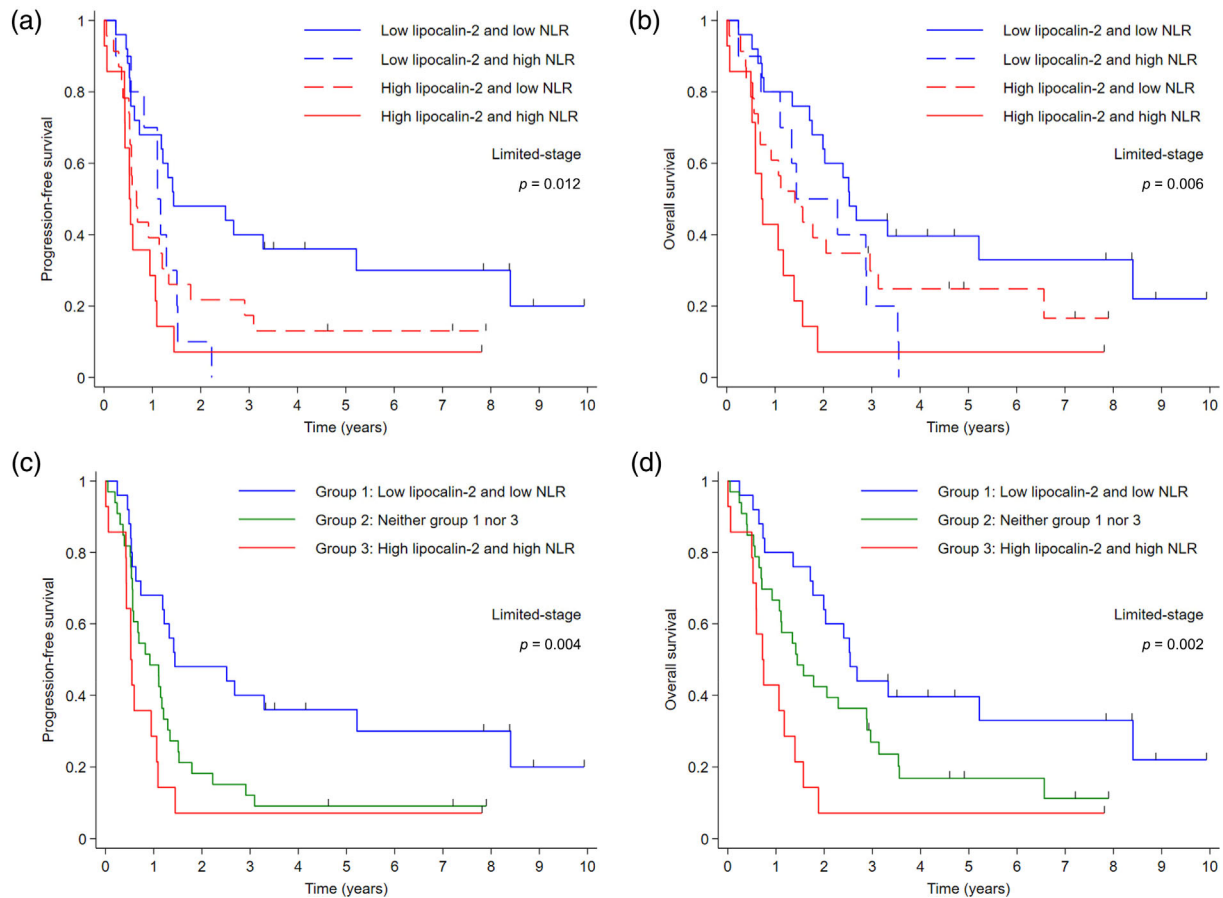
### Prognostic implications of lipocalin-2 expression

This study had a median follow-up duration of 93 months for the LD cohort. Figure 2 presents the PFS and OS rates in the LD cohort, stratified by lipocalin-2 expression. The high lipocalin-2 group had significantly worse PFS (median PFS: 7.0 vs. 15.9 months; 95% confidence interval: 6.3–12.8 vs. 13.2–26.7;  $p = 0.015$ ) and OS (median OS: 12.9 vs. 30.3 months; 95% confidence interval: 20.6–39.9 vs. 7.8–18.9;  $p = 0.035$ ) compared with the low lipocalin-2 group. However, lipocalin-2 expression lost statistical significance when combined with other clinical factors in multivariate analysis (Table 2).

