Hemosiderin-Laden Macrophages in Bronchoalveolar Lavage: Predictive Role for Acute Exacerbation of Idiopathic Interstitial Pneumonias

Toru Arai (1), Tomoko Kagawa, Yumiko Sasaki, Reiko Sugawara, Chikatoshi Sugimoto, Kazunobu Tachibana, Yoshiki Fujita, Seiji Hayashi, and Yoshikazu Inoue

1 Clinical Research Center, National Hospital Organization Kinki-Chuo Chest Medical Center, Sakai City, Osaka, Japan
2 Department of Internal Medicine, National Hospital Organization Kinki-Chuo Chest Medical Center, Sakai City, Osaka, Japan
3 Department of Respiratory Medicine, Gifu Prefectural Tajimi Hospital, Tajimi City, Gifu, Japan
4 Department of Laboratory Medicine, National Hospital Organization Hyogo-Chuo National Hospital, Sanda City, Hyogo, Japan
5 Department of Internal Medicine, Aihara Dai-ni Hospital, Osaka City, Osaka, Japan

Correspondence should be addressed to Toru Arai; toarai1192296@gmail.com

Received 18 July 2021; Revised 20 November 2021; Accepted 4 December 2021; Published 20 December 2021

Academic Editor: Jian-sheng Li

Background. Hemosiderin-laden macrophages (HLMs) have been identified in the bronchoalveolar lavage fluid (BALF) of patients with idiopathic pulmonary fibrosis (IPF). This retrospective study examined the ability of HLMs in BALF to predict the acute exacerbation (AE) of chronic idiopathic interstitial pneumonias (IIPs).

Methods. Two hundred and twenty-one patients with IIP diagnosed by bronchoscopy were enrolled in the study (IPF, n = 87; IIPs other than IPF, n = 134). Giemsa stain was used to detect HLMs in BALF specimens. Prussian blue stain was used to quantify HLMs in BALF, and a hemosiderin score (HS) was given to the specimens containing HLMs.

Results. Twenty-four patients had a positive HS (range: 7–132). The receiver-operating characteristic curve analysis identified the cutoff HS value for predicting the AE of IIPs to be 61.5. Seven cases had a higher HS (≥61.5) and 214 had a lower HS. AE occurred significantly earlier in the higher HS group (4/7 cases) than in the lower HS group (41/214 cases) during a median observation period of 1239 days (log-rank test, p = 0.026). Multivariate Cox proportional hazard regression analysis showed that a higher HS was a significant predictor of AE in addition to IPF, percent predicted forced vital capacity, and modified Medical Research Council score. The C-statistics for the prediction of AE did not significantly improve by all the above parameters with HS as compared without HS.

Conclusions. A higher HS was a significant predictor of AE in IIPs but did not significantly improve the predictive ability of other parameters.

1 Introduction

Idiopathic pulmonary fibrosis (IPF) is a fibrotic lung disease with a usual interstitial pneumonia (UIP) pattern, a poor prognosis, and an unknown etiology [1, 2]. IPF is characterized by the progressive worsening of dyspnea and lung function, however, some patients with IPF experience rapid, and often, fatal deterioration [3–7]. These episodes, known as the acute exacerbations (AEs) of IPF (AE-IPF), are often of unclear etiology. The AEs of fibrotic lung disease were originally reported in IPF but have since been documented in various types of idiopathic interstitial pneumonias (IIPs) [8, 9]. Our group has recently described the frequency and prognosis of AE in IIPs (AE-IIPs) [9, 10].

Iron deposition in the lung can be caused by exogenous factors, including smoking [11] and dust exposure, and endogenous factors, such as occult hemorrhage [12]. The transformation of excess iron into hemosiderin by macrophages is a mechanism that attenuates iron-induced oxidative stress and its inflammatory and fibrogenic effects. Therefore, an increase in the hemosiderin-laden macrophage (HLM) level in bronchoalveolar lavage fluid (BALF) is assumed to reflect the excessive iron deposition in the lungs [12].
Diffuse alveolar hemorrhage can be diagnosed by the quantification of HLMs in BALF [13]. Golde et al. devised a hemosiderin score (HS) that is reportedly useful for the diagnosis of alveolar hemorrhage, whether idiopathic or infectious in origin [13]. In patients with acute respiratory distress syndrome (ARDS), which is characterized pathologically by diffuse alveolar damage, a higher HS at the time of diagnosis heralds a poor prognosis [14].

Several studies have identified a greater deposition of hemosiderin and higher HLM levels in BALF in the patients with IPF [12, 15] than in controls. Puxeddu et al. found that the HLM level in BALF was significantly higher in the patients with IPF than in controls and that a smoking history had no significant effect on the numbers of HLMs [12]. Furthermore, a significant correlation was found between the HLM levels in BALF and pulmonary hypertension (PH) detected by echocardiography [16] or right heart catheterization [17] in patients with IPF.

