

Higher circulatory HMGB1 accelerates postoperative acute exacerbation of interstitial lung disease in lung cancer

Kakuhiro Yamaguchi (✉ yamaguchikakuhiro@gmail.com)

Hiroshima University <https://orcid.org/0000-0002-9117-1934>

Satoshi Nakao

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Hiroshi Iwamoto

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Atsushi Kagimoto

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Yoshinori Handa

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Shinjiro Sakamoto

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Yasushi Horimasu

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Takeshi Masuda

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Takahiro Mimae

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Shintaro Miyamoto

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Taku Nakashima

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Yasuhiro Tsutani

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Kazunori Fujitaka

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Yoshihiro Miyata

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Hironobu Hamada

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Morihito Okada

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Noboru Hattori

Hiroshima Daigaku Igakubu Daigakuin Ishiyaku Hokengaku Kenkyuka

Research

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Abstract

Background: Postoperative acute exacerbation of interstitial lung disease (AE-ILD) is a fatal complication in patients with lung cancer and ILD, and it needs to be overcome to improve the long-term outcomes of these patients. However, the molecular target for predicting and preventing this fatal complication remains unclear. High-mobility group box 1 (HMGB1), which is reported to increase due to surgical procedure, activates a pro-inflammatory response associated with acute lung injury. This study aimed to elucidate the association between postoperative AE-ILD and circulatory HMGB1, especially focusing on its predictive potential.

Methods: This study included 152 patients with lung cancer and ILD, who underwent radical surgery between January 2011 and August 2019. We measured HMGB1 serum levels, and investigated the factors affecting HMGB1 and the predictive potential of HMGB1 for postoperative AE-ILD.

Results: Postoperative AE-ILD was developed in 17 (11.2%) of 152 patients with lung cancer and ILD. HMGB1 serum levels in patients with AE-ILD were significantly higher than those in patients without (median [IQR]: 5.39 [3.29-11.70] ng/mL vs 3.55 [2.07-5.62] ng/mL). Logistic regression analysis revealed that HMGB1 higher levels and longer operative time was independently associated with a higher incidence of postoperative AE-ILD. Furthermore, when HMGB1 and operative time were incorporated into previously reported risk scoring system, the concordance index was 0.876 which is statistically higher than 0.715 calculated by reported scoring system only.

Conclusions: Baseline levels of serum HMGB1 could be a promising biomarker for predicting postoperative AE-ILD, especially when combined with operative time. HMGB1 may be a molecular target of this fatal complication to be overcome.

Background

High-mobility group box 1 (HMGB1) is a nuclear protein that was characterized to be involved in DNA organization and the regulation of transcription [1]. HMGB1 is released passively during cellular necrosis by almost all cells. It is also secreted actively by immune cells, including monocytes, macrophages, and dendritic cells [2]. Circulatory HMGB1 binds to pattern recognition receptors on the cell surface, such as the receptor for advanced glycation end products (RAGE) and toll-like receptor (TLR). Their interactions result in activating pro-inflammatory response associated with acute lung injury [3, 4]. We have previously reported that higher levels of circulatory HMGB1 at the baseline are associated with the earlier development of acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF) [5]. Additionally, when AE-IPF is developed, HMGB1 levels are significantly elevated compared to IPF patients without AE [5, 6]. Although the trigger of AE-IPF is not sufficiently elucidated, these data suggest that not only higher baseline levels of HMGB1 but also its additional elevation may trigger lung injury superimposed on interstitial lung disease (ILD).

Postoperative AE-ILD is triggered by surgical procedure [7]. Previous reports have shown that postoperative AE-ILD occurs in 9.3% of patients, with a mortality rate of 43.9%, in patients with lung cancer and ILD [8]. The pathophysiology of this fatal adverse event is still unclear. However, several studies have demonstrated that HMGB1-induced lung injury is accelerated during surgery. For example, mechanical ventilation induces elevated expression of HMGB1 in lung tissue and bronchoalveolar lavage fluid (BALF), and anti-HMGB1 antibody can cancel the ventilation-induced lung injury [9, 10]. These observations let us speculate that surgery-induced secretion of HMGB1 would trigger postoperative AE-ILD, especially in patients with higher levels of HMGB1. Thus, baseline levels of circulatory HMGB1 can be a predictive blood marker of this severe complication.

