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Chronic Hypersensitivity Pneumonitis with a Usual Interstitial Pneumonia (UIP)-like Pattern: Correlation between Histopathological and Clinical Findings

Running Head: Clinicopathological Findings of Chronic HP

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ABSTRACT

Background: Hypersensitivity pneumonitis (HP) is an interstitial lung disease caused by the inhalation of environmental antigens. The relationship between clinical, radiological, and histopathological findings of chronic HP remains unclear.

Methods: Sixteen patients with proven chronic bird-related HP with a UIP-like pattern were analyzed retrospectively. Histopathological findings were semi-quantitatively assessed and compared to clinical and radiological findings. We also evaluated the histopathological findings affecting prognosis.

Results: The extent of centrilobular fibrosis was negatively correlated with PaO_2 (r = -0.55, P = 0.03). The extent of bridging fibrosis was positively correlated with $\dot{V}_{50}/\dot{V}_{25}$ (r = 0.60, P = 0.02). Patients with a greater extent of fibroblastic foci (FF) had more radiologic reticulation (P = 0.01), honeycombing (P = 0.01), and traction bronchiectasis (P = 0.02), and had significantly shorter survival time (P = 0.01) than patients with a lesser extent of FF. Multivariate analysis showed that the extent of FF was a significant prognostic factor (hazard ratio, 2.36; 95 % confidence interval, 1.02 to 5.48; P = 0.04).

Conclusions: Our findings demonstrated that the extent of FF was significantly associated with reticulation, honeycombing, and traction bronchiectasis on HRCT. Moreover, the extent of FF could be a useful predictor of mortality in chronic HP with a UIP-like pattern.

Introduction

Hypersensitivity pneumonitis (HP) is a diffuse interstitial lung disease that results from exaggerated immune response to the inhalation of various organic or inorganic particles. HP has been traditionally classified into acute, subacute, and chronic forms.[1] Patients with chronic HP are categorized into two groups, recurrent or insidious, according to their clinical courses.[2] An epidemiological survey of chronic HP in Japan revealed that bird-related HP was the most prevalent form of HP, accounting for 60 % of 222 chronic HP cases.[3] Bird-related HP is caused by the inhalation of avian antigen. The amount of avian antigen in the environment can help establish the diagnosis as well as the clinical course.[4,5]

The histopathological patterns in chronic HP include organizing pneumonia, cellular non-specific interstitial pneumonia (NSIP), fibrotic NSIP, or usual interstitial pneumonia (UIP) applying the 2002 ATS/ERS criteria for the classification of idiopathic interstitial pneumonias.[6] Patients with a UIP-like pattern, who accounts for approximately half of chronic HP in Japan, presents with extensive lung fibrosis and has a poor prognosis.[6] Centrilobular fibrosis, bridging fibrosis, organizing pneumonia, bronchiolitis, granulomas, and giant cells have recently been identified as characteristic histopathological findings of chronic HP in addition to a UIP pattern.[7,8] However, the relationship between histopathological and clinical findings in chronic HP remains

incompletely understood. In this study we sought to determine the clinical implications of the histopathological findings in chronic HP with a UIP-like pattern.

Materials and Methods

Subjects

This study was conducted in accordance with the amended Declaration of Helsinki and approved by the Medical Research Ethics Committee of Tokyo Medical and Dental University (No. 1884). We were unable, however, to obtain the informed written consent of the study subjects. For adherence to the Ethical Guidelines for Medical and Health Research Involving Human Subjects, we publicly disclosed the details of the implementation of this study to ensure that the subjects had the opportunity to withdraw. Sixteen patients who had chronic HP and a UIP pattern on surgical lung biopsy between December ’94 and October ’07 were included. All had positive inhalation provocation tests with avian antigen. They also satisfied the following diagnostic criteria for chronic HP: (1) recurrence of HP symptoms triggered by an environmental stimulus or laboratory-controlled inhalation of antigen; (2) positive results for avian antibodies and/or a positive lymphocyte proliferation test when exposed to antigen; (3) histologic evidence of pulmonary fibrosis with or without granulomas; either (4) progressive deterioration of a restrictive impairment in pulmonary function for 1 year or (5)

97 persistent respiratory symptoms associated with HP for more than 6 months.[9] All cases
98 have been previously reported[10,11], however semi-quantitative assessment of
99 histopathological findings and detailed clinicopathological analysis focused on the cases
100 with a histologic UIP pattern have been performed for the first time in the current study.
101
102 *Study design*
103 Demographic findings, Krebs von den Lungen 6 (KL-6), surfactant protein D
104 (SP-D), partial pressure of oxygen in arterial blood (PaO_2), immunological findings,
105 bronchoalveolar lavage (BAL) fluid, pulmonary function tests (PFTs), and high
106 resolution computed tomography (HRCT) findings at diagnosis were extracted from the
107 medical records. Follow-up data on PFTs, treatment, acute exacerbation, and survival
108 were also obtained. The correlations between these clinical and histopathological
109 findings were evaluated.
110
111 *Immunological findings*
112 Antibodies to avian antigen in serum and BAL fluid were measured by an
113 enzyme-linked immunosorbent assay. The lymphocyte stimulation test to the avian
114 antigen was performed as previously reported.[12] Briefly, peripheral blood mononuclear
115 cells were cultured with pigeon sera and the incorporation of ^{3}H-thymidine was

116 estimated.

117

118 *BAL*

119 BAL was performed using three 50 mL aliquots of a sterile 0.9 % saline
120 solution. The cellular composition of the BAL fluid was determined using a cytospin
121 smear with Diff-Quik stain by counting 200 cells. Lymphocyte phenotypes were
122 analyzed by flow cytometry with monoclonal antibodies for CD4 and CD8.