A higher modified Medical Research Council (mMRC) score for shortness of breath and a lower forced vital capacity (FVC) are the known predictors of AE-IPF [18] and AE-IIP [9]. The neutrophil counts in BALF are known to increase during AE-IPF [19], and the neutrophil level in BALF at the time of the diagnosis of IPF is a significant predictor of AE-IPF and AE-IIP [9]. Furthermore, the activation of macrophages is thought to be associated with AE-IPF [20]. In chronic obstructive pulmonary diseases, the percentage of positive HLMs in sputum was associated with the number of AEs in the previous 2 years [21]. Therefore, we hypothesized that HLMs in BALF would predict AE-IIPs.

The aim of this single-center observational study was to determine whether HLMs in BALF in the diagnosis of IIP can predict the subsequent AE and the ability of multivariate models with/without HLMs to predict AE-IIP.

2. Patients and Methods

A search of the National Hospital Organization Kinki-Chuo Chest Medical Center database of bronchoalveolar lavage (BAL) for 2005–2009 identified 231 consecutive cases of IIP with or without transbronchial lung biopsy. Bronchoscopy is performed at our institution when IIP is suspected, provided the patient can tolerate the pulmonary function tests. BAL was performed before treatment in all cases [9].

Two patients who had been found to have AE at the time of the initial diagnosis of IIP were excluded [9]. Eight further patients were excluded because their BALF specimens could not be evaluated by Prussian blue stain. Finally, 221 patients with IIPs (IPF, n = 87; non-IPF, n = 134) were enrolled (Figure 1). Most of these cases have been reported previously [9]. IPF was diagnosed according to the American Thoracic Society (ATS)/European Respiratory Society (ERS)/Japanese Respiratory Society (JRS)/Latin American Thoracic Association guideline [2]. IIPs were diagnosed according to the ATS/ERS statement [22]. IIPs other than IPF, including those diagnosed by surgical lung biopsy (SLB) [22] and those that were unclassifiable without SLB, were classified as non-IPF (Table 1). IIPs found histologically to be pleuroparenchymal fibroelastosis with UIP were diagnosed as IPF (n = 2). Four cases of IPF diagnosed from specimens obtained during surgery for lung cancer were included in the cases diagnosed by SLB.

High-resolution computed tomography scans were independently reviewed by the same chest radiologist who was blinded to the clinical findings. Based on the pattern observed, the cases were classified according to the IPF guidelines [2] as "UIP" (n = 59), "possible UIP" (n = 97), or "inconsistent with UIP" (n = 65).

The study protocol was approved by the Kinki-Chuo Chest Medical Center review board (approval number 463; date of approval, May 5, 2014). The requirement for informed consent was waived in view of the retrospective observational nature of the research and the anonymity of the data.

2.1. HLMs in BALF at the Time of Diagnosis of IIPs. BAL was performed using three 50-ml- aliquots of saline as described elsewhere [23]. Giemsa stain was used for the analysis of cells and detection of hemosiderin in BALF specimens. Hemosiderin-positive specimens were then stained with Prussian blue to quantify HLMs in BALF using a modified version of the HS described by Golde [13]. The hemosiderin content of 500 alveolar macrophages was graded 0–4, and the sum of grades was divided by 5, corresponding to the sum of 100 cells. Each slide was scored three times, with the average providing the modified HS. Cases that were hemosiderin-negative by Giemsa staining were given an HS of 0.

2.2. Diagnosis of AE-IIPs. AE-IIPs were diagnosed based on the following modified Japanese Respiratory Society criteria [24]: (i) within one month after the chronic clinical course of IIPs, the following three conditions are satisfied: (i) progressively worsening dyspnea, (ii) new ground-glass opacities evident on high-resolution computed tomography scans superimposed on a background reticular or honeycomb pattern, and (iii) a reduction in resting the partial pressure of oxygen in arterial blood (PaO₂) by more than...
10 Torr compared with previous measurements and (2) the obvious causes of acutely impaired respiratory function, such as infection, pneumothorax, cancer, pulmonary embolism, or congestive cardiac failure, are excluded.

2.3. Clinical Findings at Diagnosis. Demographic and clinical data, including age, sex, body mass index (BMI), smoking status, mMRC scores [25], pulmonary function tests, and serum markers at the time of diagnosis of the IIPs, were obtained from the medical records. The pulmonary function tests were performed using a Chestac 8080 device (CHEST M.I., INC., Tokyo, Japan).

