Serum high-mobility group box 1 as a predictive marker for cytotoxic chemotherapy-induced lung injury in patients with lung cancer and interstitial lung disease

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Highlights

- HMGB1 is an inflammatory protein associated with lung cancer and lung injury
- Lung injury by chemotherapy is common in lung cancer with interstitial lung disease
- Elevated HMGB1 levels may be the first blood marker to predict this adverse event
- Interstitial lung disease may be a risk factor of the disease by increasing HMGB1
- Tumor burden also contributes to disease development by increasing HMGB1

Abstract

Background: High-mobility group box 1 (HMGB1) is a pro-inflammatory protein, that is associated with tumorigenesis, interstitial lung disease (ILD), and acute lung injury. Chemotherapy-induced lung injury is a common and serious adverse event in patients with lung cancer and ILD, but its pathogenesis and predictive biomarkers are not known. This study aimed to investigate the predictive potential of serum HMGB1 levels for cytotoxic chemotherapy-induced lung injury in these patients.

Methods: From 743 patients with advanced lung cancer, we enrolled 83 consecutive patients with ILD and background-matched 83 patients without ILD. Additionally, 83 healthy subjects were included. After measuring baseline levels of serum HMGB1 in three groups, we evaluated the predictive values of baseline HMGB1 levels for cytotoxic chemotherapy-induced lung injury in patients with lung cancer and ILD.

Results: Higher levels of serum HMGB1 were independently associated with higher tumor burden, as assessed by total tumor size, and the presence of ILD. Twenty-five (30.1%) of patients with lung cancer and ILD experienced cytotoxic chemotherapy-induced lung injury within one year. Univariate Cox proportional hazards model

showed that higher levels of HMGB1 and higher tumor burden were associated with disease onset. Moreover, multivariate analysis revealed that only HMGB1 was independently associated with this severe complication in patients with lung cancer and ILD.

Conclusions: HMGB1 is a potential predictive blood biomarker for cytotoxic chemotherapy-induced lung injury in patients with lung cancer and ILD. This study also suggests a potential pathogenesis of this serious adverse event that tumor- and ILD-derived HMGB1 accelerates lung injury.

Keywords: lung cancer, interstitial lung disease, lung injury, high-mobility group box 1, soluble receptor for advanced glycation end products, biomarker

Abbreviations: ALAT, Latin American Thoracic Association; ATS, American Thoracic Society; AUC, area under the curve; CT, computed tomography; ERS, European Respiratory Society; FVC, forced vital capacity; HMGB1, high-mobility group box 1; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; IQR, interquartile range; JRS, Japanese Respiratory Society; PS, performance status; RAGE, receptor for

advanced glycation end products; RECIST, Response Evaluation Criteria in Solid Tumors; ROC, receiver operating characteristic; sRAGE, soluble receptor for advanced glycation end products; UIP, usual interstitial pneumonia

1. Introduction

Interstitial lung disease (ILD) is a risk factor of lung cancer, and ILD is observed in 7.5% to 15.2% of patients with lung cancer at diagnosis [1, 2]. Treatment options are limited in patients with lung cancer and ILD due to the high incidence of treatment-related exacerbation of ILD [3]. Cytotoxic chemotherapy is usually selected as the treatment of choice in these patients with advanced lung cancer, but cytotoxic chemotherapy-induced lung injury has been observed in 13.3–34.9% of patients with lung cancer and ILD [4-9]; furthermore, the pathogenesis and predictive blood markers of this potentially fatal adverse event remain unclear.

High-mobility group box 1 (HMGB1) is one of the damage-associated molecular patterns released by injured cells. HMGB1 binds to cell surface receptors, such as the receptor for advanced glycation end product (RAGE), and this interaction accelerates pro-inflammatory intracellular signaling associated with tumorigenesis and acute lung injury, which includes acute exacerbation of ILD [10, 11]. It was shown that HMGB1 was highly expressed in lung cancer tissue [12], and circulatory HMGB1 levels were shown to be increased by larger tumor size and decreased by surgical resection of the tumor [13]. Additionally, a previous report showed that the presence of ILD elevated HMGB1 circulatory levels, and importantly, these higher levels at the

baseline could predict earlier onset of acute exacerbation of ILD [11]. Based on these observations, we hypothesized that the levels of circulatory HMGB1 would be increased in patients with lung cancer and ILD, and HMGB1 could be a predictive blood biomarker for cytotoxic chemotherapy-induced lung injury in these patients.

To elucidate this hypothesis, first, we measured serum HMGB1 levels in the following three groups: lung cancer patients with ILD, those without ILD, and healthy controls. Second, we evaluated the association of HMGB1 serum levels with tumor burden and the presence of ILD among lung cancer patients with and without ILD. Third, the potential of HMGB1 as a predictive biomarker of cytotoxic chemotherapy-induced lung injury in patients with lung cancer and ILD was analyzed. Finally, because circulatory soluble RAGE (sRAGE) acts as a decoy receptor for HMGB1 and inhibits HMGB1-associated inflammation [14-17], we evaluated the association of sRAGE with HMGB1 and cytotoxic chemotherapy-induced lung injury.

2. Material and Methods

2. 1. Study population and design

As shown in Supplementary Fig. 1, the present study included 83 consecutive advanced lung cancer patients with ILD and background-matched 83 patients without

ILD from 743 patients who were diagnosed and treated at the Hiroshima University Hospital between October 2003 and December 2018. Eighty-three healthy subjects were also enrolled. Lung cancer patients without ILD were matched to those with ILD for age, sex, smoking history, performance status, stage, and histological type. Advanced lung cancer was defined as unresectable stage III/stage IV/postoperative recurrence of disease, using the TNM classification [18], and patients who were treated with cytotoxic chemotherapy after blood draw were included for the present study. In patients with lung cancer and ILD, this study excluded those who received radical thoracic radiation therapy and those who were treated with tyrosine kinase inhibitors targeted to oncogenic driver mutation. This study was approved by the Ethics Committee of Hiroshima University Hospital (M326) and all of the participants provided written informed consent.

