Predictors of Acute Exacerbation in Biopsy-proven Idiopathic Pulmonary Fibrosis

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Abstract

***Background*:** Acute exacerbation (AE) is a major cause of death in patients with idiopathic pulmonary fibrosis (IPF). Current evidence on AE-IPF was largely based on clinical, rather than pathological, analyses.

Methods: We investigated AE incidence and its predictors using clinical, radiological, and pathological data of patients diagnosed with IPF by multi-disciplinary discussion. This study, a secondary analysis of previous research, included 155 patients with IPF who underwent surgical lung biopsy (SLB). Cumulative AE incidence was evaluated by the Kaplan–Meier method. Predictors of AE-IPF were analyzed with a Fine-Gray subdistribution hazard model. Sub-analysis was performed using propensity score-matching analysis.

***Results*:** In this cohort, median age was 66 years and median percent-predicted forced vital capacity 82.8%. The cumulative AE incidence rates at 30-days and one-year post-SLB were 1.9% and 7.6%, respectively. On multivariable analysis, a lower percent-predicted diffusing capacity of the lung for carbon monoxide (%DL_{CO}) (hazard ratio 0.98 per 1% increase, $P = 0.02$) and fibroblastic foci (FF)-

present (vs. absent; hazard ratio 3.01, $P = 0.04$) were independently associated with higher incidence of AE. The propensity score-matching analysis with adjustment for age, gender, and %DL_{CO} revealed that the cumulative AE incidence rate was significantly higher in the FF-present subgroup than in the FF-absent subgroup (1-year incidence rate, 10.5% vs. 0%, respectively; $P = 0.04$ by Gray's test).

***Conclusions*:** FF and %DL_{CO} were independent predictors of AE in patients with biopsy-proven IPF. FF may be associated with the pathogenesis of AE-IPF.

Key words:

Acute exacerbation; diffusing capacity of the lung for carbon monoxide; fibroblastic foci; idiopathic pulmonary fibrosis

Short title:

Predictors of AE in biopsy-proven IPF

Abbreviations:

AE, acute exacerbation; CI, Confidence Interval; DL_{CO}, diffusing capacity of the lung for carbon monoxide; $FEV_{1.0}$, forced expiratory volume 1.0 (sec); FF, fibroblastic foci; FVC, forced vital capacity; GAP, Gender–Age–Physiology; HR, hazard ratio; HRCT, high-resolution computed tomography; IIP, idiopathic interstitial pneumonia; ILD, interstitial lung disease; IPF, idiopathic

pulmonary fibrosis; IQR, interquartile range; KL-6, Krebs von den Lungen-6; MDD, multi-disciplinary discussion; mMRC, modified Medical Research Council dyspnea scale; SLB, surgical lung biopsy; UIP, usual interstitial pneumonia.

1. Introduction

Idiopathic pulmonary fibrosis (IPF), histopathologically characterized by usual interstitial pneumonia (UIP) pattern, is a relentless interstitial lung disease (ILD) with a poor prognosis [1–6]. Some patients with IPF may present with slowly progressive respiratory failure but others may develop acute exacerbation (AE) unpredictably during the course of their chronic illness [7, 8]. AE is the most critical determinant of mortality in patients with IPF [9]. Reportedly, median survival after onset of AE is less than 3–4 months [9]. Therefore, delineation of the incidence and predictors of AE-IPF are major unmet needs for clinicians who attempt to prevent and manage IPF.

Prior work has reported risk factors that predict AE-IPF based on the analysis of clinical/physiological features, including low forced vital capacity (FVC) and low diffusing capacity of the lung for carbon monoxide (DL_{CO}) [9–11]; it remains unclear whether specific pathological features, including fibroblastic foci (FF) and honeycombing, are associated with AE-IPF. Recently, we have developed a cloud-based integrated database containing the clinical, radiological, pathological data, along with outcomes, for a large number of patients who had undergone surgical lung biopsy (SLB) and had been diagnosed with an idiopathic interstitial pneumonias (IIP) by multi-disciplinary discussion (MDD) [12]. We conducted secondary analyses using this database to investigate AE incidence and its predictors in patients with biopsy-proven IPF.

2. Patients and methods

80 **2.1. Study subjects**

81 We screened 465 patients with biopsy-proven IIP who had been registered in the cloud-based
82 integrated database [12]. The diagnosis/classification of an IIP was based on MDD using the clinical
83 and radiological data obtained within the three months prior to SLB and pathological data from SLB
84 (between April 2009 and March 2014). The study flow chart is presented in Figure 1.
85 Patients were included in the present study if they met a criteria of IPF based on the 2011
86 international guideline [4]. Patients were excluded from the study based on following criteria:
87 insufficient data (e.g. information regarding AE, pathological findings, treatment) or identification of
88 an evident cause of ILD after IPF diagnosis [e.g. a connective tissue disease (CTD), or an
89 inhalational/environmental exposure]. Consequently, 155 patients with biopsy-proven IPF were
90 enrolled in this retrospective study, which was approved by the institutional review boards of
91 Okinawa Chubu Hospital and Hamamatsu University School of Medicine (approval numbers 2018-
92 121 and 18-282, respectively).
93
94 **2.2. Definitions of AE**
95 AE was defined as events meeting all of the following criteria: 1) unexplained worsening or
96 development of dyspnea within the previous 30 days; 2) new bilateral ground-glass abnormality
97 and/or consolidation superimposed on a background reticular or honeycomb pattern on HRCT since
98 the preceding visit; and 3) exclusion of any known causes of acute worsening, including infection,

left heart failure, pulmonary embolism, and any identifiable cause of acute lung injury, in accordance with routine clinical practice and microbiologic studies [8, 13–14].