2.4. Measurements of Serum Markers. Serum Krebs von den Lungen (KL)-6 and surfactant protein (SP)-D levels were measured using commercially available enzyme-linked immunosorbent assay kits (KL-6: Eizai, Tokyo, Japan; SP-D: Kyowa Medex, Tokyo, Japan). The KL-6 and SP-D cutoff levels were 500U/mL and 110ng/mL, respectively [26].

2.5. Histological Findings in SLB Specimens. SLB specimens with a higher HS, described in the following section, were evaluated for the presence of HLMs and vascular lesions, including capillary multiplication [27].

| Parameters                             | Lower HS (n = 214) | Higher HS (n = 7) | p value |
|----------------------------------------|-------------------|------------------|---------|
| Sex, male/female                       | 150/64            | 6/1              | 0.677   |
| Age, yrs                               | 69 (62–74)        | 62 (56–74)       | 0.018   |
| BMI, kg/m²                             | 24.0 (21.5–26.3)  | 28.0 (24.5–31.6) | 0.024   |
| Smoking, CS or ES/NS                   | 159/55            | 7/0              | 0.197   |
| Smoking CS/ES/NS                       | 39/120/55         | 1/6/0            | 0.319   |
| Iron dust exposure, yes/no             | 10/204            | 2/5              | 0.807   |
| Autoantibody*, yes/no                  | 26/188            | 0/7              | 1.000   |
| Diagnosis, IPF/non-IPF                 | 84/130            | 3/4              | 1.000   |
| HRCT, UIP/possible/inconsistent        | 56/96/62          | 3/1/3            | 0.291   |
| SLB, yes/no                            | 51/163            | 2/5              | 0.674   |
| SLB-diagnosed cases                    |                   |                  | 0.345   |
| IPF                                    | 43                | 1                |         |
| NSIP                                   | 7                 | 1                |         |
| LIP                                    | 1                 | 0                |         |
| mMRC, < 2/≥ 2                          | 137/77            | 6/1              | 0.426   |
| %FVC, %                                | 78.7 (64.6–93.9)  | 103.5 (70.5–115.2)| 0.115   |
| %DLco, %                               | 52.3 (35.6–67.2)  | 50.9 (39.6–78.9) | 0.766   |
| KL-6, ×100 U/mL                        | 8.95 (5.47–15.03) | 7.90 (3.81–9.52) | 0.271   |
| SP-D, ×10 ng/mL                        | 17.3 (9.8–26.6)   | 18.8 (14.6–24.9) | 0.643   |
| Neu in BAL, %                          | 2.2 (0.8–7.2)     | 2.40 (0.5–4.6)   | 0.835   |
| PT                                     | 1.01 (0.97–1.06)† | 0.98 (0.95–1.84)‡| 0.771   |
| APTT                                   | 28.0 (25.0–30.4)¹ | 27.8 (25.0–36.6)⁴| 0.478   |
| Fibrinogen                             | 308.3 (270.6–382.4)⁵ | 286.0 (267.5–383.7)⁴ | 0.845   |
| Prednisolone use before AE, yes/no     | 55/159            | 0/7              | 0.197   |
| Immunosuppressant before AE, yes/no    | 24/190            | 2/5              | 0.193   |
| Observation period, days               | 1214 (363–2028)   | 1284 (346–2144)  | 0.990   |
| Occurrence of AE, yes/no               | 41/173            | 4/3              | 0.033   |
| PaO₂/FiO₂ ratio at AE, ≤200/>200      | 25/16             | 2/2              | 1.000   |
| AE-occurred cases/IIP diagnosis        |                   |                  |         |
| IPF                                    | 26/84             | 2/3              |         |
| NSIP                                   | 7                 | 1/1              |         |
| LIP                                    | 0/1               | 0/0              |         |
| Non-IPF w.o. SLB                       | 15/122            | 1/3              |         |
| Median survival days after AE**        | 34                | 549              | 0.121   |

Abbreviations: AE, acute exacerbation; APTT, activated partial thromboplastin time; BAL, bronchoalveolar lavage; BMI, body mass index; CS, current smoker; DLco, diffusing capacity of carbon monoxide; ES, ex-smoker; FVC, forced vital capacity; HRCT, high-resolution computed tomography; HS, hemosiderin score; IIPs, idiopathic interstitial pneumonias; IPF, idiopathic pulmonary fibrosis; KL-6, Krebs von den Lungen-6; LIP, lymphocytic interstitial pneumonia; Neu, neutrophils; NS, nonsmoker; mMRC, modified Medical Research Council Score for shortness of breath; NSIP, nonspecific interstitial pneumonia; PT, prothrombin time; SLB, surgical lung biopsy; SP-D, surfactant protein-D; UIP, usual interstitial pneumonia; W, the pulmonary function tests were performed using a Chestac 8080 device (CHEST M.I., INC., Tokyo, Japan).
3. Results

3.1. Incidence of AE in IIPs. AE occurred in 45 cases (IPF, n = 28; non-IPF, n = 17) in 221 patients with IIPs (20.4%) during a median observation period of 1239 days. AE occurred in 14 (IPF, n = 13; nonspecific interstitial pneumonia (NSIP), n = 1) of the 53 cases diagnosed by SLB.