### Prognostic implications of combining lipocalin-2, neutrophil-lymphocyte ratio, and pectoralis muscle index

Further analyses were conducted to assess the predictive impact of lipocalin-2 in conjunction with the PMI and NLR, which are both indicative of systemic inflammation. In the LD cohort, the cutoff value of NLR was determined as 3 to differentiate patients into low and high NLR groups (Figure 3c). Patients characterized by low lipocalin-2

expression and NLR exhibited the most favorable PFS and OS. Conversely, those with high levels of lipocalin-2 expression and NLR had the worst survival outcomes (Figure 3a,b). Further stratification into three distinct cohorts based on the combined status of lipocalin-2 expression and NLR more clearly delineated these observations, as depicted in Figure 3c (median PFS: 17.3 vs. 11.0 vs. 6.3 months in groups 1, 2, and 3, respectively;  $p = 0.004$ ) and Figure 3d (median OS: 30.5 vs. 17.3 vs. 8.6 months in groups 1, 2, and 3, respectively;  $p = 0.002$ ). Similar patterns were observed (Figure 4) when evaluating the combined status of lipocalin-2 expression and PMI. The median PFS was 15.9, 11.0, and 3.7 months for group 1 (characterized by low lipocalin-2 expression and high PMI; indicative of nonsarcomatous status), group 2 (neither group 1 nor group 3 lipocalin-2 expression and PMI status), and group 3 (characterized by high lipocalin-2 and low PMI; indicative of sarcomatous status), respectively ( $p < 0.001$ ; Figure 4c). Median OS followed a similar trend, with values of 30.5, 16.9, and 4.8 months for groups 1, 2, and 3, respectively ( $p < 0.001$ ; Figure 4d). Multivariate analysis confirmed the significant survival benefit associated with the combination of lipocalin-2 expression and NLR, or lipocalin-2 expression and PMI. The type of chemotherapy regimen did not significantly impact survival outcomes (Table 2), consistent with previous literature.<sup>21</sup>



**FIGURE 3** Kaplan–Meier survival curves according to the combined status of lipocalin-2 expression and neutrophil-lymphocyte ratio (NLR) in patients with limited-stage small cell lung cancer. (a) Progression-free survival (PFS) and (b) overall survival (OS) for patients stratified by the combination of lipocalin-2 and NLR. (c) PFS and (d) OS for patients stratified into three groups based on the combined status of lipocalin-2 and NLR.