2. 2. Diagnostic criteria for ILD and cytotoxic chemotherapy-induced lung injury

The diagnostic criteria for ILD were the existence of bilateral reticulation and consolidation or ground-glass attenuation on pre-treatment computed tomography (CT), which was performed within one month from starting the treatment. The usual interstitial pneumonia (UIP) pattern was defined as UIP and probable UIP in the CT

pattern of the American Thoracic Society, European Respiratory Society, Japanese Respiratory Society, and Latin American Thoracic Association (ATS/ERS/JRS/ALAT) clinical practice guideline of idiopathic pulmonary fibrosis (IPF) [19]. These were independently classified by two pulmonologists blinded to the patient clinical details. The concordance rate was 84.3% (70/83). In 13 cases with conflicting results, the third experienced pulmonologist additionally and independently evaluated the CT images, and the final decision was made by the majority vote.

Cytotoxic chemotherapy-induced lung injury was diagnosed using the criteria modified from those for IPF, to suit the practical aspects of cancer chemotherapy [20, 21]. The criteria were as follows:

i. Acute worsening or development of dyspnea within one month.

ii. CT showing new ground-glass abnormality bilaterally and/or consolidation.

iii. No indication of apparent heart failure, pulmonary invasion of lung cancer, or pulmonary infection; no improvement with antibiotic treatment and negative sputum and/or blood cultures.

iv. Development of cytotoxic chemotherapy-induced lung injury within one month of administration of the last cytotoxic chemotherapy.

2. 3. Measurement of serum biomarkers and tumor burden

Serum samples were collected before chemotherapy administration at our hospital and stored at -80 °C. Serum levels of HMGB1 and sRAGE were measured using commercially available enzyme-linked immunosorbent assay kits according to manufacturer's instructions (HMGB1 ELISA Kit II [Shino-Test Corporation, Tokyo] and Human RAGE Quantikine ELISA Kit [R&D Systems, Minneapolis, MN, USA], respectively).

Tumor burden was quantified as the sum of the diameters (longest axis for non-nodal lesions and short axis for nodal lesions) of measurable target lesions using unidimensional Response Evaluation Criteria in Solid Tumors (RECIST)-defined measurements. Measurable target lesions (≥10 mm in the longest diameter for non-nodal lesions and ≥15 mm in the short axis for nodal lesions) were selected on baseline CT scans, allowing up to two lesions per organ and up to five lesions in total [22].

2. 4. Statistical analysis

Values were expressed as medians (interquartile ranges [IQRs]). Differences among groups were evaluated using Pearson's chi-squared and Kruskal–Wallis tests. If there was a significant difference in the Kruskal–Wallis test for multiple comparisons,

the Mann–Whitney *U* test with Bonferroni correction was performed for individual comparisons. Spearman's correlation coefficient was performed to investigate the correlation of HMGB1 serum levels with tumor burden and sRAGE serum levels, and linear regression analysis was performed in order to find out factors that affect serum levels of HMGB1. Receiver operating characteristic (ROC) curve analysis was performed to define the optimal cut-off levels of serum HMGB1, tumor burden, and serum sRAGE for predicting the development of cytotoxic chemotherapy-induced lung injury within one year of starting cytotoxic chemotherapy. Disease development was evaluated using Kaplan–Meier analysis and log-rank test. Death due to cancer progression was considered a censoring event. Cox proportional hazards analysis was used to identify significant predictors for the development of the disease. $P < 0.05$ were considered to indicate statistical significance. All data analyses were performed using JMP, version 14.1.0 (SAS Institute Inc., Cary, NC, USA).

3. Results

3. 1. Cohort characteristics

Although there were missing data, a significant difference only in the baseline forced vital capacity (FVC) was observed in 74 of 83 lung cancer patients with ILD and

61 of 83 lung cancer patients without. Healthy subjects were significantly younger and had smoked significantly less than lung cancer patients with or without ILD, and had significantly higher FVC than lung cancer patients with ILD (Table 1). Of the 83 lung cancer patients with ILD, 77 (92.8%) had idiopathic interstitial pneumonia, 5 had collagen vascular disease (3, rheumatoid arthritis; 1, systemic sclerosis; 1, mixed connective tissue disease), and the other one patient had asbestosis. Seven patients were receiving immunosuppressive treatment (4, only steroid therapy; 3, combination steroid therapy and immunosuppressant), two were receiving anti-fibrotic drugs, and three received home oxygen therapy before and during the cancer treatment. Additionally, two patients received palliative irradiation for bronchial stenosis due to lung cancer within one year of starting cytotoxic chemotherapy. However, no patient developed lung injury during the observation period.

3. 2. Baseline serum concentrations of HMGB1

Serum levels of HMGB1 in lung cancer patients with ILD were significantly higher than those in patients without ILD (5.34 ng/mL [IQR, 2.79–8.46] and 3.77 ng/mL [IQR, 2.48–5.87], P=0.008), and these levels were significantly higher than those among the healthy controls (2.24 ng/mL [IQR, 1.61–3.55], P<0.001 each) (Fig.

1). Additionally, among lung cancer patients with ILD and those without, Spearman's correlation coefficient revealed that baseline levels of serum HMGB1 were significantly and positively correlated with tumor burden (r_s=0.438, P<0.001) (n=166) (Fig. 2). Univariate linear regression analysis showed that older age, higher pack-year smoking history, the presence of ILD, and higher tumor burden were significantly associated with higher levels of serum HMGB1. Multivariate linear regression analysis revealed that only the presence of ILD and higher tumor burden were significantly and independently associated with higher levels of serum HMGB1 when adjusted by confounders (Table 2).