3.2. Distribution of Hemosiderin Score (HS). The HS were positive in 24 (10.9%) of the 221 IIP cases. The median HS in these cases was 40.5 (interquartile range, 19.0–64.5).

3.3. HS Cutoff Value That Predicted AE in HS-Positive Cases. The receiver-operating characteristic curve analysis identified the HS cutoff value that predicted AE to be 61.5. IIPs with an HS ≥ 61.5 were defined as the higher HS group (n = 7, 3.2%) and IIPs with an HS < 61.5 (n = 214, 96.8%) as a lower HS group.

3.4. Patient Demographics in the Lower and Higher HS Groups. The patient demographics were summarized in Table 1. BMI was significantly higher in the higher HS group than in the lower HS group. Other parameters at the time of diagnosis of IIPs and treatment for IIPs were similar between the two groups.

3.5. Relationship between HS and AEs in IIPs. The incidence of AE was significantly higher in the higher HS group (4/7 cases, 57.1%) than in the lower HS group (41/214 cases, 19.2%) by Fisher’s exact test (Table 1; p = 0.033). AEs also occurred significantly earlier in the higher HS group (p = 0.026, log-rank test; Figure 2). The severity of AE, indicated by the PaO2/FiO2 ratio and survival after AE, was similar between the higher and lower HS groups (Table 1; p = 1.000, Fisher’s exact test and p = 0.121, log-rank test, respectively).

3.6. Predictors of AE in IIPs

3.6.1. Cox Proportional Hazard Regression Analysis. The univariate analysis showed that a higher HS was a significant predictor of AE in addition to BMI, IPF, mMRC (≥ 2), % FVC, percent predicted diffusing capacity of carbon monoxide (%DLco), KL-6 level, and percentage of neutrophils in BALF (Table 2). The multivariate analysis of these parameters using the stepwise method revealed that a higher HS was a significant predictor of AE (Table 3).

3.6.2. Predictive Models of Occurrence of AE-IIPs. The values for the C-statistics using IPF, mMRC, %FVC with (model 2) or without (model 1) the HS were 0.7932 (95% confidence interval [CI] 0.7170–0.8351) and 0.7702 (95% CI 0.6877–0.8361), respectively. There was no significant difference in the value of the C-statistics between the two models using DeLong’s method (p = 0.1539; Table 4).

3.7. Histological Findings in SLB-Diagnosed Cases with a Higher HS. Two of the SLB-diagnosed IIP cases (IPF, n = 1; NSIP, n = 1) had a higher HS (≥ 61.5; Table 1). Only the patient with NSIP experienced AE and died 9 days later. The histological findings for the two cases were re-evaluated. The aggregation of HLMs in the peripheral air spaces was observed in the patient with IPF; however, there was no marked aggregation of HLMs in the peripheral air spaces. (Table 4).
capillary multiplication in this case. Both HLM aggregation and capillary multiplication were observed in the patient with NSIP but not always in close proximity (Figure 3). Stenosis of arterioles and venules was not observed in either case.

4. Discussion

This study is the first to investigate the ability of HLMs in BALF to predict AE-IIPs. In this study, multivariate Cox proportional hazard regression analysis identified a higher HLM level in BALF (HS ≥ 61.5) to be an independent predictor of AE-IIPs. However, the evaluation of the C-statistics showed that a higher HLM level in BALF could not improve the prediction of AE-IIPs and that its clinical impact was limited. There was no association between the severity of AE-IIPs and the HLM level in BALF or between the mortality and HLM level in BALF. The pathophysiology of HLMs and their relationship with AE-IIPs require further investigation.

Occult hemorrhage is an important endogenous cause of iron deposition in the lung and HLMs in the alveolar spaces and is associated with pulmonary hypertension [16, 17]. The Golde score was found to be significantly higher in patients with pulmonary veno-occlusive disease than in those with idiopathic pulmonary arterial hypertension [28]. Therefore, postcapillary vascular abnormalities are probably more important than arterial lesions in patients with occult alveolar hemorrhage.