This study aimed to elucidate the association between serum levels of HMGB1 and the development of postoperative AE-ILD in patients with lung cancer and ILD. First, baseline levels of serum HMGB1 were compared between the patients with and without postoperative AE-ILD. Second, the logistic regression model was used to identify the independent risks of this severe complication. Third, the difference in the incidence of postoperative AE-ILD based on the identified risks was analyzed, especially focusing on HMGB1 and factors associated with surgical procedure. Last, the utility of HMGB1 was evaluated when it combined with already reported risk scoring system using a surgical procedure, male sex, serum levels of Krebs von den Lungen-6 (KL-6), history of AE, preoperative use of steroids, radiological ILD pattern, and vital capacity (VC).

Methods

Study design and population

This cohort study consecutively included lung cancer patients with ILD who prospectively collected blood samples before surgery and underwent radical surgery for lung cancer between January 2011 and August 2019 at the Hiroshima University Hospital. The staging of lung cancer was according to the TNM Classification of Malignant Tumors, 8th edition [11]. This study was approved by the Ethics Committee of Hiroshima University Hospital (Gen-38 and E-1707). All participants provided written informed consent for obtaining the blood sample before surgery, and an opt-out method was performed to measure HMGB1 serum levels by using the already gathered samples.

Diagnostic criteria for ILD and postoperative AE-ILD

The diagnostic criteria for ILD were the existence of bilateral reticulation and consolidation or ground-glass attenuation on computed tomography (CT). The CT pattern was classified by using the American Thoracic Society, European Respiratory Society, Japanese Respiratory Society, and Latin American Thoracic Association (ATS/ERS/JRS/ALAT) clinical practice guideline of IPF; it was classified as usual interstitial pneumonia (UIP) pattern, possible UIP pattern, and inconsistent with UIP pattern [12].

Postoperative AE-ILD caused by lung resection was defined based on the criteria proposed by previous studies for the disease and the ATS/ERS/JRS/ALAT statement as follows: (i) acute worsening or

development of dyspnea and oxygen desaturation; (ii) CT showing new ground-glass abnormality bilaterally and/or consolidation; (iii) no evidence of other alternative causes, such as pulmonary infection, cardiac failure, pulmonary embolism, and pneumothorax; and (iv) development of AE-ILD within 30 days after lung resection [12–15].

Measurement of serum HMGB1 concentrations

Serum samples were collected before surgery at our hospital and stored at -80 °C. Serum levels of HMGB1 were measured using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (HMGB1 ELISA Kit II [Shino-Test Corporation, Tokyo]).

Statistical analysis

Values were expressed as mean \pm standard deviation or median (interquartile range [IQR]) according to their distribution. Normally and non-normally distributed variables were evaluated using the t-test and Mann-Whitney U tests, respectively. Pearson's chi-squared tests were also conducted. Bonferroni correction was applied for multiple comparisons. Linear regression analysis was conducted to study the independent effects of age, sex, smoking history, lung function parameters, ILD pattern, serum levels of KL-6, preoperative steroid use, and history of AE; on serum HMGB1 levels. Univariate and multivariate logistic regression analyses were conducted to identify independent risk factors for postoperative AE-ILD development and to estimate the respective odds ratios and their 95% confidence intervals (CI). Confounders in the multivariate analysis were selected by stepwise method from all factors in univariate analysis. The receiver operating characteristic (ROC) curve analysis was performed to identify the optimal cut-off levels of serum HMGB1 and operative time for predicting the development of postoperative AE-ILD. C-statistics were performed to evaluate and compare logistic models that analyzed the predictive accuracy for postoperative AE-ILD. All statistical analyses were performed using JMP version 14.1.0 (SAS Institute Inc., Cary, NC, USA). A *P*-value of less than 0.05 was considered statistically significant.

Results

Patient characteristics

The subjects were 157 patients with lung cancer and ILD who underwent radical surgery and had blood samples collected before surgery. After excluding 5 patients who had another primary cancer or active autoimmune disease, 152 patients with lung cancer and ILD were included in the analysis (Table 1).