3. 3. Predictive potential of HMGB1 and tumor burden for cytotoxic chemotherapy-induced lung injury

Of the 83 patients with lung cancer and ILD, 25 (30.1%) had cytotoxic chemotherapy-induced lung injury during the observation period. The frequency and severity of the different regimens and treatment lines are summarized in Supplementary Table 1. ROC curve analysis revealed that the optimal cut-off levels for predicting cytotoxic chemotherapy-induced lung injury were 5.04 ng/mL for serum HMGB1 (area under the curve [AUC]=0.68, specificity=55.2%, sensitivity=80.0%) and 70.7 mm for

tumor burden (AUC=0.61, specificity=53.5%, sensitivity=80.0%) (Supplementary Fig. 2). Kaplan–Meier analysis revealed that not only patients with HMGB1 higher levels but also those with higher tumor burden had a significantly earlier onset of cytotoxic chemotherapy-induced lung injury (Fig. 3a, b). Univariate Cox proportional hazards model revealed that serum HMGB1 and tumor burden higher than 70.7 mm were significant predictors of the disease onset. Multivariate Cox proportional hazards model revealed that only serum HMGB1 was the independent predictor when adjusted by confounders, including tumor burden (Table 3).

3. 4. Correlation of sRAGE with the risk of chemotherapy-induced lung injury stratified by HMGB1 levels

Among lung cancer patients with ILD and those without ILD, the median serum level of sRAGE was 751.3 pg/mL (IQR, 505.5-1064.7), which was significantly and negatively correlated with that of HMGB1 (n=166) (r_s=-0.153, P=0.049) (Supplementary Fig. 3). In patients with lung cancer and ILD, Kaplan–Meier analysis revealed that patients with sRAGE levels >606.9 pg/mL, which was obtained by ROC curve analysis (AUC=0.66, specificity=60.3%, sensitivity=76.0%) (Supplementary Fig. 2), had a significantly lower development rate of cytotoxic chemotherapy-induced lung

injury (Supplementary Fig. 4); that was particularly noticeable in patients with HMGB1 levels ≥5.04 ng/mL (n=46), but not in those with HMGB1 levels <5.04 ng/mL (n=37) (Fig. 3c, d).

4. Discussion

Chemotherapy-induced lung injury is a common and important adverse event in patients with lung cancer and ILD, and this study was conducted to investigate the first predictive blood biomarker for this life-threatening condition based on the hypothesis shown in the graphical abstract (Figure 4). We found an independent association between serum HMGB1 levels and the onset of chemotherapy-induced lung injury in patients with lung cancer and ILD. Additionally, exploratory analysis revealed that higher levels of serum sRAGE, a decoy receptor for RAGE ligands, including HMGB1, was associated with decreased incidence of cytotoxic chemotherapy-induced lung injury in patients with higher HMGB1 levels. These results indicate that HMGB1 is a promising biomarker and may have a role in the pathogenesis of cytotoxic chemotherapy-induced lung injury in patients with lung cancer and ILD.

This study found that higher tumor burden was correlated with higher levels of serum HMGB1; furthermore, these two factors were associated with earlier onset of

cytotoxic chemotherapy-induced lung injury in the univariate analysis. Notably, serum HMGB1 was shown as an independent predictor of chemotherapy-induced lung injury after adjusting for potential confounders, including tumor burden. These data indicate that the circulatory levels of HMGB1 play a promising role in the development of cytotoxic chemotherapy-induced lung injury in patients with lung cancer and ILD, and higher tumor burden may accelerate this severe complication via increasing circulatory HMGB1 (Figure 4).

This study also demonstrated that the presence of ILD accompanied with lung cancer was associated with higher levels of HMGB1 in circulation, which helps to explain the association between the presence of ILD and the risk of cytotoxic chemotherapy-induced lung injury [4-9]. We and others have previously demonstrated that increased levels of serum and bronchoalveolar lavage fluid HMGB1 were observed in patients with ILDs compared to those in healthy subjects, and these higher levels in serum were associated with the earlier development of acute exacerbation of ILD [11, 23]. A previous report has shown that increased levels of serum HMGB1 in patients with ILD may reflect the increased HMGB1 expression in the nuclei of infiltrating inflammatory cells, alveolar macrophages, and injured epithelial cells [23]. These data indicate that the presence of ILD could also promote the augmented pro-inflammatory condition in the

lungs of lung cancer patients by increasing HMGB1 levels (Figure 4).

We also evaluated serum levels of sRAGE in this study, because we previously found that increased serum levels of sRAGE were associated with a reduced risk of acute exacerbation in patients with IPF [17]. As a result, sRAGE higher levels were associated with reduced incidence of cytotoxic chemotherapy-induced lung injury in patients with higher levels of HMGB1. These data suggest that the effect of heightened HMGB1 could be canceled by high levels of sRAGE and support the hypothesis that HMGB1 is involved in the pathogenesis of chemotherapy-induced lung injury in patients with lung cancer and ILD. Further investigation is needed to clarify the possibility of blocking HMGB1 as a therapeutic target.

This study has limitations. First, the present study was conducted in a single facility, and the sample size was relatively small. Second, non-target lesion by the RECIST criteria, e.g., pleural dissemination, could not be quantified as regards tumor burden. Third, the treatment regimen was not the same when patients developed chemotherapy-induced lung injury because there was no standard of care for patients with lung cancer and ILD; this is due to the lack of a randomized study evaluating the differences in the risk of lung injury for each cytotoxic drug. Recently, Haruna et al. showed that blood HMGB1 levels increased with Docetaxel treatment [24]. This

observation suggests that treatment with cytotoxic chemotherapy may further increase HMGB1 levels (Figure 4). However, it is not clear whether each cytotoxic drug might have similar effects. Finally, the mechanism for the pathogenesis of drug-induced lung injury could generally be classified into two, the allergic mechanism and the cytotoxic mechanism. However, it is practically difficult to do this because no consensus diagnostic method exists. A prospective study is necessary to confirm the results of this study and evaluate serial changes in HMGB1 after chemotherapy to elucidate the mechanism of the association between HMGB1 and cytotoxic chemotherapy-induced lung injury.