The pathological examinations of vascular lesions in patients with an end-stage IPF who underwent lung transplantation found extensive vascular changes in the fibrotic areas in all cases but mainly in the muscular pulmonary arteries and arterioles [28]. Only mild changes were
observed in architecturally preserved areas in these vessels. The main findings in these areas were those of the occlusion of pulmonary venules, which has been associated with alveolar capillary multiplication [27]. There have also been reports of similar changes in the capillaries, including increased microvessel density [29] or alveolar septal capillary density [16]. Iron deposition was found in interstitial and alveolar macrophages in the lung parenchyma of patients with the occlusion of venules and small pulmonary veins. Therefore, postcapillary vascular lesions may be associated with alveolar hemorrhage and iron deposition in patients with IPF. However, in a study about the time course of HLMs by Sherman et al., HLMs were rarely observed in the lung biopsy specimens obtained twelve days after acute hemorrhage [30]. Therefore, HLMs could have been cleared from the lung during this time. Alveolar hemorrhage associated with postcapillary vascular lesions in IPF possibly occurred continuously from the standpoint of transient HLM presence in the lung [30].

Kim et al. evaluated the density of alveolar septal capillaries in the nonfibrotic areas of SLB specimens. Univariate analysis revealed that the right ventricular systolic pressure measured by echocardiography was significantly associated with the alveolar septal capillary density and histologically scored iron deposition [16]. Iron deposition alone was a significant predictor of the right ventricular systolic pressure by multivariate analysis. Multivariate analysis in a study by Fukihara et al. also found that the ratio of HLMs to total macrophages, an indicator of iron deposition, was a significant predictor of pulmonary vascular resistance [17]. Therefore, HLMs reflect the microhemorrhage and are significantly correlated with the severity of pulmonary hypertension.

It has been hypothesized that the increased microvascular density or alveolar capillary multiplication in less fibrotic areas may represent a compensatory response to hypovascularity in the areas of dense fibrosis, i.e., honeycomb areas [29]. Ebina et al. suggested that alveolar-capillary multiplication in less fibrotic areas is caused by vascular endothelial growth factor (VEGF) produced by hyperplastic type II alveolar epithelial cells [31]. Le Cras et al. demonstrated in a transgenic mouse model that the overexpression of VEGF induced the hyperpermeability of the alveolar-capillary endothelium, which resulted in microhemorrhages and HLMs in the lung [32]. Therefore, VEGF-induced hyperpermeability of the alveolar-capillary endothelium may cause microhemorrhages in patients with IIP in the absence of pulmonary hypertension.

In this study, pulmonary hypertension was not evaluated by the right heart catheterization at the time of the diagnosis of IIP. Although a negative correlation between the right ventricular systolic pressure and %DLco has been reported [33], there was no significant deterioration of %DLco in our higher HS group at the time of BALF sampling, unlike in the lower HS group. Therefore, our patients with a higher HS did not necessarily have pulmonary hypertension, and occult hemorrhages may have occurred independently. In some patients with a higher HS, the hyperpermeability caused by intra-alveolar VEGF may have been associated with the occurrence of HLMs and occult hemorrhage.

The reason for the increased frequency of AE in our patients with a higher HS is unclear. The generation of iron-dependent reactive oxygen species (ROS) is a key feature in iron overload fibrosing diseases and the experimental models of pulmonary fibrosis [34]. It results in damage to the epithelial and endothelial cells [35, 36], which leads to chronic pulmonary fibrosis. Moreover, the HS in BALF from the patients with IPF reportedly correlates with iron deposition in the alveolar spaces and ROS levels [15]. Recently, Lee et al. reported that murine alveolar macrophages cultured with iron molecules were converted to HLMs and generated more ROS [37].

AE-IPF and ARDS share many clinical and pathological features [7, 38]. Hence, the uncontrolled inflammation, activation of coagulation pathways, and altered permeability of the endothelial and epithelial alveolar barriers observed in ARDS [39] are also pathophysiologically important in AE-IPF and AE-IIPs. Furthermore, ROS generated by inflammation and various stimuli are associated with increased permeability in ARDS [40]. It has been reported that the blood levels of free radicals in AE-IPF are higher than those in stable IPF [41]. Thus, the generation of ROS might be more strongly augmented by various stimuli in IPF with a higher HS. Therefore, both ROS-induced epithelial and endothelial cell damage and hyperpermeability may lead to AE in patients with IIP and a higher HS.

Alveolar-capillary multiplication and increased local VEGF levels may be associated with AE. The proliferating capillary cells can be injured more easily by ROS and other stimuli. This type of alveolar injury induces inflammation and hyperpermeability along with the increased production of VEGF in the alveolar epithelial cells, whereby new radiological shadows appear on nonhoneycomb areas in the lung with IPF.