Table 1
Baseline characteristics

	All subjects	AE-ILD (+)	AE-ILD (-)	<i>P</i> -value*
Subjects, n (%)	152	17 (11.2)	135 (88.8)	
Age, years	72.9 ± 7.5	71.7 ± 7.5	73.1 ± 7.5	0.487
Sex, male/female	126/26	17/0	109/26	0.045
Smoking history, pack-years	50.0 (30.0–67.5)	60.0 (39.0–95.0)	47.5 (30.0–65.6)	0.211
VC, % predicted	92.4 (80.8–101.6)	79.3 (73.9–92.6)	93.2 (83.8–101.8)	0.014
DLco, % predicted	56.5 ± 17.4	51.2 ± 21.1	57.2 ± 16.8	0.237
ILD pattern				0.267
UIP	52	9	43	
Possible UIP	67	5	62	
Inconsistent with UIP	33	3	30	
KL-6, U/mL	432.0 (284.7–700.0)	670.0 (360.0–1139.0)	421.0 (283.0–669.0)	0.028
Preoperative steroid use, +/-	15/137	2/15	13/122	0.676
History of AE, +/-	9/143	3/14	6/129	0.064
Histology				0.689
Adenocarcinoma	68	6	62	
Squamous cell carcinoma	56	7	49	
Small cell carcinoma	11	2	9	
Others	17	2	15	
cStage, 0/1/2/3/4	122/21/7/2	13/3/0/1	109/18/7/1	0.300

*All *P*-values are evaluated by comparing between patients with and without postoperative AE-ILD using the t-test and Mann-Whitney U tests for normally and non-normally distributed variables and using Pearson's chi-squared test.

Data are presented as mean ± standard deviation or median (interquartile range) according to their distribution.

AE-ILD, acute exacerbation of interstitial lung disease; DLco, diffusing capacity for carbon monoxide; ILD, interstitial lung disease; KL-6, Krebs von den Lungen-6; UIP, usual interstitial pneumonia; VC, vital capacity;

	All subjects	AE-ILD (+)	AE-ILD (-)	<i>P</i> -value*
Surgical procedure				0.838
Wedge resection	41	4	37	
Segmentectomy	33	3	30	
Lobectomy	78	10	68	
Operative time, min	149 (108–196)	200 (138–252)	146 (106–181)	0.022
Bleeding volume, mL	66 (24–110)	155 (73–301)	60 (23–100)	0.003
*All <i>P</i> -values are evaluated by comparing between patients with and without postoperative AE-ILD using the t-test and Mann-Whitney U tests for normally and non-normally distributed variables and using Pearson's chi-squared test.				
Data are presented as mean ± standard deviation or median (interquartile range) according to their distribution.				
AE-ILD, acute exacerbation of interstitial lung disease; DLco, diffusing capacity for carbon monoxide; ILD, interstitial lung disease; KL-6, Krebs von den Lungen-6; UIP, usual interstitial pneumonia; VC, vital capacity;				

The patients with postoperative AE-ILD were male dominant and had significantly lower VC (% predicted), higher levels of serum KL-6, longer operative time, and higher bleeding volume than the patients without postoperative AE-ILD (Table 1). There was a significant correlation between operative time and bleeding volume ($r_s = 0.640$, $P < 0.001$).

Baseline concentration of HMGB1 and its predictive potential for postoperative AE-ILD

Overall, 17 patients developed postoperative AE-ILD. Serum levels of HMGB1 were significantly higher in patients with postoperative AE-ILD than in those without (median [IQR]: 5.39 [3.29–11.70] ng/mL vs 3.55 [2.07–5.62] ng/mL, $P = 0.031$) (Fig. 1). Higher levels of HMGB1 were significantly and independently associated with younger age, higher pack-year smoking history, and lower VC (Table 2). Univariate logistic regression analysis revealed that lower VC, history of AE, higher levels of serum KL-6, longer operative time, higher bleeding volume, and higher levels of HMGB1 were significantly associated with the development of postoperative AE-ILD. Multivariate stepwise analysis revealed that lower VC, history of AE, higher levels of serum KL-6, longer operative time, and higher levels of HMGB1 independently elevated the risk of this fatal complication (Table 3).

Table 2
 Linear regression analysis to elucidate the factors associated with HMGB1 serum levels

Variables	β	<i>t</i>	<i>P</i>-value
Univariate analysis			
Age, years	-0.165	-2.04	0.043*
Sex, male	0.127	1.57	0.118
Smoking history, pack-years	0.243	3.06	0.003*
VC, %predicted	-0.179	-2.23	0.027*
DLco, % predicted	-0.099	-1.22	0.226
ILD pattern, UIP + possible UIP	0.044	0.54	0.593
KL-6, U/mL	0.013	0.16	0.876
Preoperative steroid use, +	-0.008	-0.10	0.917
History of AE, +	0.169	2.10	0.037*
Multivariate analysis			
Age, years	-0.170	-2.19	0.030*
Smoking history, pack-years	0.210	2.62	0.010*
VC, % predicted	-0.164	-2.08	0.040*
History of AE, +	0.088	1.08	0.284
* <i>P</i> < 0.05 Linear regression model			
AE-ILD, acute exacerbation; DLco, diffusing capacity for carbon monoxide; HMGB1, high-mobility group box 1; ILD, interstitial lung disease; KL-6, Krebs von den Lungen-6; UIP, usual interstitial pneumonia; VC, vital capacity;			