5. Conclusions

Increased HMGB1 levels in the circulation were independently associated with earlier development of cytotoxic chemotherapy-induced lung injury in patients with lung cancer and ILD, while higher tumor burden and the presence of ILD were associated with heightened HMGB1. Our results suggest that serum HMGB1 is a promising biomarker and a potential therapeutic target for this serious adverse event in patients with lung cancer and ILD.

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Table 1. Demographic data of the study cohort

	Lung cancer with ILD	Lung cancer without ILD	Control
Subjects, n	83	83	83
Age, years	71 (67–78)	71 (63–75)	52 (50–55) ***
Sex			
Male	74	69	71
Female	9	14	12
Smoking history, pack-years	50.0 (32.0–80.0)	46.0 (29.8–75.0)	10.0 (0.0–30.0) ***
FVC, % predicted ‡	87.4 (70.6-100.0)	94.3 (78.6-106.5) ###	95.6 (85.4-107.4) ###
PS			
0–1	66	72	-
≥2	17	11	-
Stage			
III	19	10	-
IV	59	68	-
Recurrence	5	5	-
Histological type			
Adenocarcinoma	34	47	-
Squamous cell carcinoma	15	12	-
Small cell carcinoma	29	23	-
Others	5	1	-
ILD pattern			
UIP pattern	24	-	-
Non-UIP pattern	59	-	-

* $P<0.05$, ** $P<0.01$, and *** $P<0.001$ compared to lung cancer patients with ILD and those without ILD, and # $P<0.05$, ## $P<0.01$, and ### $P<0.001$ compared to lung cancer patients with ILD, Mann–Whitney U test with Bonferroni correction for individual comparisons

‡ FVC was measured only in 74 of 83 lung cancer patients with ILD and 61 of 83

lung cancer patients without.

Data are presented as median and IQR.

Abbreviations: FVC, forced vital capacity; ILD, interstitial lung disease; IQR, interquartile range; PS, performance status; UIP, usual interstitial pneumonia

Table 2. Correlations between serum levels of HMGB1 and baseline characteristics in lung cancer patients and healthy subjects (n=249)

Variables	*β*	*T*	*P*-value
Univariate analysis			
Age, years	0.28	4.58	<0.001***
Sex, male	-0.10	-1.56	0.119
Smoking history, pack-years	0.26	4.15	<0.001***
Presence of ILD	0.29	4.74	<0.001***
Tumor burden, mm	0.47	8.32	<0.001***
Multivariate analysis			
Age, years	0.01	0.09	0.932
Sex, male	-0.06	-1.08	0.281
Smoking history, pack-years	-0.01	-0.01	0.995
Presence of ILD	0.14	2.22	0.028*
Tumor burden, mm	0.41	5.78	<0.001***

* $P<0.05$, ** $P<0.01$, and *** $P<0.001$ linear regression analysis

Abbreviations: HMGB1, high-mobility group box 1; ILD, interstitial lung disease

Table 3. Cox proportional hazards model for predicting the onset of cytotoxic chemotherapy-induced lung injury in patients with lung cancer and ILD (n=83)

Variables	HR	95% CI	*P*-value
Univariate analysis			
Age, years	0.97	0.93–1.01	0.115
Sex, male	1.79	0.53–11.15	0.392
PS ≥2	1.79	0.51–4.93	0.331
Smoking history, pack-years	0.99	0.98–1.00	0.184
UIP pattern	2.19	0.95-4.84	0.065
Tumor burden, ≥70.7 mm	3.91	1.58-11.78	0.002**
Tumor burden, mm	1.01	0.99-1.02	0.070
HMGB1, ng/mL	1.13	1.05–1.20	0.001**
sRAGE, pg/mL	0.99	0.996–0.999	0.002**
Multivariate analysis			
UIP pattern	1.65	0.68-3.87	0.264
Tumor burden, ≥70.7 mm	2.55	0.98-7.91	0.056
HMGB1, ng/mL	1.09	1.02-1.16	0.020*
sRAGE, pg/mL	0.99	0.99-1.00	0.074

* $P<0.05$, ** $P<0.01$, and *** $P<0.001$ Cox proportional hazards model

Abbreviations: CI, confidence interval; HMGB1, high-mobility group box 1; HR, hazard ratio; ILD, interstitial lung disease; PS, performance status; sRAGE, soluble receptor for advanced glycation end products; UIP, usual interstitial pneumonia

Figure legends

Figure 1. Baseline levels of serum HMGB1

In the order of lung cancer patients with interstitial lung disease (ILD), those without ILD, and healthy subjects, serum levels of high-mobility group box 1 (HMGB1) were significantly decreased. Boxes represent the 25th to 75th percentiles; solid lines within the boxes show the median values; whiskers represent the 10th and 90th percentiles; the circles represent outliers. * P<0.05, ** P<0.01, and *** P<0.001 using the Mann–Whitney U test with Bonferroni correction. IQR, interquartile range

Figure 2. Correlation between serum HMGB1 levels and tumor burden

In lung cancer patients with and without interstitial lung disease, serum levels of high-mobility group box 1 (HMGB1) were significantly and positively correlated with tumor burden (n=166) (r_s=0.438 P<0.001, using Spearman's correlation coefficient).