In our study, BMI was significantly higher in the higher HS group. Higher BMI is a risk factor for AE-IPF [18], AE-IIP [9], and ARDS [42]. Furthermore, significantly elevated serum VEGF levels have been identified in patients with metabolic syndrome [43], suggesting that a systemic
metabolic factor might induce VEGF, which is associated with a higher HS and increased likelihood of AE-IIPs. This study had several limitations. Firstly, it had a retrospective, single-center design. Secondly, the group with a higher HS was too small for the use of C-statistics to confirm that the HS improved our ability to predict AE-IIPs. However, the multivariate Cox proportional hazard regression analysis showed that a higher HS was an independent predictor of AE in patients with IIP. Patients with IIP and a higher HS require careful monitoring for an early diagnosis of AE. Thirdly, although we hypothesized that capillary multiplication would be associated with the presence of HLMs, we could not confirm this association in two patients with a higher HS in whom the diagnosis of IIP was made by SLB. Further studies are needed to confirm this association. The fourth point is that IPF was diagnosed according to the 2011 guidelines [2]. We have already published the prognosis and occurrence of AE for 2005–2009 for our patients with IIP [9], most of whom were included in the present study. Therefore, we did not reevaluate the diagnosis of IPF in these patients according to the 2018 guidelines [44].

5. Conclusions
A higher HS is a significant predictor of AE-IIPs but does not improve the ability of other parameters to predict AE. Nevertheless, patients with IIP who have a higher HS in BALF required careful monitoring to avoid AE and to be able to treat AE as early as possible after its onset.

Data Availability
The datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

Disclosure
A summary of this research was presented in the poster form at the 2017 American Thoracic Society International Conference held on May 19–24, 2017, in Washington, DC (Abstract: Am J Respir Crit Care Med 2017; 195: A1558).

Conflicts of Interest
TK, YS, RS, CS, KT, YF, and SH have no conflicts of interest. YI is an advisor to Boehringer Ingelheim, Shionogi Co. Ltd, and Asahi Kasei. TA has received lecture fee from Boehringer Ingelheim and Shionogi Co. Ltd. for activities not connected with the submitted work.

Authors’ Contributions
T.A. contributed to the study conception and design, data acquisition, radiologic findings, data analysis and interpretation, manuscript writing, and final approval of the manuscript. T.K., Y.S., and R.S. contributed to data acquisition, manuscript preparation, and final approval of the manuscript. Y.F. contributed to the cytology findings, manuscript preparation, and final approval of the manuscript. K.T., C.S., and S.H. contributed to the study design, data analysis and interpretation, manuscript preparation, and final approval of the manuscript. Y.I. contributed to study conception and design, data analysis and interpretation, manuscript preparation, and final approval of the manuscript.

Acknowledgments
This study was partially supported by a JSPS KAKENHI Grant no. JP17K09636 awarded to T.A., Y.I., and M.H., a grant from the National Hospital Organization: (H26-NHO (Kokyu)-1) awarded to Y.I. and T.A., (H28-NHO (Kokyu)-2) awarded to T.A., Y.I., and C.S., and AMED: DLD/14526278 and PAP/14526182 awarded to Y.I. and T.A., respectively. The authors are grateful to Dr. Masanori Akira, Dr. Masanori Kitaichi, and Dr. Takahiko Kasai for the assessment of radiological images, pathological evaluation of SLB specimens, and discussion regarding pathology, respectively. They also thank Dr. Shigeki Shimizu for pathological re-evaluation of SLB specimens in the higher HS group, Mr. Tomoaki Teramoto for preparing the BALF specimens, and Ms. Yuki Matsui for her secretarial work.

