【 CASE REPORT 】

The Analysis of Surgical Lung Biopsy and Explanted Lung Specimens Sheds Light on the Pathological Progression of Chronic Bird-related Hypersensitivity Pneumonitis

Satoshi Hanzawa [1], Tomoya Tateishi [1], Tamiko Takemura [2], Yoshinori Okada [3],
Yoshihito Yamada [4], Masafumi Noda [3], Yasunari Miyazaki [1] and Naohiko Inase [1]

Abstract:
Chronic hypersensitivity pneumonitis is an interstitial pneumonia caused by an immunological reaction to the chronic inhalation of an antigen. Little is known, however, about the pathological change of the pulmonary lesions. A 33-year-old man was diagnosed with chronic bird-related hypersensitivity pneumonitis based on the findings of a surgical lung biopsy and an inhalation provocation test. He underwent lung transplantation at 8 years after the diagnosis because of disease progression. We conclude that the analysis of the explant suggests that the presence of extensive fibrosis in the centrilobular and perilobular area with bridging fibrosis is a form of pathological progression of chronic hypersensitivity pneumonitis.

Key words: chronic hypersensitivity pneumonitis, lung transplantation, centrilobular fibrosis, subpleural and paraseptal fibrosis, bridging fibrosis

Introduction

Hypersensitivity pneumonitis (HP) is an interstitial pneumonia caused by an immunological reaction to an inhaled antigen. Clinically, HP is divided into acute and chronic forms. In chronic hypersensitivity pneumonitis (CHP), the fibrotic process progresses for more than 6 months (1). The precise diagnosis of CHP is always difficult because radiologic data and other clinical findings mimic the clinical picture of idiopathic interstitial pneumonias (IIPs) (2, 3). Pathological findings from surgical lung biopsy (SLB) and autopsy are therefore important for exploring the diagnosis. In the pathological observation of SLB, chronic bronchiolitis with granulomatous inflammation and bronchiolocentric distribution, in addition to perilobular fibrosis, is suggestive of CHP rather than IIPs (4). Autopsy findings also shed light on the progression of fibrosis caused by CHP (5, 6). Although the autopsy lung findings reveal the progression of

CHP, the effects of terminal events, such as bacterial and fungal infection or diffuse alveolar damage are superimposed on those specimens. We hereby describe a case of CHP that was treated with lung transplantation. The specimens obtained from the explanted lung revealed how the pathological changes progressed over an 8-year period.