Figure 3. Kaplan–Meier analysis of the onset of cytotoxic chemotherapy-induced lung injury in patients with lung cancer and ILD

Kaplan-Meier analysis revealed that patients with higher serum levels of high-mobility group box 1 (HMGB1) **(a)** and higher tumor burden **(b)** had a shorter follow-

up period before the development of cytotoxic chemotherapy-induced lung injury. Soluble receptor for advance glycation end product (sRAGE) acts as a HMGB1 inhibitor, and its serum levels higher than 606.9 pg/mL were significantly decreased the development rate of this severe complication in patients with HMGB1 levels ≥5.04 ng/mL **(c)** but not in those with HMGB1 levels <5.04 ng/mL **(d)** (using log-rank test). ILD, interstitial lung disease

Figure 4. Schematic diagram of the proposed molecular mechanism of HMGB1-induced pro-inflammatory condition, induced by lung cancer, ILD, and cytotoxic chemotherapy

Up-regulation of high-mobility group box 1 (HMGB1) by the presence of lung cancer and interstitial lung disease (ILD) induce pro-inflammatory conditions via intracellular signaling from the receptor for advanced glycation end products (RAGE). Soluble RAGE (sRAGE) exerts an anti-inflammatory effect, suppressing pro-inflammatory intracellular signaling by neutralizing HMGB1 as a decoy receptor.

Figure 1

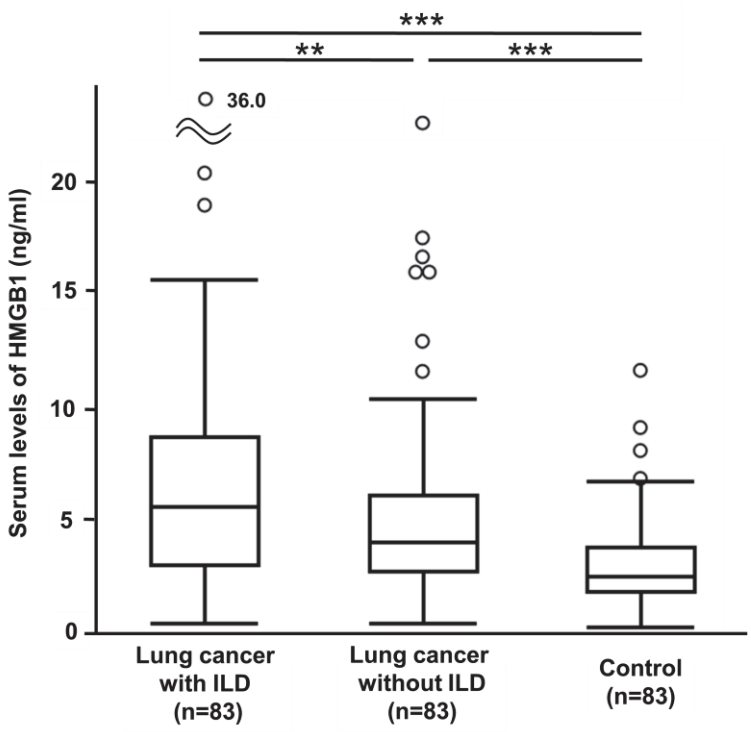

***
**
***
36.0
20
15
10
5
0
Serum levels of HMGB1 (ng/ml)
Lung cancer with ILD (n=83)
Lung cancer without ILD (n=83)
Control (n=83)

Figure 2

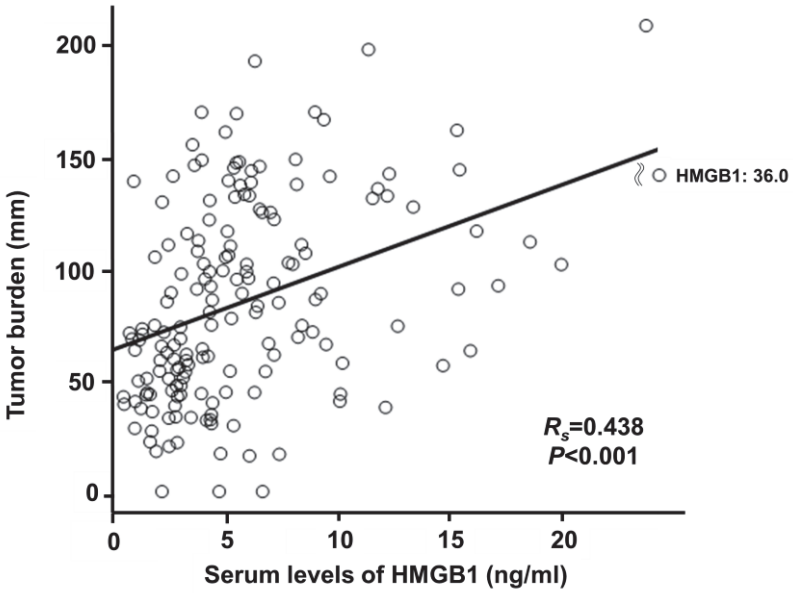
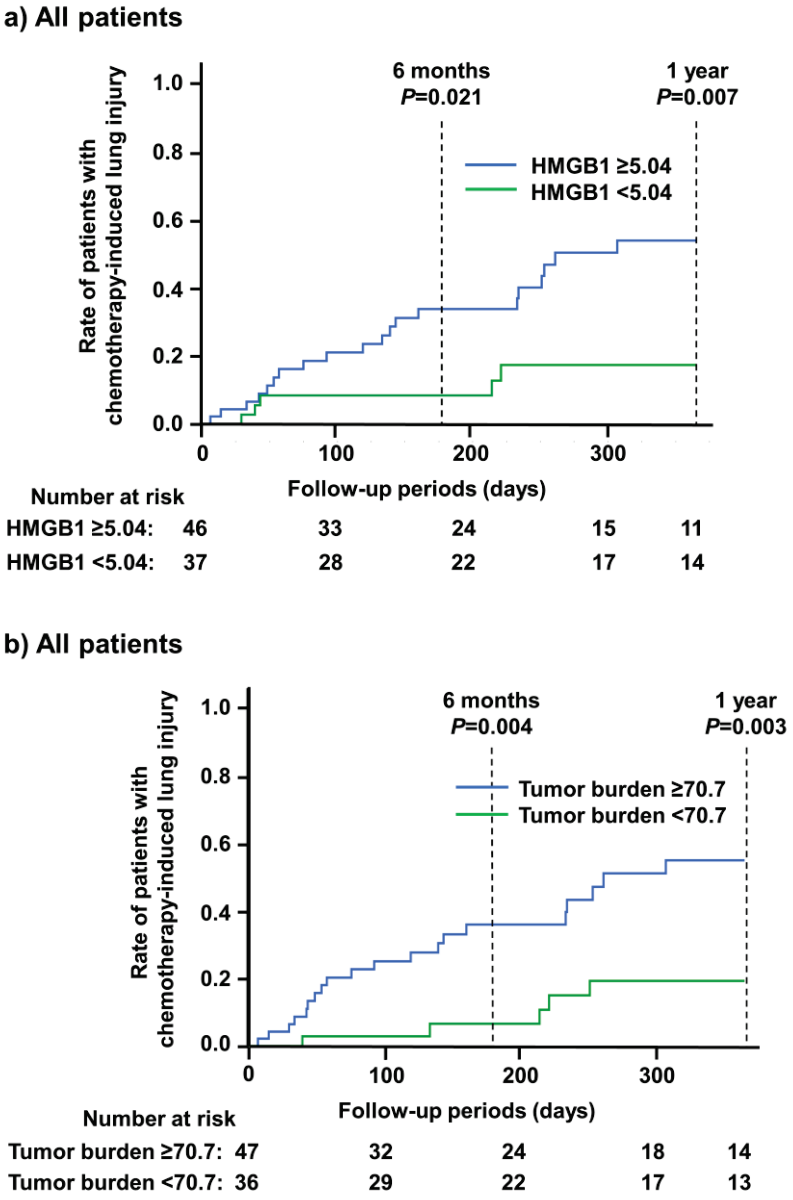
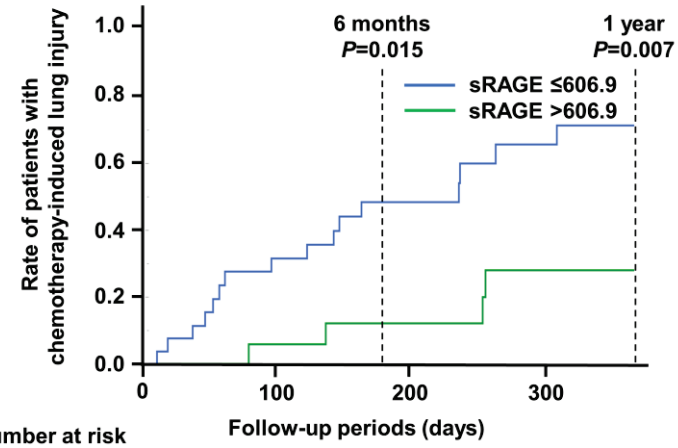