References
[1] T. E. King Jr., A. Pardo, and M. Selman, “Idiopathic pulmonary fibrosis,” The Lancet, vol. 378, no. 9807, pp. 1949–1961, 2011.
[2] G. Raghu, H. R. Collard, and J. J. Egan, “On behalf of the ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management,” American Journal of Respiratory and Critical Care Medicine, vol. 183, pp. 788–824, 2011.
[3] Y. Kondoh, H. Taniguchi, Y. Kawabata, T. Yokoi, K. Suzuki, and K. Takagi, “Acute exacerbation in idiopathic pulmonary fibrosis,” Chest, vol. 103, no. 6, pp. 1808–1812, 1993.
[4] M. Akira, H. Hamada, M. Sakatani, C. Kobayashi, M. Nishioka, and S. Yamamoto, “CT findings during phase of accelerated deterioration in patients with idiopathic pulmonary fibrosis,” American Journal of Roentgenology, vol. 168, no. 1, pp. 79–83, 1997.
[5] M. Akira, T. Kozuka, S. Yamamoto, and M. Sakatani, “Computed tomography findings in acute exacerbation of idiopathic pulmonary fibrosis,” American Journal of Respiratory and Critical Care Medicine, vol. 178, no. 4, pp. 372–378, 2008.
[6] H. R. Collard, B. B. Moore, K. R. Flaherty et al., “Acute exacerbations of idiopathic pulmonary fibrosis,” American Journal of Respiratory and Critical Care Medicine, vol. 176, no. 7, pp. 636–643, 2007.
[7] H. R. Collard, C. J. Ryerson, T. J. Corte et al., “Acute exacerbation of idiopathic pulmonary fibrosis. An international working group report,” American Journal of Respiratory and Critical Care Medicine, vol. 194, no. 3, pp. 265–275, 2016.
[8] A. Churg, N. L. Müller, C. I. S. Silva, and J. L. Wright, “Acute exacerbation (acute lung injury of unknown cause) in UIP and other forms of fibrotic interstitial pneumonias,” The American Journal of Surgical Pathology, vol. 31, no. 2, pp. 277–284, 2007.
[9] T. Arai, T. Kagawa, Y. Sasaki et al., “Heterogeneity of incidence and outcome of acute exacerbation in idiopathic
interstitial pneumonia,” Respirology, vol. 21, no. 8, pp. 1431–1437, 2016.
[10] T. Arai, K. Tachibana, C. Sugimoto et al., “High-dose prednisolone after intravenous methylprednisolone improves prognosis of acute exacerbation in idiopathic interstitial pneumonias,” Respirology, vol. 22, no. 7, pp. 1363–1370, 2017.
[11] A. B. Thompson, T. Bohling, A. Heires, J. Linder, and S. I. Rennard, “Lower respiratory tract iron burden is increased in association with cigarette smoking,” Journal of Laboratory and Clinical Medicine, vol. 117, no. 6, pp. 493–499, 1991.
[12] E. Puxeddu, A. Comandini, F. Cavalli et al., “Iron laden macrophages in idiopathic pulmonary fibrosis: the telltale of occult alveolar hemorrhage?” Pulmonary Pharmacology & Therapeutics, vol. 28, no. 1, pp. 35–40, 2014.
[13] D. W. Golde, W. L. Drew, H. Z. Klein, T. N. Finley, and M. J. Cline, “Occult pulmonary haemorrhage in leukaemia,” BMJ, vol. 26, no. 5964, pp. 166–168, 1975.
[14] F. Maldonado, J. G. Parambil, E. S. Yi, P. A. Decker, and J. H. Ryu, “Haemosiderin-laden macrophages in the bronchoalveolar lavage fluid of patients with diffuse alveolar damage,” European Respiratory Journal, vol. 33, no. 6, pp. 1361–1366, 2009.
[15] F. Sanguolo, E. Puxeddu, G. Pezzuto et al., “HFE gene variants and iron-induced oxygen radical generation in idiopathic pulmonary fibrosis,” European Respiratory Journal, vol. 45, no. 2, pp. 483–490, 2015.
[16] K.-H. Kim, F. Maldonado, J. H. Ryu et al., “Iron deposition and increased alveolar septal capillary density in nonfibrotic lung tissue are associated with pulmonary hypertension in idiopathic pulmonary fibrosis,” Respiratory Research, vol. 11, no. 1, p. 37, 2010.
[17] J. Fukihara, H. Taniguchi, M. Ando et al., “Haemosiderin-laden macrophages are an independent factor correlated with pulmonary vascular resistance in idiopathic pulmonary fibrosis: a case control study,” BMC Pulmonary Medicine, vol. 17, no. 1, p. 30, 2017.
[18] H. Kondoh, H. Taniguchi, and T. Katsuta, “Risk of acute exacerbation of idiopathic pulmonary fibrosis,” Sarcoidosis Vasculitis and Diffuse Lung Diseases, vol. 27, pp. 103–110, 2010.
[19] B. W. Kinder, K. K. Brown, M. I. Schwarz, J. H. Ix, A. Kervitsky, and T. E. King, “Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis,” Chest, vol. 133, no. 1, pp. 226–232, 2008.
[20] J. C. Schupp, H. Binder, B. Jäger et al., “Macrophage activation in acute exacerbation of idiopathic pulmonary fibrosis,” PLoS One, vol. 10, no. 1, Article ID e0116775, 2015.
[21] S. Mohan, T. Ho, M. Kjarsgaard et al., “Hemosiderin in sputum macrophages may predict infective exacerbations of chronic obstructive pulmonary disease: a retrospective observational study,” BMC Pulmonary Medicine, vol. 17, no. 1, p. 60, 2017.