### Association of lipocalin-2 expression with treatment response

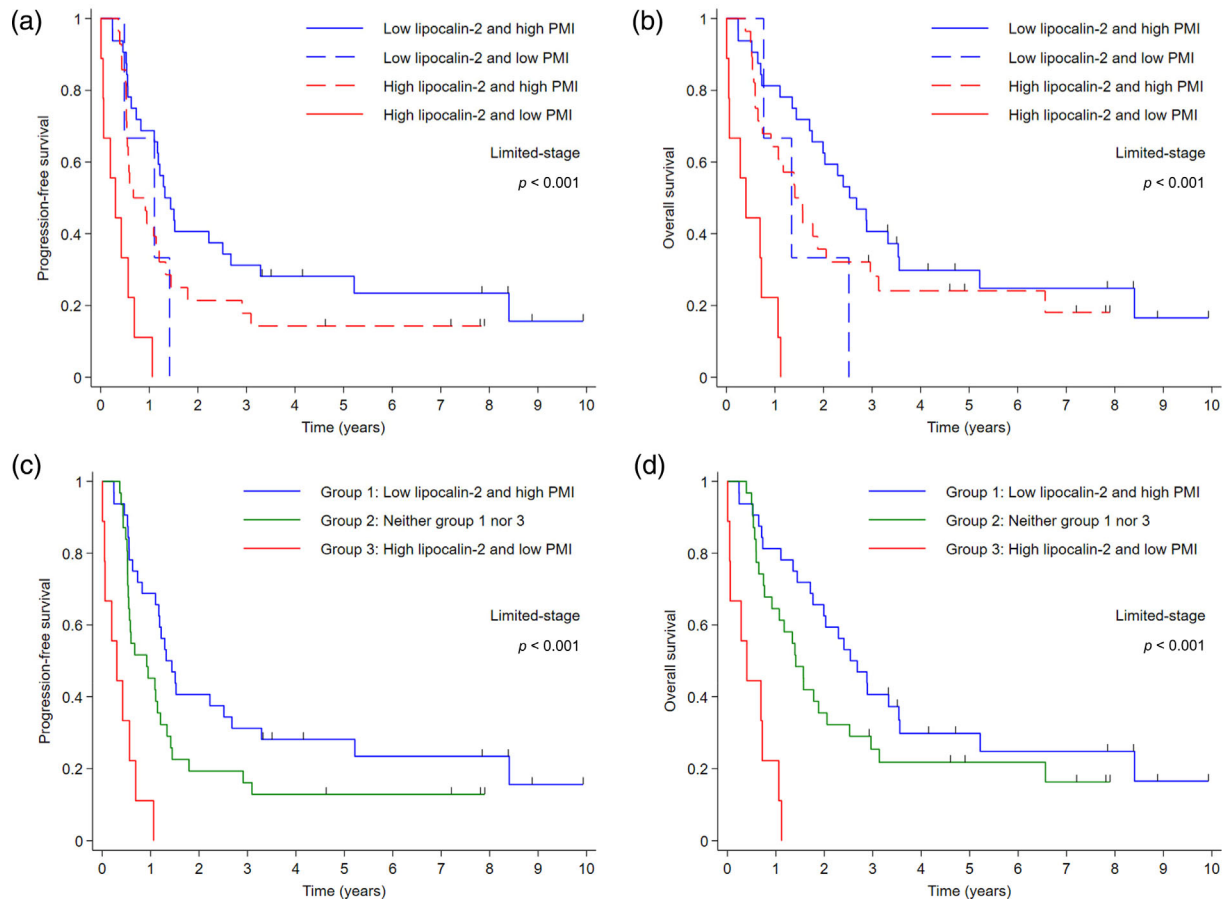
Table 3 presents the relationship between treatment response and the status of lipocalin-2, NLR, and PMI in patients with LD. Patients exhibiting high lipocalin-2 expression were less likely to achieve a complete response (CR; rate of 18.9%) compared with those with low lipocalin-2 levels (CR rate of 34.3%;  $p = 0.035$ ). However, the difference between the high and low lipocalin-2 groups was not statistically significant (83.8% vs. 97.1%,  $p = 0.108$ ) when considering the objective response rate (ORR), which encompasses both complete and partial responses.

Further analysis incorporating the NLR and lipocalin-2 expression revealed a stronger association with treatment response. Group 1 exhibited the highest CR rate of 48.0%, which was markedly above those of groups 2 (18.2%) and 3 (7.1%;  $p = 0.001$ ). Despite the ORR differences not reaching statistical significance across groups ( $p = 0.090$ ), the trend from groups 1 to 3 suggests that the combination of lipocalin-2 expression and NLR might provide a more reliable predictive tool for assessing treatment response. The treatment response analysis relative to lipocalin-2 expression

and PMI further supports the efficacy of using a combination of markers. Groups 1 and 2 displayed high ORRs (96.9% and 96.8%, respectively) compared with a significantly lower rate in group 3 (44.4%,  $p < 0.001$ ). In addition, group 1 showed a higher CR rate (34.4%) than group 2 (25.8%), and group 3 did not achieve CR ( $p = 0.002$ ).

### DISCUSSION

In this study, we investigated the prognostic value of lipocalin-2 in patients with SCLC, revealing significant associations between lipocalin-2 expression and survival outcomes. We found that high lipocalin-2 expression was potentially associated with poorer PFS and OS in the LD cohort. In addition, our analysis demonstrated that when lipocalin-2 is considered with other markers of systemic inflammation, such as NLR and PMI, it provides an enhanced prediction of clinical outcomes. Specifically, patients with low lipocalin-2 expression and favorable inflammatory profiles had the best survival outcomes, while those with both high lipocalin-2 expression and adverse inflammatory profiles had the worst survival outcomes.



**FIGURE 4** Kaplan–Meier survival curves according to the combined status of lipocalin-2 expression and pectoralis muscle index (PMI) in patients with limited-stage small cell lung cancer. (a) Progression-free survival (PFS) and (b) overall survival (OS) for patients stratified by the combination of lipocalin-2 and PMI. (c) PFS and (d) OS for patients stratified into three groups based on the combined status of lipocalin-2 and PMI.