2.3. Data collection

Data pertaining to the following variables were collected from the cloud-based integrated database [12]: Clinical data, including age, gender, smoking history, %FVC, %DL_{CO}, serum Krebs von den Lungen-6 (KL-6) levels, arterial oxygen pressure (PaO_2), modified Medical Research Council dyspnea scale (mMRC), and gender-age-physiology (GAP) index [15]; high-resolution computed tomography (HRCT) data; pathological data; treatment details; information regarding AE; survival outcomes.

2.4. Review of HRCT patterns

Chest HRCT images obtained within the 3 months prior to SLB had been classified as UIP, possible UIP, and inconsistent with UIP patterns according to the 2011 international guideline [4] by expert chest radiologists at the previous study [12].

2.5. Review of pathological patterns and findings

In the current study, the number of patients with IPF whose SLB specimens were taken from a single lobe was 19 (12.3 %) out of 155 patients with IPF and remaining 136 (87.7 %) were sampled from two

or more lobes. SLB specimens had been evaluated according to the 2011 international guidelines [4] by expert lung pathologists during the previous study [12]. Pathological patterns were classified as definite UIP, probable UIP, and possible UIP. FF was defined as convex sub-epithelial foci of proliferating fibroblasts and myofibroblasts, typically existing among chronic fibrotic lesions. Honeycombing was diagnosed by a combination of chronic fibrosis with alveolar remodeling and variably sized cystic airspaces, often lined by bronchiolar epithelium and filled with mucus and inflammatory cells. The lung pathologists evaluated whether each of these two pathological findings was present or absent in any of SLB specimens from each patient.

2.6. Statistical analysis

Data were expressed as median with [interquartile range (IQR)] or frequency and percent (%). The Mann–Whitney U-test was used for comparing medians. Fisher's exact test was used for comparing proportions among groups. The period of observation was calculated from the date of SLB until the date of the last visit or death. AE-free survival was calculated from the date of SLB until the date of AE onset or until the last visit in patients who did not develop AE. Patients were censored if AE did not develop by October 2017, and death was treated as a competing event. Patient survival was evaluated by the Kaplan–Meier method. Cox proportional hazards analyses with time-dependent covariates were performed to identify prognostic factors associated with mortality. Age and gender were included in the model as potential prognostic factors, as well as any other variables with *P*-

values of < 0.10 in univariate analyses. A cumulative incidence curve for AE was constructed, treating death as a competing event. Subdistribution hazard analyses were performed, according to the method of Fine and Gray, to identify predictive factors associated with AE development. Age, gender, and all variables with *P*-values of < 0.10 in univariate analyses were included as potential predictors in the multivariable analyses. Propensity scores, which are predicted probabilities of each patient being assigned to either of two subgroups, were calculated using a logistic regression model that was adjusted for clinical variables. Propensity score matching was performed using the following algorithm: 1:1 nearest neighbour matching with a ± 0.05 caliper with no replacement. In all analyses, $P < 0.05$ was considered statistically significant. All data were analysed using commercially available software (JMP version 13.2.1, SAS Institute Inc., NC, USA) and R software version 2.15.1 (The R Foundation for Statistical Computing, Austria).

3. Results

3.1. Characteristics

Baseline characteristics of 155 patients with biopsy-proven IPF are summarized in Table 1.

Table 1. Baseline characteristics

	N=155
Age at SLB, years	66 (61–70)
Men / women	109 (70.3)/ 46 (29.7)
Smoking, current / ex / never	17 (11.0)/ 92 (59.4)/ 46 (29.6)
%FVC, %	82.8 (72.3–95.0)
%DLco *, %	66.8 (52.8–82.6)

KL–6, U/mL	1082 (714–1642)
PaO_2, Torr	85.4 (76.8–92.8)
mMRC §, grade 0/ 1/ 2/ 3/ 4	58 (40.3)/ 57 (39.6)/ 22 (15.3)/ 7 (4.9)/ 0 (0)
GAP stage †, Ⅰ / Ⅱ / Ⅲ	102 (76.1)/ 30 (22.4)/ 2 (1.5)
UIP pattern on HRCT, definite/ possible / inconsistent	27 (17.4)/ 120 (77.4)/ 8 (5.2)
Pathological UIP pattern, definite/ probable/ possible	43 (27.7)/ 99 (63.9)/ 13 (8.4)
Pathological finding	
FF, present/ absent	26 (16.8)/ 129 (83.2)
Honeycombing, present/ absent	51 (32.9)/ 104 (67.1)
Observation period, month	45.6 (18.6–59.5)
Death ¶	57 (36.8)
AE-free period, month	44.1 (16.8–57.3)
AE development	43 (27.7)
within 30 days from SLB	3 (1.9)
Treatment for IPF ‡	90 (58.0)
Corticosteroids	29 (18.7)
Anti-fibrotic	64 (41.3)
Pirfenidone	62
Nintedanib	3

Data are presented as n (%), median (interquartile range).

* n = 134; § n = 144; † n = 134; ¶ causes of death; Chronic respiratory failure, n = 24; AE, n = 18; lung cancer, n = 8; infection, n = 3; others, n = 4; ‡ prior to first AE in patients who developed AE, during observation period in those who did not develop AE.