Table 3
Logistic regression analysis elucidating the risk of postoperative AE-ILD

Variables	Odds	95% CI	P-value
Univariate analysis			
Age, years	0.975	0.911–1.045	0.475
Sex, male	Not calculated		
pack-year	1.010	0.999–1.020	0.076
VC, % predicted	0.965	0.936–0.994	0.020*
DLco, % predicted	0.979	0.950–1.010	0.180
NSCLC vs SCLC	0.536	0.106–2.715	0.451
Stage I vs II-III	0.775	0.233–2.573	0.677
History of AE-ILD, +	4.607	1.037–20.475	0.045*
ILD pattern, UIP + possible UIP	1.221	0.328–4.546	0.766
KL-6, U/mL	1.001	1.000–1.001	0.043*
Preoperative steroid use, +	1.251	0.257–6.089	0.781
Surgical procedure, Segmentectomy/lobectomy	1.227	0.376–4.000	0.735
Operative time, min	1.010	1.003–1.018	0.008*
Bleeding volume, mL	1.003	1.001–1.005	0.008*
HMGB1, ng/mL	1.141	1.050–1.239	0.002*
Multivariate stepwise analysis [#]			
VC, % predicted	0.957	0.918–0.997	0.034*
History of AE, +	23.436	1.601–343.133	0.021*
KL-6, U/mL	1.001	1.000–1.002	0.049*
Preoperative steroid use, +	5.663	0.228–140.924	0.290

* $P < 0.05$ Logistic regression analysis

[#] Confounders in the multivariate analysis were selected by stepwise method from all factors in univariate analysis.

AE-ILD, acute exacerbation of interstitial lung disease; CI, confidence interval; DLco, diffusing capacity for carbon monoxide; HMGB1, high mobility group box 1; ILD, interstitial lung disease; KL-6, Krebs von den Lungen-6; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; UIP, usual interstitial pneumonia; VC, vital capacity;

Variables	Odds	95% CI	P-value
Operative time, min	1.016	1.004–1.028	0.011*
Bleeding volume, mL	1.002	0.999–1.004	0.161
HMGB1, ng/mL	1.132	1.008–1.271	0.036*
* $P < 0.05$ Logistic regression analysis			
# Confounders in the multivariate analysis were selected by stepwise method from all factors in univariate analysis.			
AE-ILD, acute exacerbation of interstitial lung disease; CI, confidence interval; DLco, diffusing capacity for carbon monoxide; HMGB1, high mobility group box 1; ILD, interstitial lung disease; KL-6, Krebs von den Lungen-6; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; UIP, usual interstitial pneumonia; VC, vital capacity;			

The risk stratification based on HMGB1 and operative time

To elucidate the influence of the surgical procedure as the trigger of postoperative AE-ILD, the incidence was evaluated focusing on HMGB1 baseline levels and operative time identified as the independent risk factor of the disease in this study. ROC curve analysis revealed that the optimal cut-off level of serum HMGB1 and operative time for predicting postoperative AE-ILD was 3.82 ng/mL (AUC 0.661, specificity 51.9%, and sensitivity 76.5%) and 200 min (AUC 0.671, specificity 58.9%, and sensitivity 80.0%). Each of HMGB1 higher than 3.82 ng/mL and operative time longer than 200 min was associated with a higher incidence of postoperative AE-ILD (Fig. 2a, 2b). Additionally, the patients with both HMGB1 higher than 3.82 ng/mL and operative time longer than 200 min had the highest risk of the disease compared to those with either HMGB1-high or operative time-long and those with neither (Fig. 2c).

Comparisons of C-statistics in predictive models for postoperative AE-ILD

The C-statistics showed that HMGB1 alone had modest predictive accuracy for postoperative AE-ILD (concordance index [c-index] = 0.661) (Supplementary Table S1). However, when numerical variables of HMGB1 were combined with covariates calculated by previously reported risk scoring system, i.e., 5 × (history of AE) + 4 × (surgical procedures) + 4 × (UIP appearance in CT scan) + 3 × (male sex) + 3 × (preoperative steroid use) + 2 × (serum KL-6 level higher than 1000 U/mL) + 1 × (vital capacity lower than 80%) [14], the c-index tended to increase to 0.799 ($P = 0.109$), which was higher than 0.715 calculated by covariates only (Fig. 3). Additionally, when numerical variables of HMGB1 and operative time were incorporated into a model with covariates, the c-index was 0.876, which is statistically and significantly higher than that calculated by covariates only (Fig. 3).