200
150
100
50
0
Tumor burden (mm)
0
5
10
15
20
Serum levels of HMGB1 (ng/ml)
HMGB1: 36.0
R_s=0.438
P<0.001

Figure 3

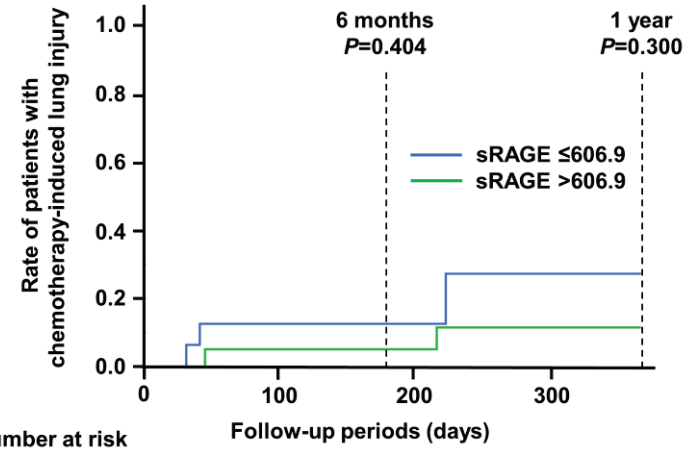

a) All patients
6 months
P=0.021
1 year
P=0.007
HMGB1 ≥5.04
HMGB1 <5.04
Rate of patients with chemotherapy-induced lung injury
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
Follow-up periods (days)
Number at risk
HMGB1 ≥5.04: 46 33 24 15 11
HMGB1 <5.04: 37 28 22 17 14
b) All patients
6 months
P=0.004
1 year
P=0.003
Tumor burden ≥70.7
Tumor burden <70.7
Rate of patients with chemotherapy-induced lung injury
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
Follow-up periods (days)
Number at risk
Tumor burden ≥70.7: 47 32 24 18 14
Tumor burden <70.7: 36 29 22 17 13