SLB, surgical lung biopsy; %FVC, percent predicted forced vital capacity; $\%DL_{CO}$, percent predicted diffusing capacity of the lung carbon monoxide; KL-6, Krebs von den Lungen-6; PaO_2, arterial oxygen pressure; mMRC, modified Medical Research Council dyspnea scale; GAP, Gender–Age–Physiology index; HRCT, high-resolution computed tomography; UIP, usual interstitial pneumonia; FF, fibroblastic foci; AE, acute exacerbation; IPF, idiopathic pulmonary fibrosis.

The median age at the time of SLB was 66 years, and 70.3% of the patients were men. With respect to pulmonary function tests, the median %FVC and $\%DL_{CO}$ were 82.8% and 66.8%, respectively. Almost all of the patients were categorized as GAP Ⅰ (76.1%) or Ⅱ (22.4%).

Out of the 155 patients, 43 (27.7 %) developed AE after a median AE-free period of 44.1 months

and 3 (1.9 %) developed within 30 days after SLB. The one-year cumulative AE incidence rate was 7.6% (Figure 2A). Among 155 patients, 57 (36.8 %) died during a median observation period of 45.6 months. Twenty-four deaths occurred due to chronic respiratory failure, eighteen were attributed to AE, eight were attributed to lung cancer that developed after IPF diagnosis, and seven were attributed to other causes. The 5-year cumulative survival rate was 56.9% (Figure 2B).

3.2. Predictors of AE

The results of sub-distribution hazards analysis of AE development are presented in Table 2.

Table 2. Results of Fine-Gray sub-distribution hazards analysis for AE development

	HR	95% CI	*P*-value
Univariate			
Age at SLB, years	1.02	0.98–1.06	0.30
Men (vs. women)	0.88	0.47–1.67	0.70
Smoking, current or ex (vs. never)	1.08	0.56–2.09	0.81
%FVC, per 1% increase	0.99	0.98–1.01	0.40
%DL_{CO} †, per 1% increase	0.98	0.97–0.99	0.02**
KL–6, per 100 U/mL increase	1.00	0.99–1.02	0.85
PaO_2, per 1 Torr increase	1.00	0.98–1.03	0.82
mMRC §, grade 2/3/4 (vs. grade 0/1)	1.61	0.77–3.37	0.21
UIP pattern on HRCT, definite (vs. possible/inconsistent)	1.60	0.80–3.20	0.19
Pathological UIP pattern, definite (vs. probable/possible)	0.55	0.25–1.19	0.13
Pathological finding			
FF-present (vs. absent)	2.32	0.89–6.04	0.08*
Honeycombing-present (vs. absent)	1.58	0.79–3.13	0.20
Treatment for IPF ¶			
Corticosteroids, yes (vs. no)	0.94	0.45–1.93	0.86
Anti-fibrotic treatment, yes (vs. no)	0.98	0.54–1.77	0.95
Multivariate			
Age, years	1.01	0.97–1.05	0.65
Men (vs. women)	1.24	0.58–2.64	0.58

%DLCO, per 1% increase	0.98	0.97–0.99	0.02**
FF-present (vs. absent)	3.01	1.06–8.55	0.04**

*$P < 0.10$ (potential predictor), ** $P < 0.05$, † n = 134, § n = 144; ¶ prior to first AE in patients who developed AE or during observation period in those who did not develop AE.
AE, acute exacerbation; HR, hazard ratio; CI, confidence interval; SLB, surgical lung biopsy; %FVC, percent predicted forced vital capacity; %DL_{CO}, percent predicted diffusing capacity of the lung carbon monoxide; KL-6, Krebs von den Lungen-6; PaO_2, arterial oxygen pressure; mMRC, modified Medical Research Council dyspnea scale; HRCT, high-resolution computed tomography; UIP, usual interstitial pneumonia; FF, fibroblastic foci; IPF, idiopathic pulmonary fibrosis.

In univariate analyses, presence of pathological FF was positively associated with AE development, and higher %DL_{CO} was negatively associated with AE. On multivariable analysis, lower %DL_{CO} [hazard ratio (HR) 0.98 per 1% increase, $P = 0.02$] and FF-present (vs. absent; HR 3.01, $P = 0.04$) remained independently associated with incidence of AE.

3.3. Subgroup-analysis based on FF-grade

The baseline characteristics of patients with biopsy-proven IPF disaggregated into the FF-present subgroup and the FF-absent subgroup are presented in Supplementary Table 1. The median PaO_2 level in the FF-present subgroup was significantly higher than that in the FF-absent subgroup (86.5 Torr vs. 83.4 Torr, respectively; $P = 0.04$). However, no significant between-group differences were observed with respect to other characteristics. Cumulative AE incidence in the FF-present subgroup was significantly higher than that in the FF-absent subgroup ($P = 0.048$; Figure 3A).

On the basis of propensity scores that were adjusted for age, gender, and %DL_{CO}, 19 matched pairs were made between the FF-present or FF-absent subgroups. The characteristics of the two matched subgroups were well-balanced (Supplementary Table 2). Cumulative AE incidence in the matched FF-present subgroup was significantly higher than that in the FF-absent subgroup ($P = 0.04$; Figure 3B).

3.4. Prognostic factors

The results of Cox proportional hazards analysis (with time-dependent covariates) of mortality are presented in Table 3.