Discussion

Postoperative AE-ILD is a fatal complication, and thus it is important to increase its predictive accuracy before surgery. This study showed that baseline levels of serum HMGB1 in patients with postoperative AE-ILD were significantly higher than those without AE-ILD. Additionally, higher levels of HMGB1 were significantly and independently associated with a higher incidence of this fatal complication. Lastly, HMGB1 had the potential to improve the predictive accuracy of risk scoring system, which had been already reported by Sato et al [8, 14]. These data indicate that HMGB1 can be a promising biomarker to predict the postoperative AE in patients with lung cancer and ILD.

This study firstly showed that higher levels of HMGB1 were significantly associated with a higher incidence of postoperative AE-ILD in patients with lung cancer and ILD. We and others previously reported that HMGB1 levels in serum and BALF in patients with IPF were significantly elevated than those in healthy subjects [5, 16]. Additionally, we previously reported that higher levels of HMGB1 were associated with earlier onset of AE in patients with IPF [5]. HMGB1 itself accelerates pro-inflammatory signaling via interacting with RAGE and TLR4 [3, 4], and intratracheal administration of HMGB1 induces acute lung injury [17]. At the same time, some reports showed that injured alveolar epithelial cells highly expressed HMGB1 [16], indicating that one of the HMGB1 sources in patients with ILD is injured lung tissue. This study also showed that there was a negative correlation between HMGB1 and VC. These data suggest that higher levels of HMGB1 reflect not only pro-inflammatory conditions in the lung but also pre-existing lung damage. Thus, its higher levels can predict the development of postoperative AE-ILD.

This study showed that the patients, both with higher HMGB1 and longer operative time, had a significantly higher risk of this fatal complication than patients with either higher HMGB1 or longer operative time and patients with neither. When operative time becomes longer, it requires a longer time of artificial ventilation, which increases the expression of HMGB1 in BALF and lung tissue in a tidal volume- and time-dependent manner [9, 10]. Exposure to hyperoxia during artificial ventilation also promotes increasing expression of HMGB1 in a time-dependent manner [18]. This mechanical ventilation- and hyperoxia-induced lung injury is canceled by inhibition of HMGB1 [9, 18]. Additionally, higher bleeding volume, which was correlated with operative time and showed potential association with postoperative AE-ILD in this study, is also reported to increase the expression of HMGB1 in plasma and lung [19]. These data indicate that surgery accelerates inflammatory response via additional HMGB1 secretion, which results in the development of postoperative AE-ILD, especially in patients with HMGB1 higher levels.

This study also showed that HMGB1, especially when combined with operative time, could improve the predictive accuracy of postoperative AE-ILD based on previously reported risk scoring systems in patients with lung cancer and ILD [14]. Surgical resection is generally performed as a standard radical treatment for lung cancer. However, it is associated with higher postoperative morbidity of complications when the patient has concurrent ILD [20]. Preventing this fatal complication is crucial to improve the long-term outcome in patients with lung cancer and ILD who underwent surgical resection. One potential preventive strategy is that patients with higher HMGB1 accompanied with reported risks would be treated with wedge resection, because it generally needs shorter operative time and implies less bleeding volume than segmentectomy/lobectomy. Another potential strategy is to use HMGB1-inhibitory drugs. Some existing

drugs are reported to inhibit HMGB1-induced inflammatory signal via decreasing HMGB1 secretion and capturing HMGB1 [21]. Further investigations are needed to elucidate the utility of circulatory HMGB1 as a predictive biomarker and molecular target for preventing postoperative AE-ILD in patients with lung cancer and ILD.

This study has several limitations. It was a single-institution study, and the number of patients with postoperative AE-ILD was relatively small. These may substantially impact the generalizability of the study findings. Multicenter studies with a larger sample size are needed to validate the predictive value and the optimal cut off levels of HMGB1.

Conclusions

Higher levels of circulatory HMGB1 are associated with postoperative AE-ILD in patients with lung cancer and ILD. The combination of HMGB1 and operative time might improve the predictive accuracy of this fatal complication. These data confirm that HMGB1 is associated with the molecular mechanism of acute lung injury superimposed on ILD by focusing on surgery as a trigger of AE.