c) Patients with HMGB1 ≥5.04 ng/mL



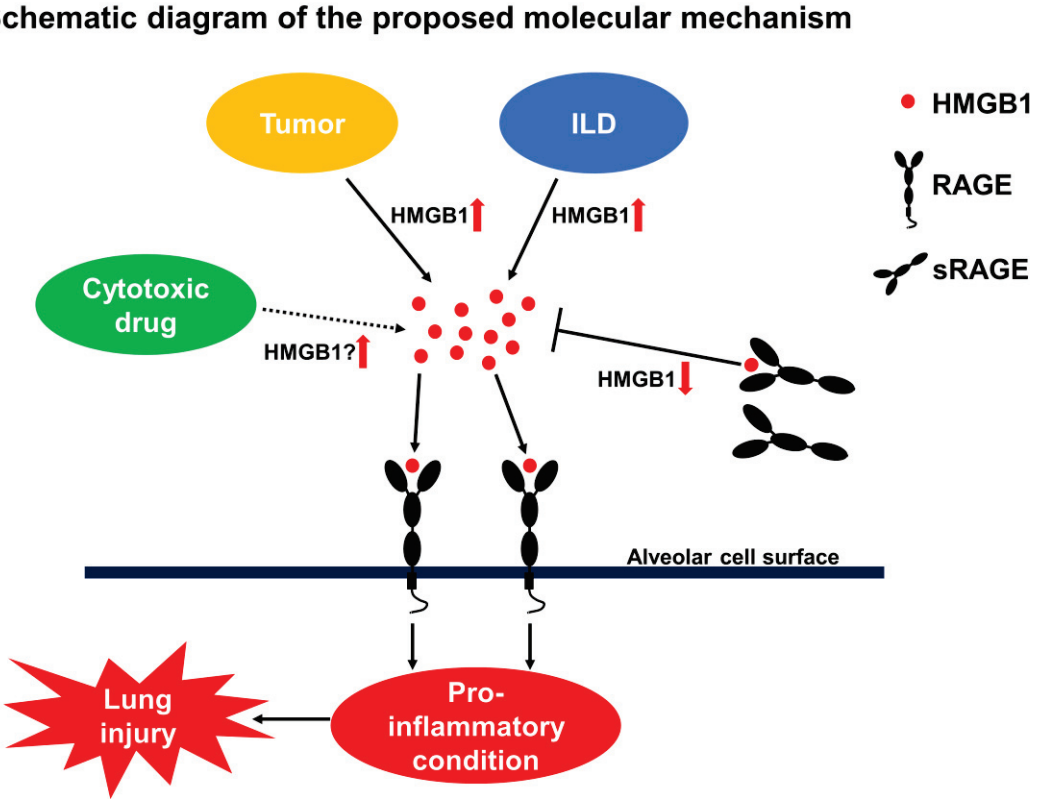
Number at risk					
sRAGE ≤606.9:	26	18	11	7	5
sRAGE >606.9:	20	16	14	9	6

d) Patients with HMGB1 <5.04 ng/mL



Number at risk					
sRAGE ≤606.9:	16	11	7	4	3
sRAGE >606.9:	21	18	16	14	11

Figure 4.


Schematic diagram of the proposed molecular mechanism
Tumor
ILD
HMGB1
HMGB1
HMGB1
RAGE
sRAGE
Cytotoxic drug
HMGB1?
HMGB1
Alveolar cell surface
Lung injury
Pro-inflammatory condition

Supplementary Table 1. Chemotherapy regimens and treatment lines for cytotoxic chemotherapy-induced lung injury within one year of starting chemotherapy

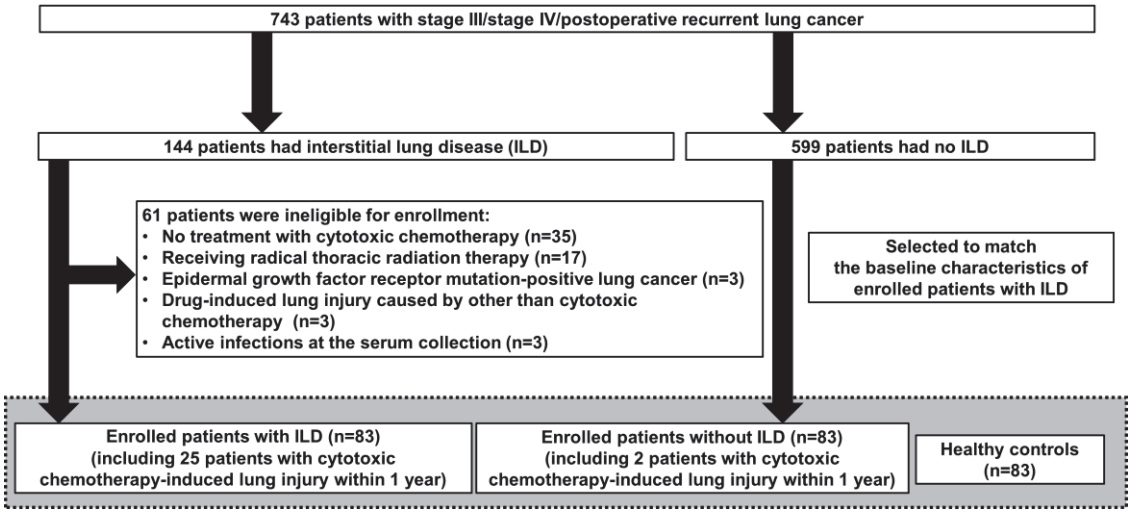
	No. of patients with lung injury, n	No. of patients without lung injury, n	Grade of lung injury*, 1–2/3–4/5
First line			
Platinum agents+ETP	3	27	1/1/1
Platinum agents+PEM	1	2	0/0/1
Platinum agents+S-1	0	2	0/0/0
Platinum agents+GEM	0	2	0/0/0
CBDCA+PTX	4	13	1/0/3
CBDCA+nab-PTX	0	4	0/0/0
CDDP+DTX	0	2	0/0/0
S-1	2	3	0/1/1
VNR	2	7	0/1/1
DTX	0	4	0/0/0
PEM	1	3	1/0/0
GEM	0	1	0/0/0
Second line			
Platinum agents+ETP	0	2	0/0/0
CBDCA+PTX	0	2	0/0/0
CDDP+PEM+BEV	0	1	0/0/0
CDDP+VNR	0	1	0/0/0
S-1	1	4	0/0/1
DTX	3	6	2/1/0
PEM	1	2	0/1/0
AMR	3	7	0/1/2
nab-PTX	0	2	0/0/0
NGT	1	1	0/1/0
CPT-11	0	1	0/0/0
Third line			
S-1	0	3	0/0/0
VNR	1	1	0/0/1

DTX	1	1	0/1/0
PEM	1	1	0/1/0
GEM	0	2	0/0/0
AMR	0	1	0/0/0
nab-PTX	0	1	0/0/0
PTX	0	1	0/0/0
CPT-11	0	1	0/0/0
Nivolumab	0	1	0/0/0
Fourth line			
S-1	0	1	0/0/0
VNR	0	1	0/0/0
Atezolizumab	0	1	0/0/0
Fifth line			
PEM	0	1	0/0/0

*According to the Common Terminology Criteria for Adverse Events version 4.0.