Table 3. Results of Cox regression hazard analysis for mortality

	HR	95% CI	*P*-value
Univariate			
Age at SLB, years	1.05	1.02–1.09	<0.01**
Men (vs. women)	1.47	0.78–2.77	0.24
Smoking, current or ex (vs. never)	1.12	0.62–2.01	0.72
%FVC, per 1% increase	0.98	0.97–0.99	0.01**
%DL_{CO} †, per 1% increase	0.99	0.97–1.01	0.11
KL–6, per 100 U/mL increase	0.99	0.98–1.02	0.77
PaO_2, per 1 Torr increase	0.99	0.97–1.02	0.52
mMRC §, grade 2/3/4 (vs. grade 0/1)	1.56	0.84–2.93	0.16
UIP pattern on HRCT, definite (vs. possible/inconsistent)	2.48	1.42–4.33	<0.01**
Pathological UIP pattern, definite (vs. probable/possible)	0.66	0.35–1.24	0.19
Pathological finding			
FF-present (vs. absent)	2.20	0.93–5.08	0.07*
Honeycombing-present (vs. absent)	1.65	0.90–3.06	0.09*
Treatment for IPF ¶			
Corticosteroids, yes (vs. no)	1.10	0.56–2.14	0.78
Anti-fibrotic treatment, yes (vs. no)	0.95	0.55–1.65	0.86
AE development, yes (vs. no)	21.1	12.2–36.6	<0.01**

Multivariate			
Age at SLB, years	1.06	1.01–1.11	0.04**
Men (vs. women)	2.32	1.10–4.88	0.03**
%FVC, per 1% increase	0.97	0.95–0.99	<0.01**
UIP pattern on HRCT, definite (vs. possible/inconsistent)	1.69	0.90–3.16	0.09*
FF-present (vs. absent)	2.90	1.07–7.88	0.04**
Honeycombing-present (vs. absent)	1.49	0.73–3.07	0.28
AE development, yes (vs. no)	20.2	11.5–35.3	<0.01**

*$P < 0.10$ (potential prognostic factor), ** $P < 0.05$, † n = 134, § n = 144; ¶ prior to first AE in patients who developed AE or during observation period in those who did not develop AE.
HR, hazard ratio; CI, Confidence Interval; SLB, surgical lung biopsy; %FVC, percent predicted forced vital capacity; %DL_{CO}, percent predicted diffusing capacity of the lung carbon monoxide; KL-6, Krebs von den Lungen-6; PaO_2, arterial oxygen pressure; mMRC, modified Medical Research Council dyspnea scale; HRCT, high-resolution computed tomography; UIP, usual interstitial pneumonia; FF, fibroblastic foci; AE, acute exacerbation; IPF, idiopathic pulmonary fibrosis.

On univariate analysis, higher age, lower %FVC, UIP pattern on HRCT (vs. possible UIP pattern), FF-present (vs. absent), pathological honeycombing-present (vs. absent), and AE development had potential associations with mortality. On multivariable analysis, higher age (HR 1.06 per one-year increase, $P = 0.04$), men (vs. women; HR 2.32, $P = 0.03$), lower %FVC (HR 0.97 per 1% increase, $P < 0.01$), FF-present (vs. absent; HR 2.90, $P = 0.04$), and AE development (vs. no development; HR 20.2, $P < 0.01$) were independently associated with increased mortality.

4. Discussion

In this study, the 30-day and one-year cumulative incidences of AE post-SLB among patients with biopsy-proven IPF were 1.9% and 7.6%, respectively. Multivariable analyses revealed independent

associations of pathological FF with both AE and mortality. Lower %DL_{CO} was an independent physiological predictor of AE-IPF. To our knowledge, this is the first study that has identified predictive factors for AE on the basis of clinical, radiological, and pathological data, in a large cohort of patients with biopsy-proven IPF.

In IPF, the histopathologic features of the UIP pattern include patchy involvement of lung parenchyma by fibrosis, honeycombing, and FF [3, 4, 6]. Prior work has demonstrated that increased numbers of FF were associated with increased risks of lung function deterioration and mortality [16–18]. In this context, we hypothesized that FF might be associated with the risk of AE development. Our qualitative evaluation in this study was able to demonstrate that the presence of FF was independently associated with increased mortality in patients with biopsy-proven IPF. We further found that the presence or absence of FF in SLB specimens was an independent predictive factor of AE-IPF development, which is the major finding of this study. Development of AE had a strong negative association with survival among patients with biopsy-proven IPF in this study. Therefore, it is likely that the increased mortality risk in those who had FF is attributable to the higher AE incidence rate. However, in this study, most of patients with IPF (83.2%) had a pathological finding of FF-present. Whether the number/extent of FF is correlated with the risk of AE development remains unclear. Future studies are needed using more precise quantitative methods for evaluating the amount of FF (e.g., analytical imaging software, or the use of artificial intelligence to process histopathology).