Abbreviations

AE, acute exacerbation; ALAT, Latin American Thoracic Association; ATS, American Thoracic Society; BALF, bronchoalveolar lavage fluid; CI, confidence interval; CT, computed tomography; DLco, diffusing capacity for carbon monoxide; ERS, European Respiratory Society; HMGB1, high mobility group box 1; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; IQR, interquartile range; JRS, Japanese Respiratory Society; KL-6, Krebs von den Lungen-6; RAGE, receptor for advanced glycation end products; ROC, receiver operating characteristic; TLR, toll-like receptor; UIP, usual interstitial pneumonia; VC, vital capacity;

Declarations

Ethics approval and consent to participate; This study was approved by the Ethics Committee of Hiroshima University Hospital (Gen-38 and E-1707). All participants provided written informed consent for obtaining the blood sample before surgery, and an opt-out method was performed to measure HMGB1 serum levels by using the already gathered samples.

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Authors' contributions; KY and SN designed the study, performed the data analysis and interpretation, and wrote the manuscript. HI, SS, YH, TM, SM, TN, KF, HH, and NH designed and interpreted the data. AK, YH, TM, YT, YM, and MO collected samples and interpreted the data. All authors helped to draft the manuscript and approved the final manuscript.

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Figures

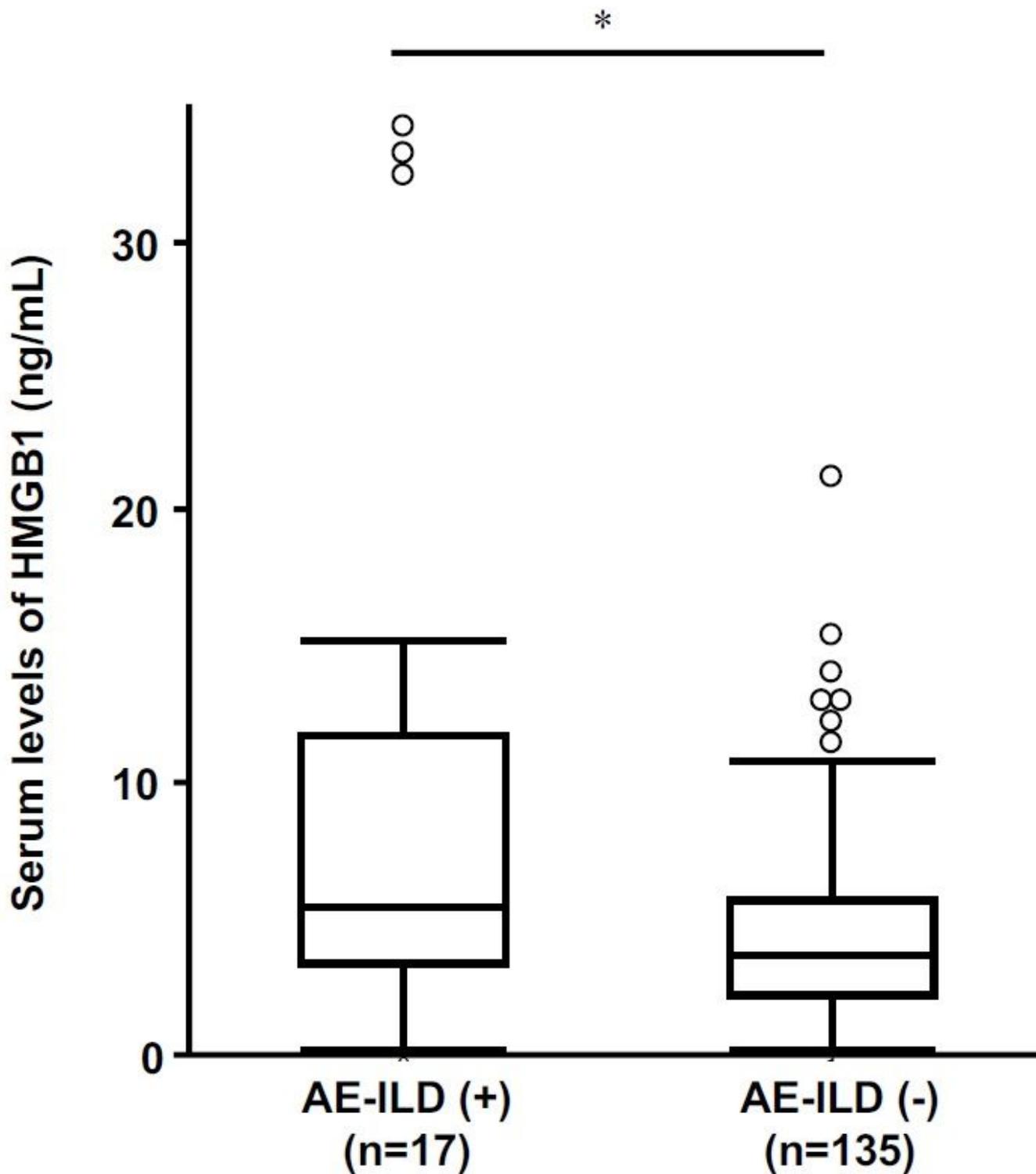


Figure 1

Baseline levels of serum HMGB1 Serum levels of HMGB1 in patients with postoperative acute exacerbation of interstitial lung disease (AE-ILD) were higher than those in patients without (median [IQR]: 5.39 [3.29-11.70] ng/mL vs. 3.55 [2.07-5.62] ng/mL, $P = 0.031$). 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. * $P < 0.05$ using the Mann-Whitney U test. IQR, interquartile range

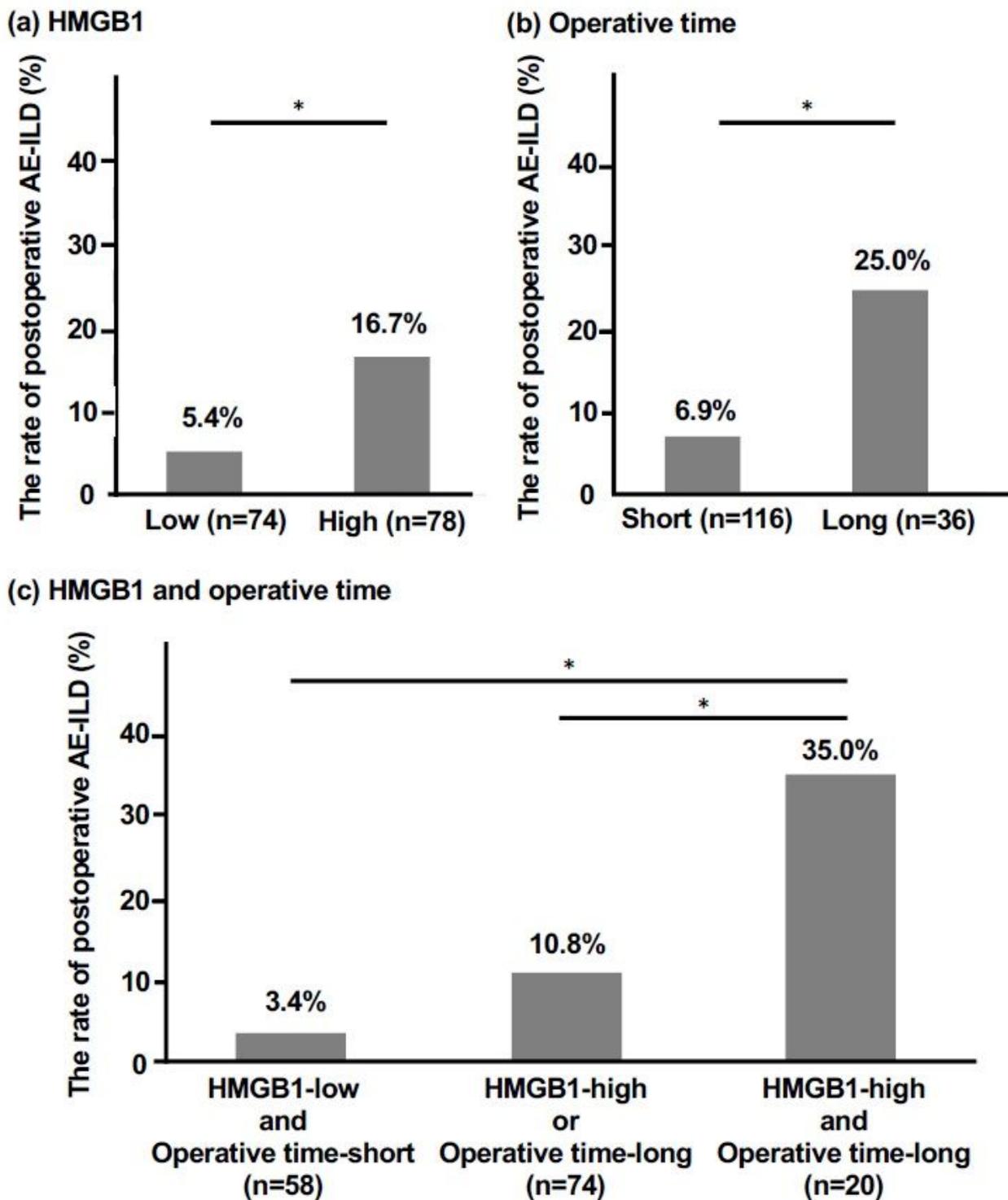
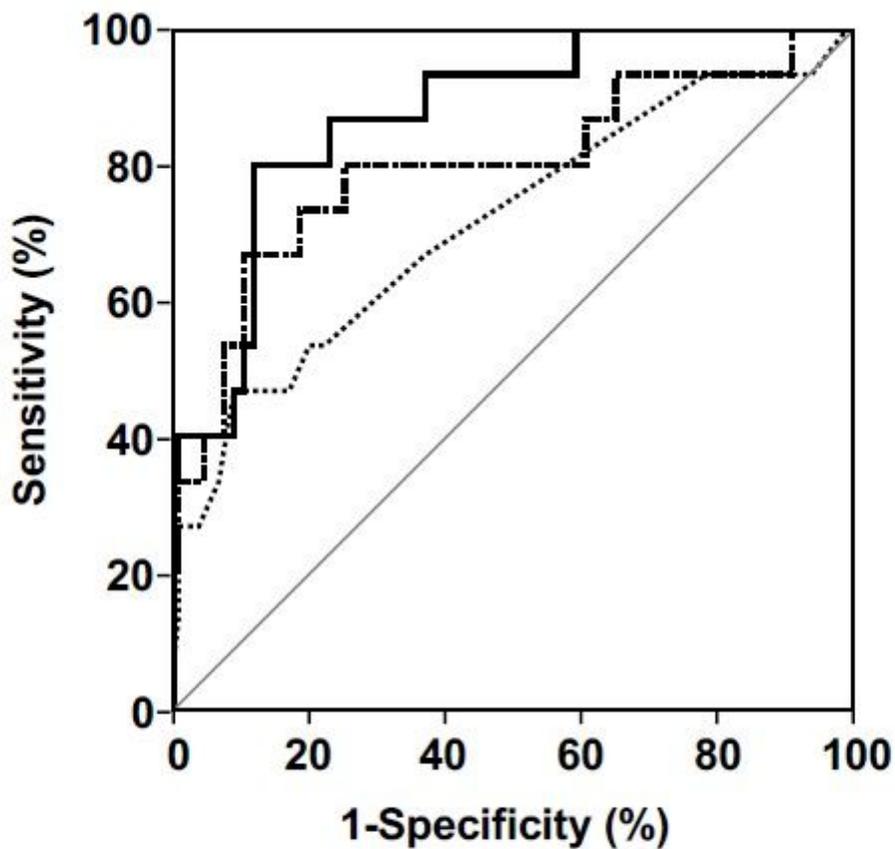