123

124 *Pulmonary function tests*

125 The PFT data included vital capacity (VC), forced vital capacity (FVC), forced
126 expiratory volume in 1 second (FEV_1), FEV_1/FVC, diffusing capacity for carbon
127 monoxide (DLco), maximum expiratory flow at 25 and 50 % ($\dot{V}_{25}$ and $\dot{V}_{50}$), and $\dot{V}_{50}/\dot{V}_{25}$.
128 The results for VC, FVC, FEV_1, $\dot{V}_{25}$, $\dot{V}_{50}$, and DLco were expressed as percentages of
129 predicted values according to the age, gender, and height of each individual. The interval
130 of PFTs differed among the patients, so the degree of FVC decline (ΔFVC) was
131 calculated as an adjusted annual rate of change. Follow-up PFTs were unavailable in 2
132 patients.

133

134 *Histopathological findings*

The UIP-like pattern was defined according to the American Thoracic Society/European Respiratory Society (ATS/ERS/JRS/ALAT) consensus statement published in 2011[13]: briefly, a patchwork appearance of subpleural or paraseptal fibrosis alternating with normal alveoli, temporal heterogeneity, architectural distortion with or without honeycombing on the histological sections, and presence of fibroblastic foci. Although some cases had additional features such as granulomas, organizing pneumonia, or lymphocytic alveolitis that might be considered atypical for UIP as seen in IPF, the basic fibrotic pattern was that of UIP. The histological sections were stained with haematoxylin and eosin, elastica van Gieson, and Alcian Blue-periodic acid-Schiff. The semiquantitative evaluation was performed by one pathologist (T.A.) who had no knowledge of the clinical, physiological, or HRCT findings. Centrilobular fibrosis, bridging fibrosis, lymphoid follicles, and fibroblastic foci (FF) were counted in each slide, and the quantity per square centimeter was calculated. The presence and extent of perilobular fibrosis (PLF), bronchiolitis, organizing pneumonia, honeycombing, granuloma, giant cells, fibroelastosis, and lymphocytic alveolitis were scored as absent (0), rare (1), occasional (2), or marked (3) in each slide, and a mean score was taken. Specimens with strong degeneration precluding assessment of the pathological findings individually were excluded from our calculations of the quantities per square centimeter or the mean scores. Centrilobular fibrosis is defined as a peribronchiolar fibrosis with

bronchiolar distortion and occlusion by scarring and smooth muscle hyperplasia.[14] PLF means fibrosis in subpleural or paraseptal region.[14] Bridging fibrosis is defined as linear fibrotic connection between centrilobular and perilobular areas or between centrilobular and adjacent centrilobular areas.[14] Representative histopathological images are shown in Figure 1.

HRCT

HRCT images were obtained at end inspiration in the supine position. The scanning protocol consisted of reconstruction of 1- to 3-mm collimation sections with a high spatial frequency algorithm at 1- or 2-cm intervals. Images were photographed at window settings appropriate for viewing the lung parenchyma (window level, -600 to -700 Hounsfield units [HU]; window width, 1200-1500 HU). The three slice images at the levels of the aortic arch, carina, and inferior pulmonary vein were extracted, and the respective slices from the right and left lungs were reviewed independently by two pulmonary specialists (M.I. and T.O.) independently who had no knowledge of the patients' clinical information. The following findings were examined separately: reticulation, honeycombing, centrilobular nodules, ground glass opacity, consolidation, emphysema, traction bronchiectasis, mosaic attenuation, and interlobular septal thickening (Figure 2). Reticulation, honeycombing, centrilobular nodules, ground glass

opacity, consolidation, and emphysema were quantified as proportions of lung parenchyma between 0 % and 100 %, and censored at 5 %. If the estimations by the two observers differed by less than < 20 %, the mean value of the two estimations was taken; otherwise, a third observer (M.K., a chest radiologist) was brought in to resolve the discrepancy. Traction bronchiectasis, mosaic attenuation, and interlobular septal thickening were scored as absent (0) or present (1). Here too, discrepancies between the two observers were resolved by the third observer. The global score was calculated as the mean of the six zones. Additionally, the predominant distribution of the findings was determined as follows: upper predominance, lower predominance, or no zonal predominance.

Statistical analysis

Statistical analyses were performed using SPSS version 17.0 (IBM Corporation, Chicago, IL, USA) and R version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria). *P* values of less than 0.05 were considered significant. The Spearman rank-correlation test was used to analyze the relationship between histopathological and clinical findings. In some analyses, patients were divided into two groups by the median-split of their histopathological scores. The Mann-Whitney U test or Fisher's exact test was used for comparisons between two groups. Univariate and multivariate

Cox proportional hazard models were used to examine the relationship between histopathological findings and survival. Multivariate analysis was performed using variables with a univariate *P*-value < 0.2. The proportional hazards assumption was tested with log-minus log plots. Cumulative survival curves and acute exacerbation-free interval curves were constructed using the Kaplan–Meier method and comparisons between two groups were based on the log-rank test.

Results

Patient characteristics

The patient characteristics are shown in Table 1. Fourteen (88 %) of the patients were male with a mean age of 58.3 years. Twelve (75 %) of the patients were current or ex-smokers with a smoking history 44.3 ± 46.5 pack-years. None of the patients had been previously diagnosed with asthma. All of the patients presented with insidious course. Surgical lung biopsies were obtained from both the upper and lower lobes in 13 patients (81 %), from the upper and middle lobes in 2 patients (13 %), and from only the lower lobes in 1 patient (6 %). The mean interval between the onset of symptoms and the time of surgical biopsy was 34.4 ± 25.9 months. The follow-up duration ranged from 1 month to 8 years. All of the patients attempted to avoid further exposure to the antigen. Fourteen patients (88 %) were treated with corticosteroids.