Annual AE incidence rates in patients with IPF have been reported to be approximately 4% to 14%, with variability due to differences in the study populations (e.g., severity, ethnicity) and the definitions of AE [9–11, 13, 14]. Prior incidence rates were mainly derived from the cohorts of patients with clinical diagnoses of IPF who exhibited an UIP pattern on HRCT, rather than those with biopsy-proven IPF who did not. The present study included only biopsy-proven IPF cases, most of which exhibited "possible UIP" pattern on HRCT, and the annual AE incidence was comparable to those reported previously [9–11, 13, 14]. Interestingly, the sub-analyses, including propensity score-matching analysis, demonstrated that the one-year cumulative AE incidence rate of the FF-present subgroup was 9–10.5% while that of the FF-absent subgroup was extremely low (0%). This suggests that patients without FF may represent a phenotype that is at reduced risk for AE or may be ILD other than IPF. In this study, patients who developed a CTD after IPF diagnosis were excluded (Figure 1) and no significant difference was observed between the FF-present and FF-absent IPF subgroups with respect to CTD-associated autoantibody status (Supplementary Table 3). Therefore, it is unlikely that patients without FF are more closely associated with autoimmunity compared with those with FF. Prior in vitro evidence suggests that activated fibroblasts/myofibroblasts play a key role in fibrogenesis in IPF, and that increased amounts of FF reflect higher fibrotic activity [19]. Although the etiology of AE-IPF remains unclear, it is hypothesized that AE may be a subsequent acceleration of an underlying chronic fibrotic process caused by acute direct stress to the lung, including infection and surgical intervention [8, 9]. Considering the role of FF in the fibrogenesis,

the results of this study generate a hypothesis that FF is associated with the pathogenesis of AE-IPF as well. Further studies are needed to evaluate this hypothesis.

AE development is thought to be more common in patients with physiologically and functionally advanced stages of IPF [9, 20]. Indeed, studies have reported risk factors of AE-IPF, including low FVC, low DL_{CO}, low 6-minute-walk distance, and increased dyspnea [9, 14, 21–23]. However, in the present study, lower FVC was not independently associated with higher AE incidence. This discrepancy might have arisen from the unique population of this study, which included only patients with relatively earlier stages of IPF (the median %FVC > 80%), excluding those who could not have safely undergone SLB. On the other hand, the present study demonstrated that lower %DL_{CO} was an independent physiological predictor of AE-IPF, in addition to the pathological predictor of FF, suggesting that physicians should be more alert to the risk of AE development in patients who had lower %DL_{CO} and/or FF-present on lung biopsy. A recent clinical trial (INPULSIS) has demonstrated that nintedanib, an anti-fibrotic agent, may reduce the risk of AE-IPF [13]. The secondary analysis of INPULSIS also suggested that nintedanib therapy improved post-AE survival rates [24]. Further study is needed to determine whether earlier pharmacological intervention may be warranted for patients with IPF who have FF and reduced %DL_{CO}.

Although SLB is necessary for the accurate diagnosis of IPF in patients who are clinically suspected of having an IIP/ILD but do not exhibit UIP pattern on HRCT [4, 6], surgical intervention-triggered AE is associated with high post-operative mortality [9]. However, there have been few

studies on SLB-related AE incidence in patients with IPF. Park *et al*. in their study reported the post-SLB AE incidence in 140 patients diagnosed with IPF by SLB from 1990 to 2003, as 1.4% [25]. Our finding that 1.9% of patients with IPF developed AE within 30 days from SLB was very similar to the decade-old study by Park *et al*. The updated data from the present study will provide valuable information for attending physicians and patients, especially those with suspected IPF, when contemplating indications for SLB.

The present study had several limitations. Firstly, the retrospective design of the study renders it vulnerable to several biases. For instance, original data of patients with biopsy-proven IPF were collected from hospitals that participated in the previous multicenter research [12], which may have introduced an element of selection bias. Secondly, the diagnosis of IPF was based on the 2011 international guideline [4], but not the 2018 international guideline [6]. Thirdly, the diagnosis of AE-IPF was made by attending physicians in each hospital, but not based on central review. In addition, a revised definition and diagnostic criteria for AE-IPF was proposed in 2016 [9]. These factors may have influenced the study results. Fourthly, we could not evaluate the reproducibility of FF-grade scored by pathologists. Finally, the treatment regimens for IPF (e.g., induction of anti-fibrotic agent) were not standardized among institutions, which may have affected outcomes in our study population.

5. Conclusions

In conclusion, the present study indicated that, in patients with biopsy-proven IPF, presence of pathologic FF and lower %DL_{CO} are independent risk factors that may prove helpful in predicting AE development, which may facilitate risk stratification of IPF patients by their treating physicians. Also, this study suggested that FF may play a role in the pathogenesis of AE-IPF. A prospective study would be required to validate these hypotheses.

Author contributions:

*T.K. and *H.H. contributed equally to this work.

T.K. and H.H. designed the research; T.K., H.H., T.F., Y.N., N.E., H.S., M.K., and T.S. contributed to the acquisition or analysis of the data; T.K. and H.H. wrote the initial and final drafts of the manuscript; T.K., H.H., T.F., Y.N., N.E., H.S., M.K., and T.S. revised the drafts of the manuscript; and all authors approved the final version of the manuscript.

Guarantor statement:

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest:

The authors have no conflicts of interest.