Case Report

A 33-year-old man was referred to a previous respiratory hospital with a 2-year history of dry cough and progressive breathlessness. On physical examination, he was afebrile and non-cyanotic with bilateral fine crackles auscultated throughout both lung fields. Severe clubbing was noted on all of his fingers and toes. A chest X-ray film showed diffuse bilateral ground glass opacities, and chest computed tomography (CT) showed diffuse nodular shadows in all lung fields (Fig. 1A and B). The patient's Krebs von den Lungen-6 (KL-6) and surfactant protein D (SP-D) levels (markers of
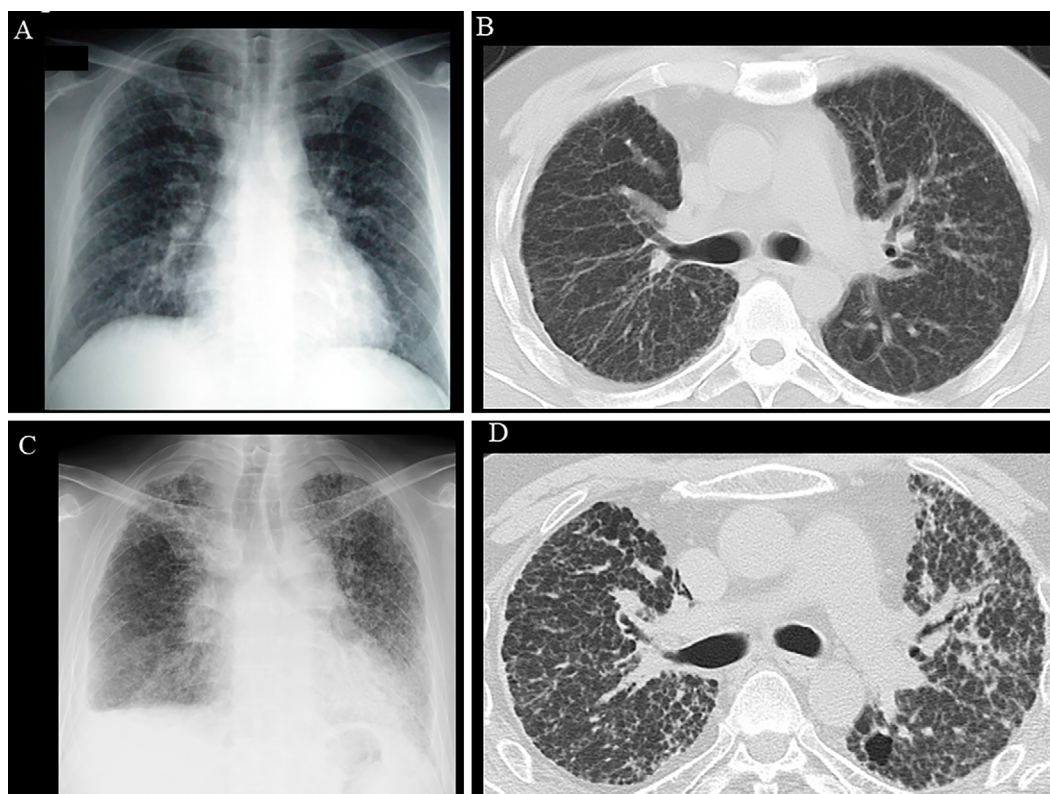
Figure 1. The radiological findings. A chest X-ray film shows bilateral ground glass opacities (A) and chest CT shows diffuse nodular shadow (B) in all lobes at 33 years of age. A chest X-ray film shows the progression of ground glass opacities and a decrease of lung volume capacity (C); chest CT shows a thickened interlobular septa (D) at 41 years of age.

interstitial pneumonia) were 721 U/mL (normal range <500 U/mL) and 215 ng/mL (normal range <110 ng/mL), respectively. A serum antibody test was weakly positive for pigeon dropping extracts, while a test for *Trichosporon asahii* was negative (Table 1) (7). The percentage of lymphocytes in the bronchoalveolar lavage fluid was 3.9%; thus, lymphocytosis was not present. However, CHP and other secondary interstitial pneumonias were not ruled out because of the younger age at onset, positive serum antibody test result for pigeon dropping extract, and the fact that the pattern on CT was inconsistent with usual interstitial pneumonia (UIP). An SLB was performed to investigate the causes of interstitial pneumonia at the previous hospital. CHP was suspected based on the findings of the SLB. Specimens obtained from the right lower lung revealed centrilobular fibrosis, subpleural and paraseptal fibrosis, and bridging fibrosis (Fig. 2A and B). The bridging fibrosis was a linear connection of fibrotic tissue between the centrilobular area and the perilobular area, as well as between the centrilobular and adjacent centrilobular areas (8). Mild lymphocyte infiltration and loose granulomas were seen in the limited alveolar areas (Fig. 2C). The patient's persistent cough went into remission soon after his admission to the previous hospital, prompting the suspicion that the causative antigen was present in his house. The result was negative, however, when a challenge test was conducted by exposing the patient to his environment. The patient was referred to our hospital to identify the

antigen. At the time of admission, he had a smoking history of 1 pack per day for 12 years, worked in a sushi restaurant and used a duvet in his house. His brother had a history of interstitial pneumonia.

Although the pathological findings suggested that the cause of the interstitial pneumonia was CHP, an environmental challenge test performed at the previous hospital had been negative. We therefore investigated the environment of his home and routine working environments and concluded that bird-related antigens such as the duvet in his house or pigeons on his commuting route were the most probable cause of his CHP. The patient underwent an inhalation provocation test using avian dropping extracts at our hospital to identify the causative antigen. He fulfilled 2 of the 6 criteria, namely, a greater than 20% decrease in the diffusing capacity of the lungs for carbon monoxide and a more than 30% increase in the peripheral white blood cell count, and an inhalation provocation test was positive (Table 2) (9). These results suggested that an avian antigen was the antigen responsible for his disease.