**TABLE 3** Treatment response of patients with limited stage by lipocalin-2, NLR, and PMI.

Treatment response	Lipocalin-2 alone			Lipocalin-2 and NLR				Lipocalin-2 and PMI			
	Low lipocalin-2 (n = 35)	High lipocalin-2 (n = 37)	p-value	Group 1 (n = 25)	Group 2 (n = 33)	Group 3 (n = 14)	p-value	Group 1 (n = 32)	Group 2 (n = 31)	Group 3 (n = 9)	p-value
CR	12 (34.3)	7 (18.9)	0.035 <sup>a</sup>	12 (48.0)	6 (18.2)	1 (7.1)	0.001 <sup>b</sup>	11 (34.4)	8 (25.8)	0	0.002 <sup>b</sup>
PR	22 (62.9)	24 (64.9)		12 (48.0)	24 (72.7)	10 (71.4)		20 (62.5)	22 (71.0)	4 (44.4)	
SD, PD, or not evaluated	1 (2.9)	6 (16.2)		1 (4.0)	3 (9.1)	3 (21.4)		1 (3.1)	1 (3.2)	5 (55.6)	
Objective response rate (CR + PR)	34 (97.1)	31 (83.8)	0.108	24 (96.0)	30 (90.9)	11 (78.6)	0.090 <sup>a</sup>	31 (96.9)	30 (96.8)	4 (44.4)	<0.001 <sup>a</sup>

Note: The variables are presented as numbers (%).

Abbreviations: CR, complete response; NLR, neutrophil-lymphocyte ratio; PD, progressive disease; PMI, pectoralis muscle index; PR, partial response; SD, stable disease.

<sup>a</sup>Cochrane-Armitage trend test.

<sup>b</sup>Cuzick's trend test.

These findings underscore the potential of lipocalin-2, in conjunction with other inflammation-related markers, to serve as a valuable prognostic tool in managing patients with SCLC.

Given the absence of direct experimental studies on lipocalin-2 in SCLC, we can infer its potential mechanisms

of action from findings in other types of cancer. For instance, murine breast cancer cells with high lipocalin-2 levels exhibit increased lung metastasis, due to the ability of lipocalin-2 to suppress the phosphoinositide 3-kinase (PI3K)/Akt pathway.<sup>22</sup> In addition, lipocalin-2 promotes tumor progression by angiogenesis through the upregulation

of vascular endothelial growth factor and the activation of the mitogen-activated protein kinase/extracellular-signal-regulated kinase pathway.<sup>23,24</sup> Moreover, lipocalin-2 inhibits ferroptosis, resulting in resistance to chemotherapy in colorectal cancer.<sup>17</sup> It also plays a role in systemic, cellular, and mucosal hypoferremia during inflammation, affecting the tumor microenvironment and potentially promoting tumor progression.<sup>11</sup> In pancreatic ductal adenocarcinoma, lipocalin-2 promotes tumor progression by modulating the secretion of proinflammatory cytokines within the tumor microenvironment.<sup>25</sup> These conditions and pathways—PI3K/Akt signaling, angiogenesis, ferroptosis, and systemic inflammation—are similarly implicated in the pathogenesis of SCLC.<sup>3–6,26–29</sup> Inflammation acts as a critical regulator, activating and modulating the PI3K/Akt pathway, angiogenesis, and ferroptosis within the tumor microenvironment.<sup>30–32</sup> In addition, cancer-related cachexia may be another factor explaining why lipocalin-2 is associated with poor prognosis in SCLC. The plasma progastrin-releasing peptide level has been reported to be associated with weight loss and increased in nonresponders to chemotherapy.<sup>33</sup> Lipocalin-2 is known for its role in appetite suppression during cancer-related cachexia,<sup>34</sup> and its expression level was inversely related to PMI level (Table 1), which is associated with sarcopenia and cachexia, in our study. These findings indicate that the lipocalin-2 may affect SCLC progression through both direct and inflammation-mediated mechanisms.