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Supplementary Table 1. Comparison between FF-present and FF-absent subgroups

	FF-present, n = 129	FF-absent, n = 26	*P* value
Age at SLB, years	66 (62–70)	65 (60–69)	0.25
Men / women	92 (71.3)/ 37 (28.7)	17 (65.4)/ 9 (34.6)	0.64
Smoking, current or ex	93 (72.7)	16 (61.5)	0.34
%FVC, %	82.8 (73.4–94.4)	81.9 (64.6–96.7)	0.54
$\%DL_{CO}$ †, %	66.0 (53.7–81.8)	67.0 (47.2–84.9)	0.75
PaO_2, Torr	86.5 (77.8–93.4)	83.4 (71.3–87.4)	0.04*
GAP stage §, I / II / III	85 (75.2)/ 26 (23.0)/ 2 (1.8)	17 (81.0)/ 4 (19.0)/ 0 (0)	0.64
UIP pattern on HRCT, definite/ possible/ inconsistent	25 (19.4)/ 99 (76.7)/ 5 (3.9)	2 (7.7)/ 21 (80.8)/ 3 (11.5)	0.13
Observation period, month	45.7 (17.6–57.6)	54.0 (23.1–76.1)	0.15
Death	51 (39.5)	6 (23.1)	0.13
AE-free period, month	43.0 (16.5–55.1)	47.7 (17.5–70.4)	0.18
AE development	39 (30.2)	4 (15.4)	0.15
Treatment for IPF ¶	74 (57.4)	16 (61.5)	0.83
Corticosteroids	23 (17.8)	6 (23.1)	0.58
Anti-fibrotic	54 (41.9)	10 (38.5)	0.83
Pirfenidone	52	10	
Nintedanib	3	0	

Data are presented as n (%), median (interquartile range).

* $P < 0.05$, † n = 134; § n = 134; ¶ prior to first AE in patients who developed AE, during observation period in those who did not develop AE.

FF, fibroblastic foci; SLB, surgical lung biopsy; %FVC, percent predicted forced vital capacity; $\%DL_{CO}$, percent predicted diffusing capacity of the lung carbon monoxide; PaO_2, arterial oxygen pressure; GAP, Gender–Age–Physiology index; UIP, usual interstitial pneumonia; HRCT, high-resolution computed tomography; AE, acute exacerbation; IPF, idiopathic pulmonary fibrosis.

Supplementary Table 2. Comparison between propensity score-matched FF-present and FF-absent subgroups

	FF-present, n = 19	FF-absent, n = 19	*P* value
Age at SLB, years	62 (59–68)	64 (60–69)	0.67
Men / women	13 (68.4)	12 (63.2)	1.00
Smoking, current or ex	12 (63.2)	12 (63.2)	1.00
%FVC, %	83.6 (78.2–93.8)	80.4 (64.6–96.6)	0.41
%DL_{CO}, %	64.2 (55.5–81)	67.8 (47.3–85.7)	0.82
PaO_2, Torr	87.5 (80.5–100)	83.5 (73.6–87.2)	0.10
GAP stage, I / II / III	16 (84.2)/ 3 (15.8)/ 0 (0)	16 (84.2)/ 3 (15.8)/ 0 (0)	1.00
UIP pattern on HRCT, definite/ possible/ inconsistent	2 (10.5)/ 17 (89.5)/ 0(0)	1 (5.3)/ 16 (84.2)/ 2(10.5)	0.31
Observation period, month	43.0 (12.0–53.8)	50.2 (24.6–83.7)	0.08
Death	5 (26.3)	4 (21.1)	1.00
AE-free period, month	36.7 (12.0–53.8)	50.2 (24.6–81.4)	0.07
AE development	5 (26.3)	2 (10.5)	0.40
Treatment for IPF ¶	8 (42.1)	12 (63.2)	0.33
Corticosteroids	5 (26.3)	4 (21.1)	1.00
Anti-fibrotic	3 (15.8)	8 (42.1)	0.15
Pirfenidone	3	8	
Nintedanib	1	0	

Data are presented as n (%), median (interquartile range).

¶ prior to first AE in patients who developed AE, during observation period in those who did not develop AE.

FF, fibroblastic foci; SLB, surgical lung biopsy; %FVC, percent predicted forced vital capacity; %DL_{CO}, percent predicted diffusing capacity of the lung carbon monoxide; PaO_2, arterial oxygen pressure; GAP, Gender–Age–Physiology index; UIP, usual interstitial pneumonia; HRCT, high-resolution computed tomography; AE, acute exacerbation; IPF, idiopathic pulmonary fibrosis.