Immunosuppressant medications were administered in conjunction with corticosteroids in 7 patients (44 %). Seven patients died from an acute exacerbation and one from sepsis during the follow-up period.

Clinical findings

The clinical findings are shown in Table 2. The mean values for %VC, %FVC, ΔFVC, %DLco, and $\dot{V}_{50}/\dot{V}_{25}$ were 77.0 %, 66.4 %, -151.5 mL/year, 60.4 %, and 4.0, respectively. The current and ex-smokers tended to have lower percentages of BAL lymphocytes (P = 0.06) and higher percentages of macrophages (P = 0.06), and had significantly lower $\%\dot{V}_{25}$ (P = 0.03; difference in location, -0.33; 95%CI, -0.67 to -0.17) than never smokers.

Histopathological findings

Each histopathological finding was semi-quantitatively assessed. Centrilobular fibrosis and bridging fibrosis were found in 14 and 11 patients (88 and 69 %, respectively). FF, lymphoid follicles, bronchiolitis, organizing pneumonia, granuloma, giant cells, PLF, honeycombing, fibroelastosis, and lymphocytic alveolitis were found in 100 %, 69 %, 44 %, 75 %, 25 %, 31 %, 81 %, 100 %, 44 %, and 81 % of the patients, respectively. The median value of FF was 2.2/cm^2. The extent of centrilobular fibrosis

230 was significantly associated with PLF (r = 0.59; 95%CI, 0.13 to 0.84; P = 0.02).

231

232 *Relationship between histopathological and clinical findings*

233 The Spearman-rank correlation test revealed a moderate correlation between

234 centrilobular fibrosis and PaO_2 (r = -0.55; 95%CI, -0.79 to -0.07; P = 0.03, Figure 3A)

235 and a moderate correlation between bridging fibrosis and $\dot{V}_{50}/\dot{V}_{25}$ (r = 0.60; 95%CI, 0.09

236 to 0.90; P = 0.02, Figure 3B). Compared to the low FF group ($< 2.2/cm^2$), the high FF

237 group ($\geq 2.2/cm^2$) had lower %DLco (P = 0.03; difference in location, -18.9; 95%CI,

238 -43.3 to -3.3) and higher incidence of acute exacerbation (P = 0.04; odds ratio, 21;

239 95%CI, 1.5 to 293.3) (Table 3). No other histologic finding was significantly correlated

240 with any clinical parameter.

241

242 *Relationship between histopathological and HRCT findings*

243 The two pulmonologists independently assessed each finding on HRCT.

244 Differences between observers were adjudicated by a third radiologist. The results are

245 summarized in Table 4. Analyzing the relationship between histopathological and HRCT

246 findings, we found that the high FF group presented with a greater extent of reticulation

247 (P = 0.01; difference in location, 13.1; 95%CI, 3.8 to 22.1; Figure 4A), honeycombing (P

248 = 0.01, difference in location, 7.1; 95%CI, 2.9 to 17.9; Figure 4B), and traction

bronchiectasis (P = 0.02, difference in location, 0.3; 95%CI, 0.2 to 0.7; Figure 4C). No other histologic finding was significantly correlated with any of the HRCT finding.

Acute exacerbation and survival

We examined the histopathological factors affecting prognosis. We checked that the log-minus log plots were approximately parallel for each variable to confirm the assumptions in the Cox proportional hazards model. A univariate analysis indicated that the extent of FF was a significant predictor of mortality (Table 5). In a multivariate analysis, we adjusted for age, sex, smoking status, and %DLco which was significantly associated with the extent of FF as shown in Table 3. The extent of FF was found to confer an elevated risk for mortality (hazard ratio, 2.36; 95 % CI, 1.02 to 5.48; P = 0.04, Table 5). The log-rank test showed that the extent of FF tended to be associated with acute exacerbation-free interval (P = 0.08, Figure 5A) and was significantly associated with survival time (P = 0.01, Figure 5B). Mean survival time for the low FF group was 92.7 months (95%CI: 84.1 to 101.2). The high FF group had a mean survival time of 34.6 months (95%CI: 17.5 to 51.7) and a median of 27.0 months (95%CI: 20.3 to 33.7).

Discussion

In this study we semiquantified the histopathological findings of chronic HP

268 with a UIP-like pattern and analyzed the relationship between the histopathological
269 findings and clinical and radiological findings. The extent of centrilobular fibrosis was
270 negatively correlated with PaO_2 and the extent of bridging fibrosis was positively
271 correlated with $\dot{V}_{50}/\dot{V}_{25}$. However, 95%CI for correlation coefficients were close to zero,
272 so further observations of a large sample will be required to confirm these results.
273 Patients with a greater extent of FF had more reticulation, honeycombing, and traction
274 bronchiectasis on HRCT, and they had a higher risk of mortality.

275 The pathogenesis of HP involves type III and IV allergic reactions in the small
276 airways and lung parenchyma[1] caused by a variety of antigens that are small enough to
277 reach the alveoli (< 2.5 μ m). Takemura et al. proposed that centrilobular fibrosis could
278 form around the bronchioles as a consequence of the deposition of inhaled antigens.[7]
279 Other studies have established that centrilobular fibrosis in chronic HP occurs in parallel
280 to fibrosis in the surrounding parenchyma[15] and that centrilobular and perilobular fibrosis
281 are often connected with each other via bridging fibrosis.[8] These earlier findings are
282 consistent with the correlations we identified between the extent of centrilobular fibrosis
283 and PLF in the present study.