Figure 2

The incidence of postoperative AE-ILD based on HMGB1 and operative time. The cut-off levels of HMGB1 and operative time were 3.82 ng/mL and 200 min, respectively. The incidence of postoperative acute exacerbation of interstitial lung disease (AE-ILD) was significantly higher (a) in patients with HMGB1-high and (b) in those with operative time-long. Additionally, when HMGB1 and operative time were combined, (c) the incidence in patients both with HMGB1-high and operative time-long was statistically the highest.

compared to that in other two groups. * P < 0.05 using the Pearson's chi-squared tests with Bonferroni correction.



	C-index	P-value
— Covariates + HMGB1 + operative time	0.876	0.044
- - - Covariates + HMGB1	0.799	0.109
..... Covariates only	0.715	Ref.

Figure 3

C-statistics for predicting postoperative AE-ILD Concordance index (C-index) for predicting postoperative acute exacerbation of interstitial lung disease (AE-ILD) was significantly improved when HMGB1 and operative time were incorporated into the model with covariates calculated by previously reported risk scoring system as follows: 5 × (history of AE) + 4 × (surgical procedures) + 4 × (usual interstitial pneumonia appearance in computed tomography scan) + 3 × (male sex) + 3 × (preoperative steroid use) + 2 × (serum Krebs von den Lungen-6 level higher than 1000 U/mL) + 1 × (vital capacity lower than 80%).

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