Supplementary Table 3. Frequency of autoantibody positivity

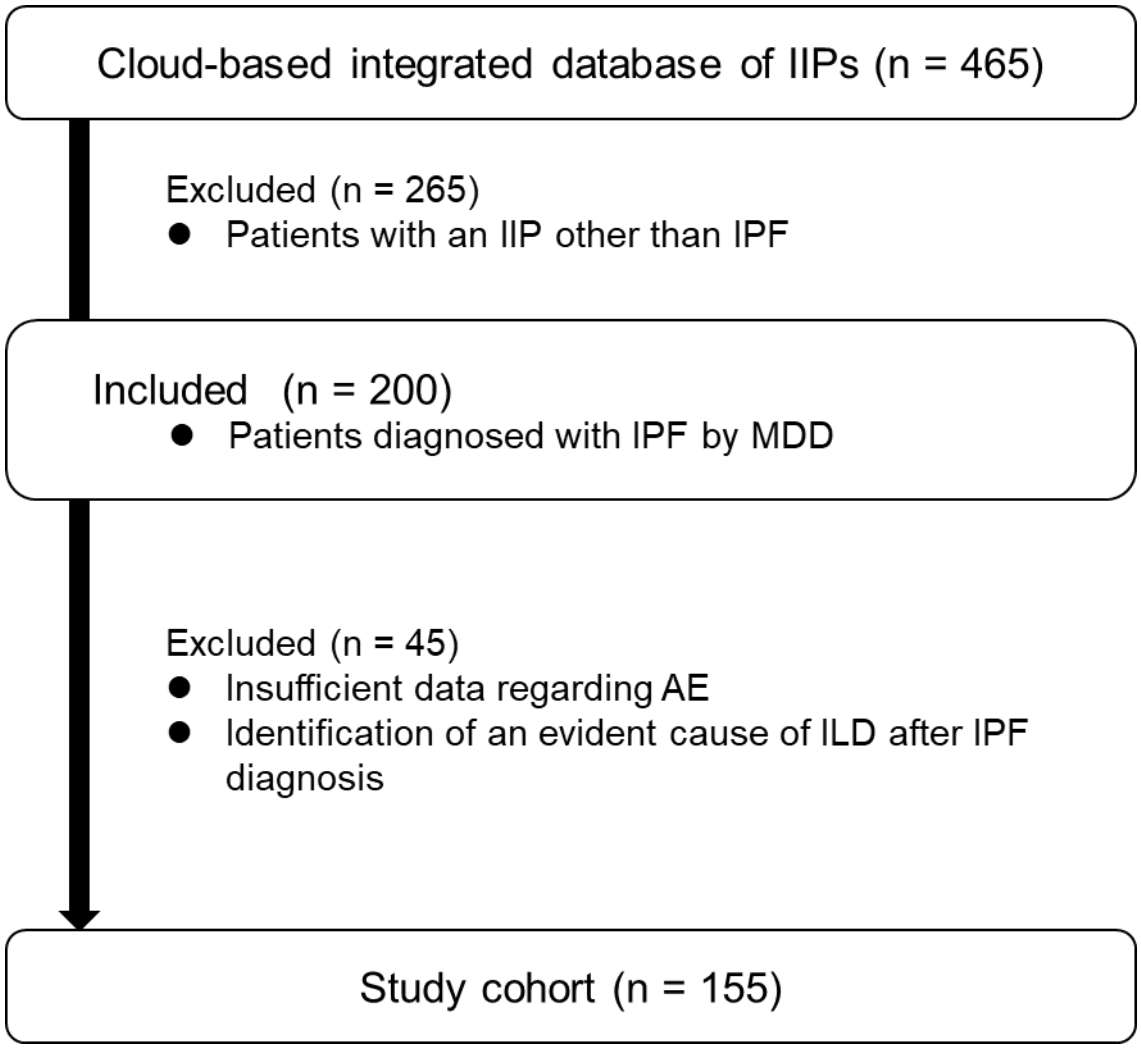
	FF-present subgroup		FF-absent subgroup		*P*-value
	ab (+)	ab (–)	ab (+)	ab (–)	
RF ≥ 2× upper limit of normal, n = 133*	7 (6.4)	103 (93.6)	1 (4.4)	22 (95.6)	1.00
ANA, n = 54*	7 (15.9)	37 (84.1)	4 (40.0)	6 (60.0)	0.19
≥1:320 titer	1 (2.3)	43 (97.7)	1 (10)	9 (90)	0.34
Centromere pattern	0 (0)	54 (100)	0 (0)	10 (100)	–
Nucleolar pattern	6 (13.6)	38 (86.4)	3 (30.0)	7 (70.0)	0.34
Anti-dsDNA, n = 87*	0 (0)	71 (100)	0 (0)	16 (100)	–
Anti-Sm, n = 88*	1 (1.4)	70 (98.6)	0 (0)	17 (100)	1.00
Anti-SSA/Ro n = 128*	1(0.9)	105 (99.1)	1 (4.6)	21 (95.5)	0.32
Anti-SSB/La, n = 120*	0 (0)	98 (100)	0 (0)	22 (100)	–
Anti-Scl-70, n = 133*	0 (0)	110 (100)	0 (0)	23 (100)	–
Anti-RNP, n = 119*	0 (0)	98 (100)	0 (0)	21 (100)	–
Anti-Jo-1, n = 134*	0 (0)	110 (100)	0 (0)	24 (100)	–
Anti-ARS, n = 14*	0 (0)	12 (0)	0 (0)	2 (100)	–
Anti-CCP, n = 104*	2 (2.3)	85 (97.7)	1 (5.9)	16 (94.1)	0.42
Anti-PM/Scl, n = 10*	0 (0)	9 (100)	0 (0)	1 (100)	–
Anti-MDA5, n = 5*	0 (0)	4 (100)	0 (0)	1 (100)	–
Any of autoantibodies, n = 146	15 (12.4)	106 (87.6)	6 (24.0)	19 (76.0)	0.21

Data are presented as n (%).

*the number of patients in whom a specific autoantibody was tested.