284 Small airway obstruction in HP has been reported previously.[16] We showed that
285 there was a significant correlation between the extent of bridging fibrosis and $\dot{V}_{50}/\dot{V}_{25}$, even
286 though $\%\dot{V}_{25}$ was also significantly associated with smoking status. $\dot{V}_{50}/\dot{V}_{25}$ may be a

notable factor reflecting characteristic pattern of fibrosis in chronic HP, although we should take account of confounding factors such as cigarette smoking, asthma, or dust inhalation.[17]

Acute exacerbation can occur in chronic HP as well as IPF/UIP,[18] and this imparts a poor prognosis.[19] Our previous study of chronic HP showed that a low DLco, reduced lymphocyte percentages in BAL fluid and a histologic UIP pattern at the time of diagnosis were potential risk factors for acute exacerbation. Importantly, FF were present in the initial lung biopsy specimens of all patients who developed acute exacerbation.[11] FF are a confirmed prognostic parameter in IPF/UIP.[20] Honeycombing and traction bronchiectasis on HRCT have been reported to be predictors of mortality in chronic HP.[21] Recently, the relationship of the extent of FF with the severity of traction bronchiectasis and the extent of reticulation and honeycombing in fibrotic lung disease including chronic HP has been shown.[22] Our current investigation also demonstrated the significant relationship between the extent of FF and the extent of reticulation, honeycombing, and traction bronchiectasis on HRCT. Moreover, we identified the extent of FF as a potential risk factor for mortality in chronic HP. While DLco and the extent of FF were significantly correlated, our multivariate analysis suggested that the latter may be an independent prognostic factor.

Conclusions

In patients with chronic HP presenting with a histologic UIP pattern, the extent of FF in the lung is significantly related to the extent of reticulation, honeycombing, and traction bronchiectasis on HRCT. Furthermore, the extent of FF may be a marker of poor prognosis.

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Abbreviations

BAL	Bronchoalveolar lavage
DLco	Diffusing capacity for carbon monoxide
FEV_1	Forced expiratory volume in one second
FF	Fibroblastic foci
FVC	Forced vital capacity
HP	Hypersensitivity pneumonitis
HRCT	High resolution computed tomography
IPF	Idiopathic pulmonary fibrosis
PaO_2	Partial pressure of oxygen in arterial blood
PFTs	Pulmonary function tests

PLF	Perilobular fibrosis
UIP	Usual interstitial pneumonia
VC	Vital capacity
$\dot{V}_{25}$	Maximum expiratory flow at 25 %
$\dot{V}_{50}$	Maximum expiratory flow at 50 %

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416

Tables and figure legends

Table 1. Patients characteristics.

Case No.	Sex	Age at diagnosis (years)	Smoking, pack-years	Biopsy site	Treatment	Acute exacerbation	Follow-up (months)
1	M	55	Ex, 10	L	PSL, CsA	+	Died (59)
2	M	34	Ex, 19	U, L	PSL, TAC, PFD	+	Alive (98)
3	M	55	Never	U, L	None	–	Alive (1)
4	M	56	C, 36	U, L	None	–	Alive (3)
5	M	42	Ex, 5	U, L	PSL	–	Alive (74)
6	M	67	Ex, 52	U, L	PSL	+	Died (9)
7	M	62	Ex, 47	U, L	PSL, PFD	–	Alive (89)
8	M	75	Ex, 63	U, L	PSL, CsA	+	Died (16)
9	F	65	Never	U, L	PSL, CsA	–	Alive (14)
10	F	75	Never	U, L	PSL	–	Alive (67)
11	M	64	C, 16	U, M	PSL	+	Died (82)
12	M	65	Never	U, L	PSL	+	Died (28)
13	M	59	Ex, 27	U, L	PSL, CsA	+	Died (27)
14	M	53	Ex, 51	U, L	PSL, CsA	+	Died (22)
15	M	67	Ex, 180	U, M	PSL	–	Alive (98)
16	M	38	C, 25	U, L	PSL, CsA	+	Died (27)
16 cases	M 88 %	mean 58.3	C or Ex 75 %, 44.3 pack-years	U, L 81 %	PSL 88 %	56 %	Died 50 %

Acute exacerbation indicates the occurrence of exacerbation during the observation period. Percentage or mean values are shown at the bottom. Ex; ex-smoker, C; current smoker, U; upper lobe, M; middle lobe, L; lower lobe, PSL; prednisolone, CsA; cyclosporine A, TAC; tacrolimus, PFD; Pirfenidone.

Table 2. Clinical findings of the patients.