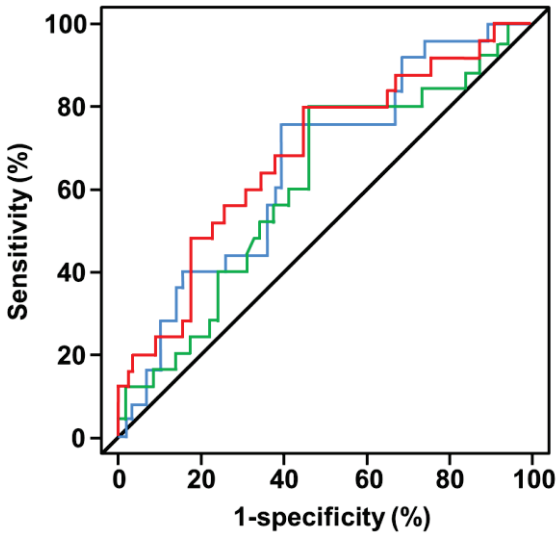
Abbreviation: AMR, amrubicin; BEV, bevacizumab; CBDCA, carboplatin; CDDP, cisplatin; CPT-11, irinotecan; DTX, docetaxel; ETP, etoposide; GEM, gemcitabine; NGT, nogitecan; PEM, pemetrexed; PTX, paclitaxel; S-1, Tegafur/Gimeracil/Oteracil; VNR, vinorelbine

Supplementary Fig. 1. Flowchart of patient enrollment



Among 743 patients with lung cancer with prospectively collected blood samples, 83 consecutive patients with lung cancer and interstitial lung disease (ILD) were enrolled. Additionally, background-matched 83 lung cancer patients without ILD and 83 healthy controls were also enrolled.

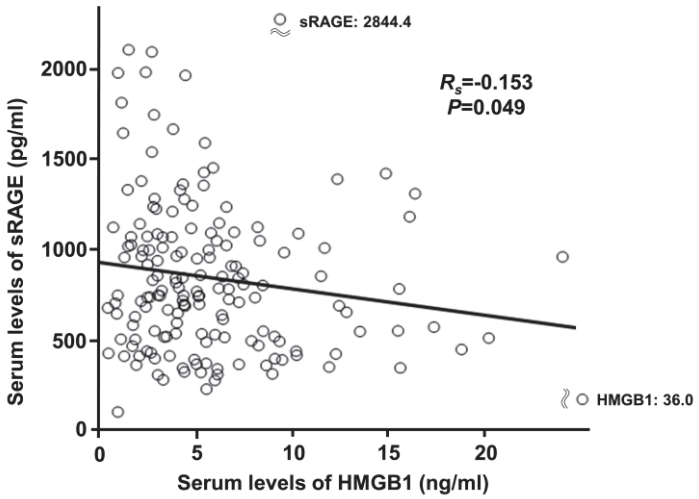
Supplementary Fig. 2. Receiver operating characteristic curve analysis for predicting the onset of cytotoxic chemotherapy-induced lung injury in patients with lung cancer and ILD (n=83)



	AUC	95% CI	Cut-off value	Specificity	Sensitivity
HMGB1	0.68	0.55-0.80	5.04 ng/mL	55.2 %	80.0 %
Tumor burden	0.61	0.47-0.73	70.7 mm	53.5 %	80.0 %
sRAGE	0.66	0.52-0.77	606.9 pg/mL	60.3 %	76.0 %

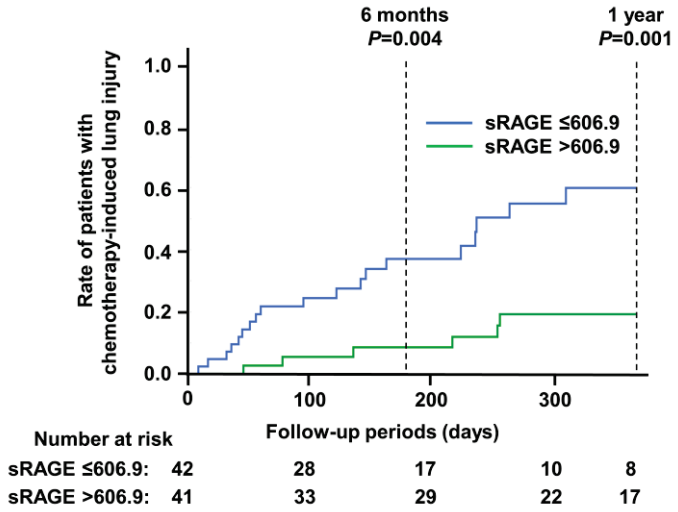
Receiver operating characteristic curve analysis revealed that the optimal cut-off levels for predicting cytotoxic chemotherapy-induced lung injury were 5.04 ng/mL for serum high-mobility group box 1 (HMGB1) (area under the curve [AUC]=0.68, specificity=55.2%, sensitivity=80.0%), 70.7 mm for tumor burden (AUC=0.61, specificity=53.5%, sensitivity=80.0%), and 606.9 pg/mL for soluble receptor for advanced glycation end products (sRAGE) (AUC=0.66, specificity=60.3%, sensitivity=80.0%). CI, confidence interval; ILD, interstitial lung disease

Supplementary Fig. 3. Correlation between serum HMGB1 and sRAGE levels



In lung cancer patients with and without interstitial lung disease (n=166), serum levels of high-mobility group box 1 (HMGB1) were significantly and negatively correlated with those of soluble receptor for advanced glycation end products (sRAGE) (r_s=-0.153, P=0.049, using Spearman's correlation coefficient).

Supplementary Fig. 4. Kaplan–Meier analysis of the onset of cytotoxic chemotherapy-induced lung injury in patients with lung cancer and ILD



Higher levels of serum soluble receptor for advanced glycation end products (sRAGE) were associated with a lower development rate of cytotoxic chemotherapy-induced lung injury (n=83) (using log-rank test). ILD, interstitial lung disease