The significant prognostic value of lipocalin-2 was valid only in the LD cohort in this study. In contrast to our study findings, limited evidence specifically identifying lipocalin-2 as a prognostic biomarker in early-stage cancer has been available until now.<sup>23</sup> Additionally, more evidence supporting the prognostic implications of lipocalin-2 in late-stage cancer and its association with more aggressive histological features has been available.<sup>35–38</sup> The unique findings observed in our study might be attributed to several factors, including differences in methodological approaches and patient cohorts across studies, which could affect the expression level and prognostic significance of lipocalin-2. In addition, the inherent heterogeneity of SCLC, especially in the ED cohort, may further complicate the interpretation of the prognostic value of lipocalin-2. ED-SCLC is characterized by a wider range of genetic mutations, diverse tumor microenvironments due to metastasis, and the development of treatment resistance through clonal evolution.<sup>39,40</sup> This heterogeneity in ED-SCLC may overshadow the prognostic significance of single biomarkers, such as lipocalin-2, as the effect of multiple interacting biological processes becomes more pronounced. Consequently, the utility of lipocalin-2 in ED-SCLC may require further investigation to elucidate its role amidst the complex interplay of methodological and biological factors.

The combined model incorporating lipocalin-2 with NLR or PMI demonstrated superior prognostic value compared with lipocalin-2 alone. The rationale for developing the combined model lies in the complex interplay between systemic inflammation and tumor biology. Systemic

inflammation promotes tumor progression through diverse mechanisms, such as angiogenesis, immune response suppression, and epithelial-mesenchymal transition.<sup>41–43</sup> NLR and PMI each may reflect different aspects of the inflammatory process and have been suggested to be prognostic factors in SCLC.<sup>3,6,20,44–46</sup> The combined model integrating novel and conventional markers related to systemic inflammation may provide a more comprehensive assessment of the inflammatory status and its potential impact on tumor progression. This approach is also supported by previous studies that have demonstrated the value of combining multiple inflammatory markers to improve prognostic stratification in patients with SCLC.<sup>6,47–49</sup>

One of the main limitations of the present study was that lipocalin-2 alone did not demonstrate independent prognostic value in the multivariate analysis. This finding suggests that the prognostic impact of lipocalin-2 may be confounded by other clinical factors. Although the combined model incorporating lipocalin-2 with NLR or PMI showed improved performance, it may only partially explain the complex interplay between lipocalin-2 and other prognostic factors. Moreover, functional translational studies are necessary to elucidate the precise mechanisms by which lipocalin-2 affects SCLC progression. Other study limitations included its retrospective nature, relatively small sample size, missing values for certain variables such as LDH, lack of a significant cutoff value for lipocalin-2 in the ED cohort, and scarcity of patients treated with immunotherapy in our cohort. Consequently, the present study findings may have limited applicability to future research involving patients treated using immunotherapy.

In conclusion, this study suggests that high lipocalin-2 expression is potentially associated with poorer survival outcomes in patients with LD-SCLC. The combined model incorporating lipocalin-2 with inflammation-related markers demonstrated improved prognostic performance, highlighting the potential of integrating multiple biomarkers in SCLC prognostication. Nevertheless, further research is needed to validate these findings in a larger number of prospective cohorts and explore the potential of lipocalin-2 as a therapeutic target. Functional studies are also warranted to elucidate the precise mechanisms by which lipocalin-2 affects SCLC progression.

## AUTHOR CONTRIBUTIONS

Conceptualization: Jung Wook Yang and Gyeong-Won Lee. Data curation: Se-Il Go, Jung Wook Yang, Woo Je Lee, Eun Jeong Jeong, Sungwoo Park and Gyeong-Won Lee. Formal analysis: Se-Il Go, Jung Wook Yang and Gyeong-Won Lee. Investigation: Se-Il Go, Jung Wook Yang, Woo Je Lee, Eun Jeong Jeong, Sungwoo Park and Gyeong-Won Lee. Methodology: Jung Wook Yang and Gyeong-Won Lee. Project administration: Gyeong-Won Lee. Resources: Jung Wook Yang, Woo Je Lee and Gyeong-Won Lee. Supervision: Gyeong-Won Lee. Visualization: Se-Il Go. Writing—original draft: Se-Il Go, Jung Wook Yang, Eun Jeong Jeong and Gyeong-Won Lee. Writing—review and editing: Se-Il Go,



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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data sets generated during and/or analyzed during this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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