PaO_2, Torr	81.1 ± 11.1
KL-6, U/mL	1833 ± 1495
SP-D, ng/mL	286.1 ± 141.4
BAL fluid	
total cell counts, 10^5/mL	3.5 ± 1.6
macrophages, %	76.5 ± 16.7
lymphocytes, %	16.2 ± 14.4
neutrophils, %	5.6 ± 8.2
eosinophils, %	1.6 ± 3.5
CD4/CD8 ratio	4.9 ± 5.9
Positive of antibodies to avian antigen	9
Positive of LST	11
%VC	77.0 ± 18.3
%FVC	66.4 ± 18.8
ΔFVC, mL/year	-151.5 ± 362.2
$\%FEV_1$	73.8 ± 17.3
FEV_1/FVC, %	82.9 ± 8.9
$\%\dot{V}_{50}$	87.6 ± 26.4
$\%\dot{V}_{25}$	66.6 ± 40.5
$\dot{V}_{50}/\dot{V}_{25}$	4.0 ± 1.7
%DLco	60.4 ± 20.4

Values are given as the mean ± SD unless otherwise indicated. KL-6; Krebs von den lungen-6, SP-D; surfactant protein D, PaO_2; partial pressure of oxygen in arterial blood, BAL; bronchoalveolar lavage, LST; Lymphocyte stimulation test. VC; vital capacity, FVC; forced vital capacity, ΔFVC; the amount of annual change in FVC, FEV_1; forced expiratory volume in 1 second, $\dot{V}_{50}$; maximum expiratory flow at 50 %, $\dot{V}_{25}$; maximum expiratory flow at 25 %, DLco; diffusing capacity for carbon monoxide. The n of KL-6 and SP-D was 15. The n of CD4/CD8 ratio was 13. The n of ΔFVC was 14.

Table 3. Comparison of clinical findings between low and high fibroblastic foci group.

Clinical findings	Low FF group ($< 2.2/cm^2$) n = 8	High FF group ($\geq 2.2/cm^2$) n = 8	*P*	Difference in location (95% CI)	
PaO_2, Torr	80.4 ± 11.8	81.8 ± 11.1	0.83	1.6	(-12.6 to 14.7)
KL-6, U/mL	1436 ± 1324	2287 ± 1648	0.23	640	(-390 to 2450)
SP-D, ng/mL	222.4 ± 125.1	358.9 ± 129.6	0.07	119	(-31 to 297)
Lymphocytes in BAL fluid, %	18.9 ± 18.4	13.6 ± 9.5	0.88	-1.4	(-22.3 to 9.8)
Neutrophils in BAL fluid, %	3.4 ± 6.9	7.8 ± 9.3	0.34	0.7	(-0.8 to 18.1)
%FVC	68.2 ± 16.7	64.5 ± 21.7	0.72	-3.6	(-24.3 to 17.4)
%DLco	71.8 ± 20.8	49.0 ± 12.6	0.03	-18.9	(-43.3 to -3.3)
				Odds ratio (95% CI)	
Acute exacerbation, n (%)	2 (25)	7 (88)	0.04	21.0	(1.5 to 293.3)

Values are given as the mean ± SD unless otherwise indicated. FF; fibroblastic foci. KL-6; Krebs von den lungen-6. SP-D; surfactant protein D. BAL; bronchoalveolar lavage. FVC; forced vital capacity. DLco; diffusing capacity for carbon monoxide. CI; confidence interval. The n of KL-6 and SP-D in high FF group was 7.

459 Table 4. HRCT findings.

460

The extent of each finding[a] (%)	
Reticulation	14.5 ± 9.9
Honeycombing	5.9 ± 7.6
Centrilobular nodules	9.2 ± 17.0
Ground grass opacities	14.4 ± 6.4
Consolidation	3.5 ± 3.1
Emphysema	1.7 ± 3.7
The score of each finding[b]	
Traction bronchiectasis	0.6 ± 0.4
Mosaic attenuation	0.1 ± 0.1
Interlobular septal thickening	0.7 ± 0.3
Distribution of findings[c], n (%)	
Upper predominance	2 (12.5)
Lower predominance	8 (50.0)
No zonal predominance	6 (37.5)

461 a

462 The extent of each finding is presented as the mean ± SD of 16 cases.

463 b

464 The score of each finding is presented as the mean ± SD of scores (absent = 0 or present

465 = 1) in 16 cases.

466 c

467 Distribution of findings is presented as the number of cases.

468

469

470

Table 5. Survival analysis of histopathological findings.

Histopathological findings	Hazard ratio	95 % Confidence interval	*P*
Univariate analysis			
Centrilobular fibrosis	1.35	0.789 to 2.299	0.27
Bridging fibrosis	2.15	0.552 to 8.381	0.27
Fibroblastic foci	1.81	1.104 to 2.975	0.02
Lymphoid follicle	1.02	0.764 to 1.370	0.88
Bronchiolitis	0.45	0.142 to 1.429	0.18
Organizing pneumonia	2.0	0.728 to 5.463	0.18
Granulomas	1.67	0.369 to 7.588	0.51
Giant cells	0.59	0.148 to 2.359	0.46
Perilobular fibrosis	0.90	0.343 to 2.347	0.83
Honeycombing	1.72	0.540 to 5.467	0.36
Fibroelastosis	0.62	0.164 to 2.337	0.48
Lymphoid alveolitis	0.66	0.227 to 1.929	0.45
Multivariate analysis[a]			
Fibroblastic Foci	2.36	1.018 to 5.479	0.04

[a] Covariates with $P < 0.2$ in univariate analysis and clinical relevant covariates such as age, sex, smoking status, and %DLco were included in multivariate analysis (Cox regression).

Figure 1. Typical lesions in the histopathological examinations of the surgical lung biopsy specimens. A. (Case 2), centrilobular fibrosis (*) (elastica van Gieson, × 4) B. (Case 5), bridging fibrosis (arrows) connects respiratory bronchioles (RB) to each other or connects respiratory bronchioles with the perilobular area. Centrilobular fibrosis is frequently close to the interlobular septum (ILS). TB; terminal bronchioles. A; pulmonary arteries. (haematoxylin and eosin, × 1.5). C. (Case 8), fibroblastic foci (**) (haematoxylin and eosin, × 10). D. (Case 1), bronchiolitis (haematoxylin and eosin, × 10). Inflammatory cells infiltrate around the terminal bronchioles.