The patient was treated with oral corticosteroids and advised to avoid using feather duvet and other potential antigens. Four years later, the disease progressed and pirfenidone was added. A few years later, at 39 years of age, the patient suffered from an acute exacerbation of interstitial pneumonia in spite of his therapies, and tacrolimus was added (Fig. 3). The radiological findings at that time showed

Table 1. Laboratory Findings on Admission.

Hematology		Biochemistry		Serology	
WBC	7,200 /μL	TP	7.9 g/dL	CRP	0.4 mg/dL
neut	68 %	Alb	4.6 g/dL	IgG	1,301 mg/dL
lymph	25.3 %	BUN	15 mg/dL	IgA	514 mg/dL
mono	4.6 %	Cre	0.62 mg/dL	IgM	96 mg/dL
eos	1.5 %	Na	139 mEq/L	KL-6	721 U/mL
baso	0.6 %	K	4.1 mEq/L	SP-D	215 ng/mL
RBC	491 /μL	Cl	103 mEq/L	RF	96 IU/mL
Hb	15.3 g/dL	Ca	9.7 mg/dL	ANA	40
Hct	46.3 %	T-Bil	1.0 mg/dL	speckled type	40
Plt	$19.6×10^4$ /μL	AST	43 IU/L	anti-DNA antibody	<2.0 IU/mL
		ALT	42 IU/L	anti-SS-A antibody	<7.0 U/mL
Arterical blood gas analysis (room air)		LDH	243 IU/L	anti-RNP antibody	<7.0 U/mL
pH	7.396	γ-GTP	222 IU/L	anti-Scl-70 antibody	(-)
PaO_2	81.8 Torr	ALP	256 IU/L	anti-Jo-1 antibody	<7.0 U/mL
$PaCO_2$	40.6 Torr			PR3-ANCA	<10 EU
HCO_3^-	24.4 mmol/L	Lymphocyte stimulation test		MPO-ANCA	<10 EU
SaO_2	97.0 %	pigeon plasma	stimulation index	ACE	15.7 U/L
		0.5%	1.15	anti-PDE IgG	negative
		1%	1.29	anti-PDE IgA	weakly positive
		2%	1.26	anti-T. asahii IgG	negative

PDE: pigeon dropping extracts

increased ground glass opacities and reticular shadows (Fig. 1C and D). The disease progressed further and he underwent left-sided lung transplantation from a brain-dead donor at 41 years of age.

The macroscopic findings of the explanted left lung showed an irregular, convex, hard, pleural surface with a roughness of 3-5 mm and bullous change of the upper lobe (Fig. 2D). No honeycomb change was observed. Microscopy revealed extensive perilobular atelectatic fibrosis throughout the upper and lower lobes (Fig. 2E) and progression of thick bridging fibrosis (Fig. 2F). Smooth muscle hyperplasia and osseous tissue were observed in the subpleural and paraseptal fibrosis as a UIP-like lesion. Numerous giant cells with cholesterol clefts were observed, along with lymphocyte, infiltration in some areas of fibrosis. A cystic lesion with dense collagen deposition was also noted (Fig. 2G). Extensive bronchiolar metaplasia with mucus, a finding absent from the earlier SLB, was seen in the lower lobe (Fig. 2H). The progression of bridging fibrosis shortened the distance between the centrilobular areas and the distance between the centrilobular areas and perilobular or subpleural areas, resulting in a collapse of the alveoli (Fig. 4).

The patient was treated with immunosuppressants to prevent acute transplant rejection and graft-versus-host disease after lung transplantation. Disseminated nocardiosis emerged as an infectious complication of the immunosuppressant therapy one year after lung transplantation; it was treated with antibiotics. At present, HP has not recurred in the implanted lung up to the present; however, only 2 years have passed since lung transplantation.

Discussion

In this report we describe a case in which lung transplantation was performed as a treatment for chronic respiratory failure. To the best of our knowledge, this is the first published pathological comparison between SLB specimens and explanted lung specimens in patient with of CHP. Our findings suggest that a review of explanted lungs may shed light on the progress of the fibrotic change in CHP.