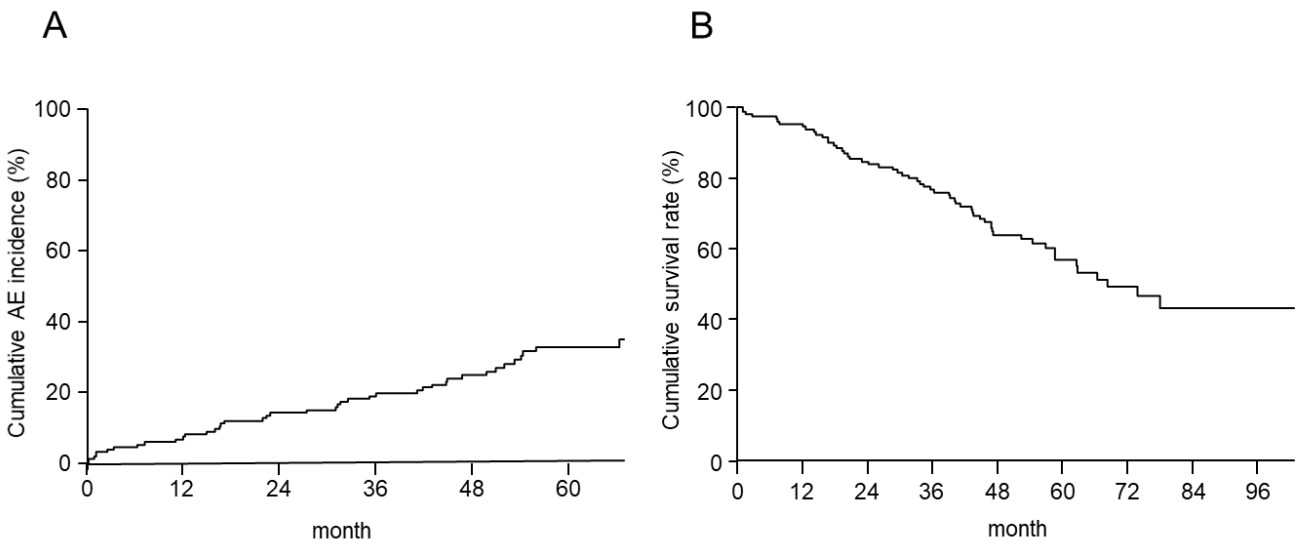
FF, fibroblastic foci; ab, autoantibody; RF, rheumatoid factor; ANA, anti-nuclear antibody; dsDNA, double stranded DNA; Sm, smith; RNP, ribonucleoprotein; ARS, aminoacyl tRNA synthetase; CCP, cyclic citrullinated peptide; PM/Scl, polymyositis/scleroderma; MDA5, melanoma differentiation-associated gene 5.

Figure 1. Study flow chart



IIPs, idiopathic interstitial pneumonias; IPF, idiopathic pulmonary fibrosis; MDD, multi-disciplinary discussion; AE, acute exacerbation; ILD, interstitial lung disease, UIP, usual interstitial pneumonia; HRCT, high-resolution computed tomography

Figure 2. Cumulative AE incidence (A) and survival (B) of patients with biopsy-proven IPF

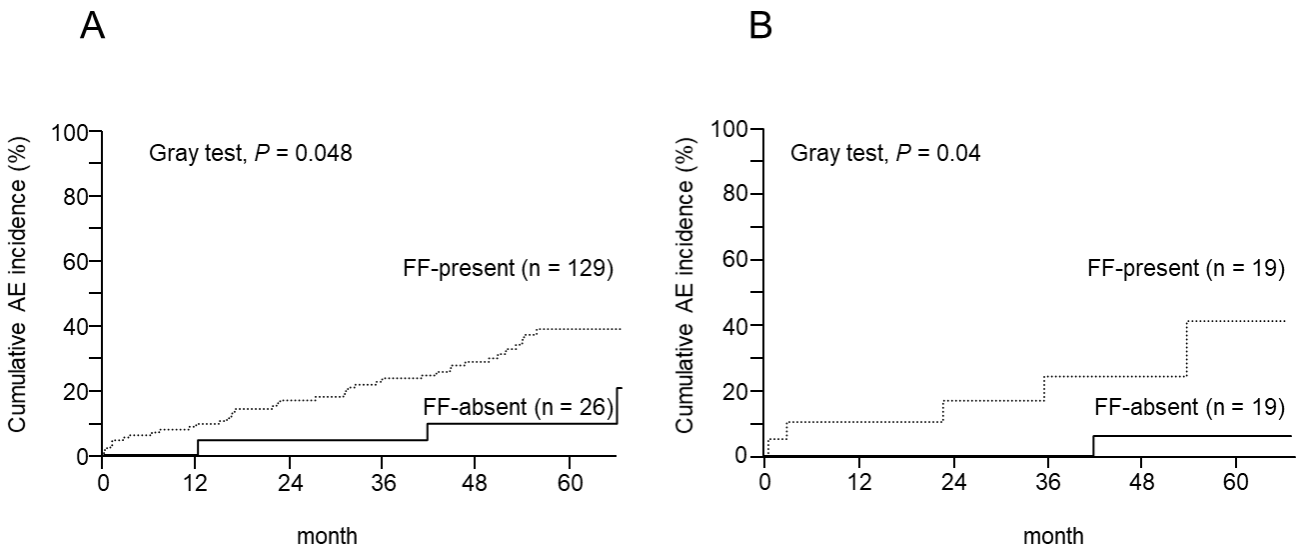


A: The 1-year cumulative AE incidence rate of the study cohort was 7.6%.

B: The 5-year cumulative survival rate of the study cohort was 56.9%

AE, acute exacerbation; IPF, idiopathic pulmonary fibrosis

Figure 3. Cumulative AE incidence rates according to FF-grade



A: Before propensity score-matching; the 1-year and 2-year cumulative AE incidence rates of the FF-present subgroup were 9.0% and 16.6%, respectively. The 1-year and 2-year cumulative AE incidence rates of the FF-absent subgroup were 0% and 4.5%, respectively.

B: After propensity score-matching; the 1-year and 2-year cumulative AE incidence rates of the FF-present subgroup were 10.5% and 16.9%, respectively. The 1-year and 2-year cumulative AE incidence rates of the FF-absent subgroup were 0% and 0%, respectively.

AE, acute exacerbation; FF, fibroblastic foci