[22] W. D. Travis, U. Costabel, D. M. Hansell et al., “An official American thoracic society/European respiratory society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias,” American Journal of Respiratory and Critical Care Medicine, vol. 188, no. 6, pp. 733–748, 2013.
[23] K. C. Meyer, G. Raghu, R. P. Baughman et al., “An official American thoracic society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease,” American Journal of Respiratory and Critical Care Medicine, vol. 185, no. 9, pp. 1004–1014, 2012.
[24] T. Arai, H. Kida, Y. Ogata et al., “Recombinant thrombomodulin for acute exacerbation in idiopathic interstitial pneumonias,” Respirology, vol. 24, no. 7, pp. 658–666, 2019.
[25] B. R. Celli, W. MacNee, A. Agusti et al., “Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper,” European Respiratory Journal, vol. 23, no. 6, pp. 932–946, 2004.
[26] Y. Inoue, B. C. Trapnell, R. Tazawa et al., “Characteristics of a large cohort of patients with autoimmune pulmonary alveolar proteinosis in Japan,” American Journal of Respiratory and Critical Care Medicine, vol. 177, no. 7, pp. 752–762, 2008.
[27] M. Colombat, H. Mal, O. Groussard et al., “Pulmonary vascular lesions in end-stage idiopathic pulmonary fibrosis: histopathologic study on lung explant specimens and correlations with pulmonary hemodynamics,” Human Pathology, vol. 38, no. 1, pp. 60–65, 2007.
[28] A. Rabiller, X. Jais, and A. Hamid, “Occult alveolar haemorrhage in pulmonary veno-occlusive disease,” European Respiratory Journal, vol. 27, no. 1, pp. 108–113, 2006.
[29] E. P. Judge, A. Fabre, H. I. Adamali, and J. J. Egan, “Acute exacerbations and pulmonary hypertension in advanced idiopathic pulmonary fibrosis,” European Respiratory Journal, vol. 40, no. 1, pp. 93–100, 2012.
[30] J. M. Sherman, G. Winnie, M. J. Thomassen, F. W. Abdul-Karim, and T. F. Boat, “Time course of hemosiderin production and clearance by human pulmonary macrophages,” Chest, vol. 86, no. 3, pp. 409–411, 1984.
[31] M. Ebina, M. ShimizuKawa, N. Shibata et al., “Heterogeneous increase in CD34-positive alveolar capillaries in idiopathic pulmonary fibrosis,” American Journal of Respiratory and Critical Care Medicine, vol. 169, no. 11, pp. 1203–1208, 2004.
[32] T. D. Le Cras, R. E. Spitzmiller, K. H. Albertine, J. M. Greenberg, J. A. Whitsett, and A. L. Akeson, “VEGF causes pulmonary hemorrhage, hemosiderosis, and air space enlargement in neonatal mice,” American Journal of Physiology-Lung Cellular and Molecular Physiology, vol. 287, no. 1, pp. L134–L142, 2004.
[33] S. D. Nathan, O. A. Shlobin, S. Ahmad, S. Urbanek, and S. D. Barnett, “Pulmonary hypertension and pulmonary function testing in idiopathic pulmonary fibrosis,” Chest, vol. 131, no. 3, pp. 657–663, 2007.
[34] P. Cheres, S. J. Kim, S. Tulasiram, and D. W. Kamp, “Oxidative stress and pulmonary fibrosis,” Biochimica et Biophysica Acta - Molecular Basis of Disease, vol. 1832, no. 7, pp. 1028–1040, 2013.
[35] A. Günther, M. Korfi, P. Mahavadi, D. von der Beck, C. Ruppert, and P. Markart, “Unravelling the progressive pathophysiology of idiopathic pulmonary fibrosis,” European Respiratory Review, vol. 21, no. 124, pp. 152–160, 2012.
[36] S. B. Wallach-Dayan, G. Izbicki, P. Y. Cohen, R. Gerstl-Golan, A. Fine, and R. Breuer, “Bleomycin initiates apoptosis of lung epithelial cells by ROS but not by Fas/FasL pathway,” Human Pathology, vol. 24, no. 7, pp. 658–666, 2007.
[40] M. Kellner, S. Noonepalle, Q. Lu, A. Srivastava, E. Zemskov, and S. M. Black, "ROS signaling in the pathogenesis of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS)," Advances in Experimental Medicine & Biology, vol. 967, pp. 105–137, 2017.

[41] Y. Matsuzawa, T. Kawashima, R. Kuwabara et al., "Change in serum marker of oxidative stress in the progression of idiopathic pulmonary fibrosis," Pulmonary Pharmacology & Therapeutics, vol. 32, pp. 1–6, 2015.

[42] G. Zhi, W. Xin, W. Ying, X. Guohong, and L. Shuying, "Obesity paradox in acute respiratory distress syndrome: a systematic review and meta-analysis," PLoS One, vol. 11, no. 9, Article ID e0163667, 2016.

[43] S. Jesmin, S. Akter, and M. M. Rahman, "Disruption of components of vascular endothelial growth factor angiogenic signaling system in metabolic syndrome. Findings from a study conducted in rural Bangladeshi women," Thrombosis & Haemostasis, vol. 109, pp. 696–705, 2013.

[44] G. Raghu, M. Remy-Jardin, J. L. Myers et al., "Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline," American Journal of Respiratory and Critical Care Medicine, vol. 198, no. 5, pp. e44–e68, 2018.