In this case, bridging fibrosis and centrilobular fibrosis were clearly seen in the explanted lung specimens but not in the specimens from the SLB. The histopathologic features of CHP are often similar to a UIP pattern, while granulomas and giant cells containing cholesterol clefts are suggestive of CHP (8, 10-12). In the present case we mainly observed centrilobular fibrosis and perilobular and subpleural fibrosis with scant normal alveoli, whereas normal alveolar structures remained in widespread areas of the SLB specimens. In the explant, however, we found extensive perilobular fibrosis with smooth muscle hyperplasia and a broad band of bridging fibrosis between the centrilobular and perilobular areas. The centrilobular lesions often took a nodular form with hyperplasia of the smooth muscle cells. These fibrotic changes and bronchiolar metaplasia decreased the normal alveolar structure. The progression of bridging fibrosis shortened the distance between the centrilobular areas and the distance between the centrilobular areas and perilobular or subpleural areas, resulting in a collapse of the alveoli. This comparison between the specimens from the SLB and the explant at lung transplantation in the same case suggests that
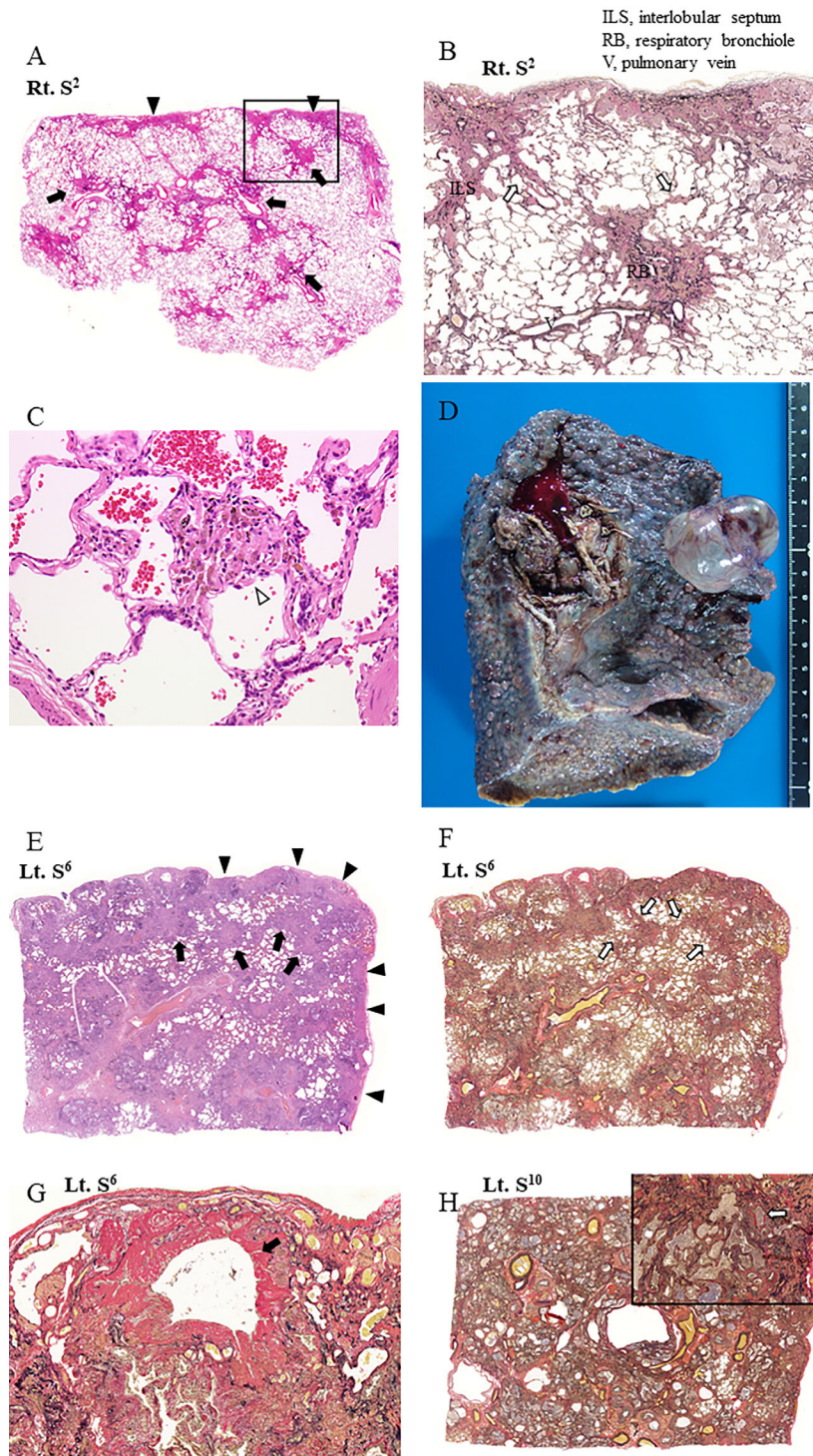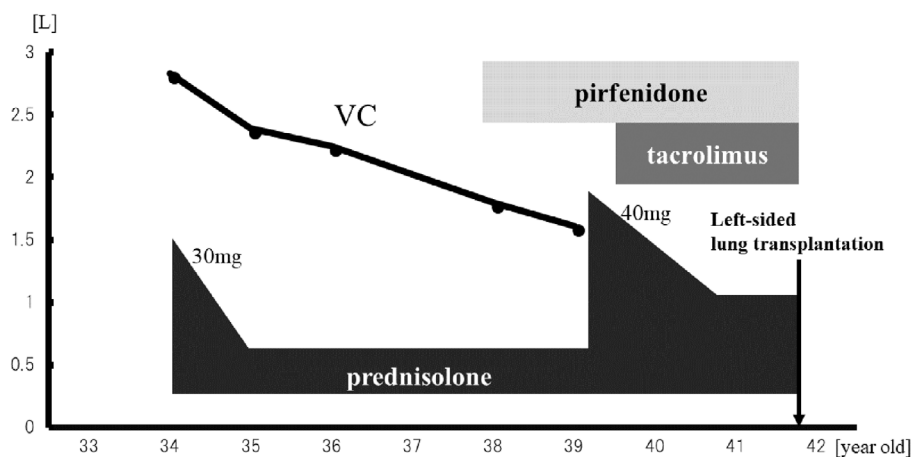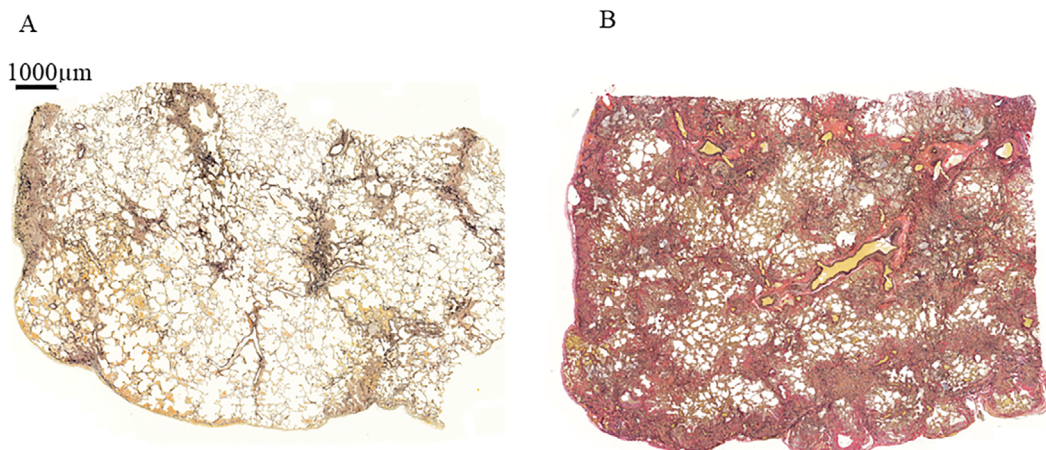
Figure 2. (A-C) Microscopic findings of surgical lung biopsy specimen. Centrilobular fibrosis (black arrows), subpleural and paraseptal fibrosis (black arrowheads) [A: panoramic view of the right S², Hematoxylin and Eosin (H&E) staining, ×40], bridging fibrosis (white arrows) (B: a square of A, elastica van Gieson staining, ×100) and loose granuloma (white arrowheads) (C: H&E staining, ×400) are seen. (D-H) Macroscopic and microscopic findings of the left lung explant. An irregular, convex, hard, pleural surface with a roughness of 3-5 mm and bullous change of the upper lobe are seen (D). UIP-like features are remarkable. Hyperplasia of the smooth muscle cells and progression of fibrosis are seen in the centrilobular areas (black arrows). The progression of subplerural and paraseptal fibrosis is seen; the pleura has a rough surface (black arrowheads) (E: H&E staining, ×40). The progression of thick bridging fibrosis is seen (white arrows) (F: elastica van Gieson staining, ×40). A cystic lesion with collagen deposition (black arrow) is seen (G: elastica van Gieson staining, ×200). Extensive bronchiolar metaplasia with mucus in the lower lobe are seen (white arrows) [H: elastic van Gieson staining, ×40 and ×200 (inset)].