Figure 2. Typical HRCT images. A. (Case 12), reticulation. B. (Case 1), honeycombing. C. (Case 2), centrilobular nodules. D. (Case 14), ground grass opacity and mosaic attenuation. E. (Case 8), consolidation (arrow). F. (Case 15), emphysema. G. (Case 6), traction bronchiectasis. H. (Case 7), interlobular septal thickening (arrows).

Figure 3. Correlations between histopathological and clinical findings. CI; confidence interval. A. centrilobular fibrosis and PaO_2. B. bridging fibrosis and $\dot{V}_{50}/\dot{V}_{25}$.

Figure 4. The relationship between histopathological and HRCT findings. A. fibroblastic foci (FF) and reticulation. Difference in location, 13.1; 95% confidence interval (CI), 3.8 to 22.1. B. FF and honeycombing. Difference in location, 7.1; 95% CI, 2.9 to 17.9. C. FF and traction bronchiectasis. Difference in location, 0.3; 95% CI, 0.2 to 0.7.

Figure 5. Kaplan-Meier curves for acute exacerbation-free interval (A) and survival (B) in patients with more than 2.2 fibroblastic foci (FF) per square centimeter (high FF group) and patients with fewer than 2.2 FF per square centimeter (low FF group).

Figure 1

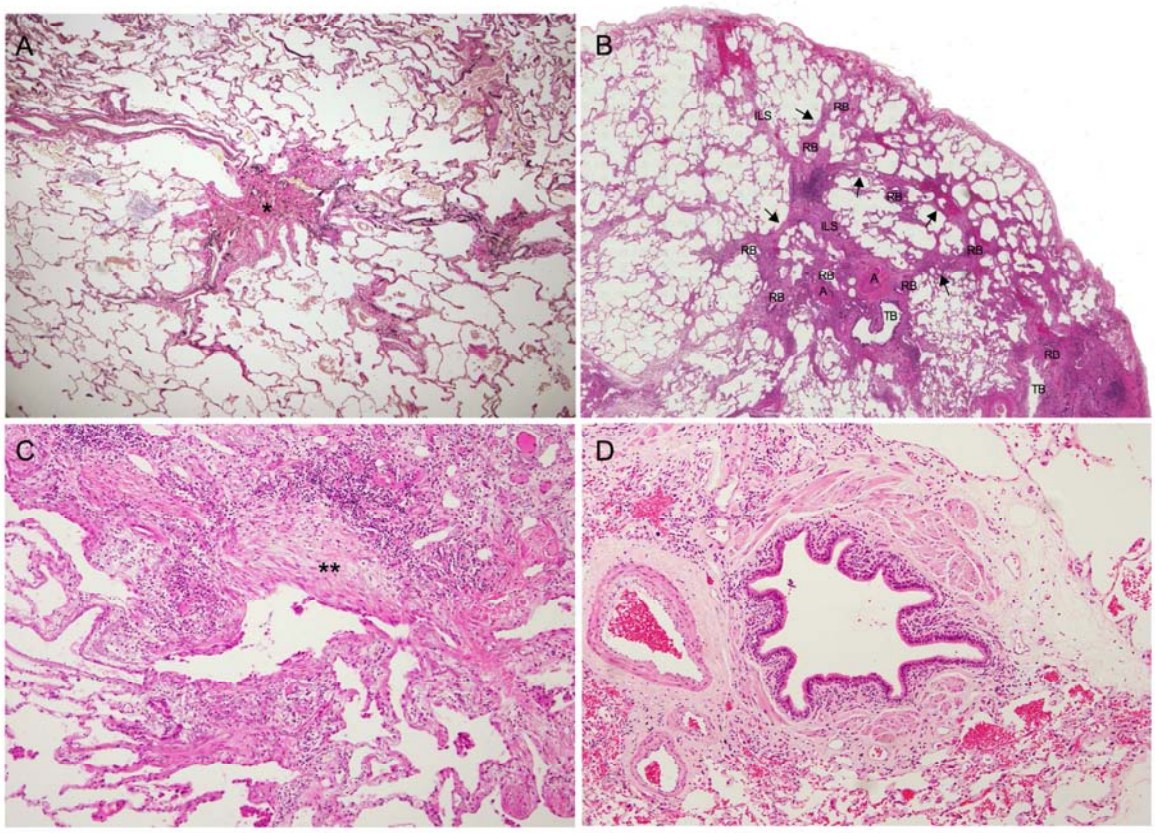


Figure 2

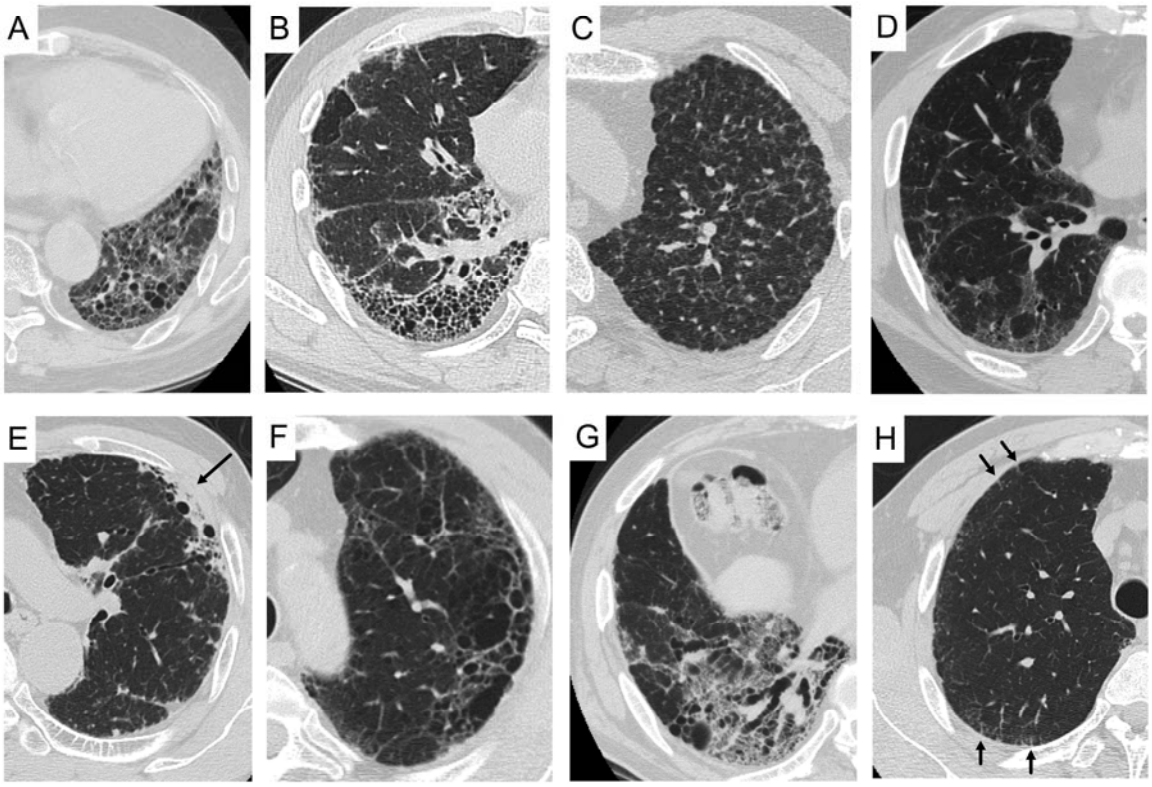


Figure 3

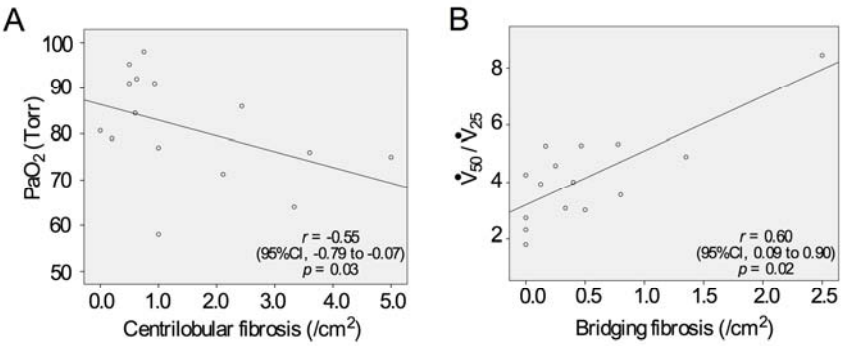


Figure 4

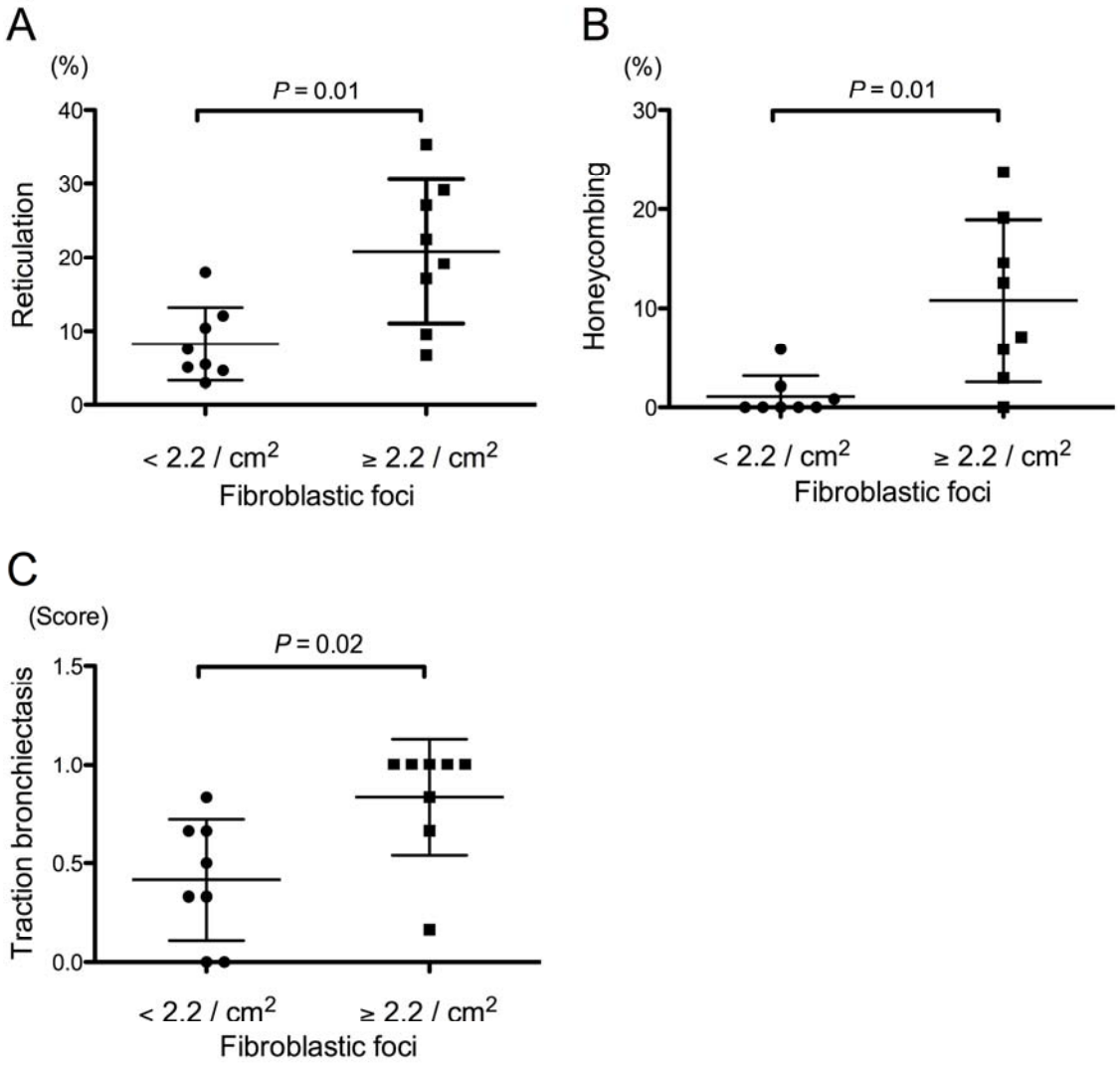
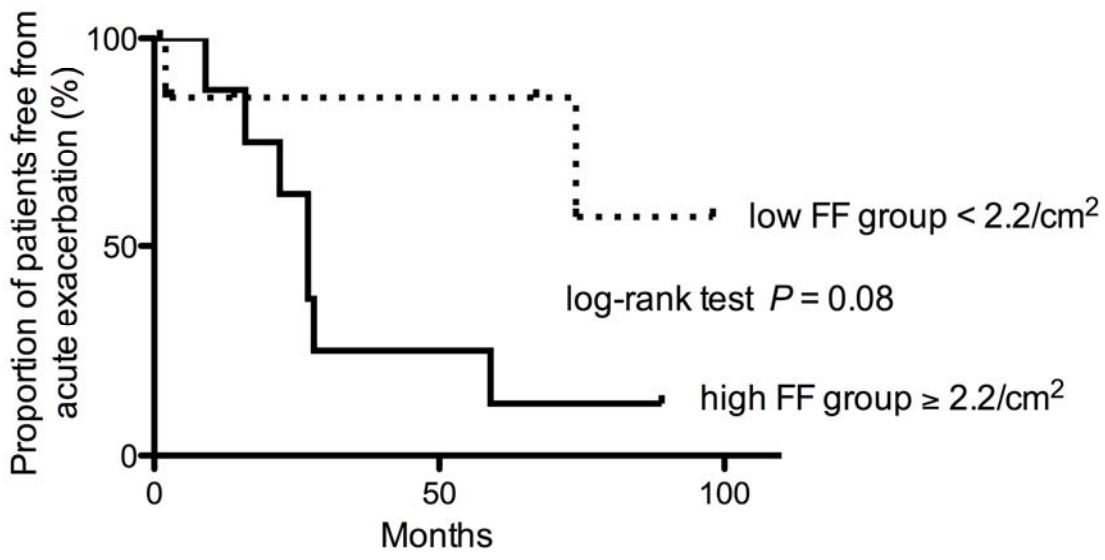
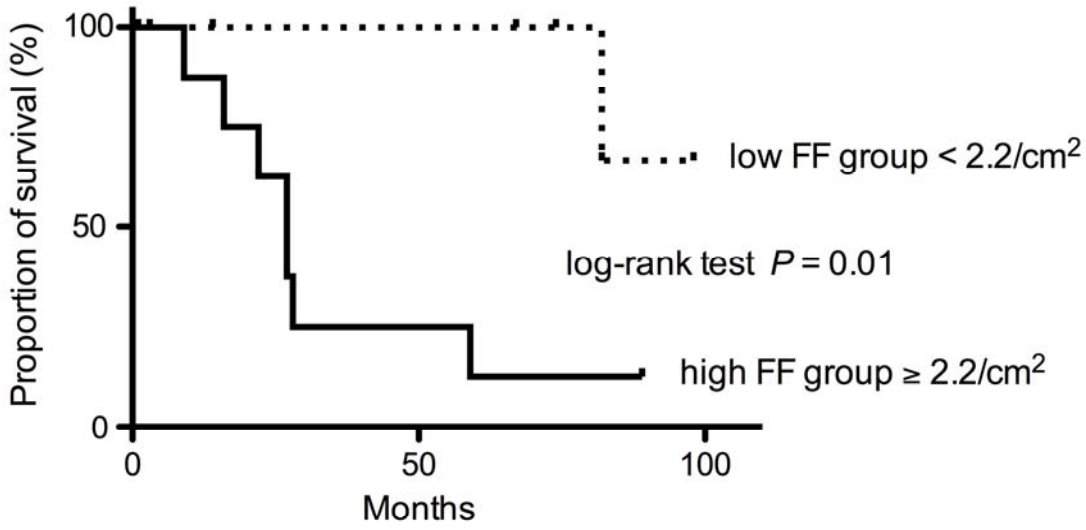


Figure 5

A


Proportion of patients free from acute exacerbation (%)
100
50
0
0
50
100
Months
low FF group < 2.2/cm2
log-rank test P = 0.08
high FF group ≥ 2.2/cm2

B


Proportion of survival (%)
100
50
0
0
50
100
Months
low FF group < 2.2/cm2
log-rank test P = 0.01
high FF group ≥ 2.2/cm2