Table 2. The Result of an Inhalation Provocating Test.

	Before inhalation	6 h after inhalation	12 h after inhalation	24 h after inhalation
Radiologic abnormalities	-	not increased	not increased	not increased
D_{LCO}	16.2	not examined	not examined	11.2
VC (L)	2.83	not examined	not examined	2.76
WBC (/μL)	5,400	12,600	12,400	6,900
CRP (mg/dL)	0.2	0.1	0.3	0.7
Body temperature*	-	not increased	not increased	not increased
Symptoms	-	no symptom	no symptom	no symptom

*The significant increase of body temperature (BT) is defined as more than 1°C increase in BT on this test. D_{LCO}: diffusing capacity of the lung carbon monoxide; VC: vital capacity

Figure 3. The clinical course up to lung transplantation. VC: vital capacity

Figure 4. Comparison between the microscopy findings of a surgical lung biopsy from Rt. S⁶ (A) and the Lt. S⁶ from explant (B). The progression of bridging fibrosis shortens the distance between the centrilobular areas or between the centrilobular areas and the perilobular or subpleural areas, and results in alveolar collapse.

the progression of the CHP lesions resulted from the centrilobular fibrosis and the perilobular or subpleural fibrosis shown by the lesion, along with the bridging fibrosis.

This process of progressive fibrosis is a typical finding in CHP. Pathologic studies of CHP by Akashi et al. showed a mixed pattern of UIP-like areas, non-specific interstitial pneumonia (NSIP)-like areas, centrilobular fibrosis, and bridging fibrosis (ex. 5). In autopsy cases with idiopathic pulmonary fibrosis (IPF/UIP), however, centrilobular and bridging fibrosis have rarely been seen. The observation of fibroblastic foci randomly distributed in the lung is regarded as an early-stage finding of IPF/UIP and is likely to convert to non-uniform interstitial fibrosis with a patchwork pattern over time. Meanwhile, an analysis of the serial histological

changes from SLB to autopsy by Ochi et al. supported the hypothesis that fibrosis distributed in the peribronchovascular area at a relatively early stage of CHP changes to bridging fibrosis (6). Ochi et al. also found that a fibrotic NSIP pattern in SLB progressed to a UIP pattern in 6 of 7 autopsy cases. The SLB specimens from our case manifested centrilobular fibrosis, while the explant specimens showed NSIP-like features (e.g., peribronchiolar metaplasia), UIP-like features (e.g., subpleural fibrosis and smooth muscle hyperplasia), and centrilobular and bridging fibrosis. We concluded that signs of UIP-like features in our case developed as a progressive form of centrilobular and perilobular fibrosis with bridging fibrosis. This process through which UIP-like features emerge has been seen in cases of CHP but not IPF/UIP.

Reviews of explanted lungs tissues shed light on the process of fibrosis in CHP, as the explanted tissues usually show fewer secondary changes than autopsy specimens. Autopsy specimens show numerous secondary changes, including diffuse alveolar damage, bacterial, fungal or viral infection, and post mortem change. These changes are sometimes superimposed on the finding of fibrosis (5, 6). We observed a slight secondary pathological change in our explant specimens, possibly due to the effects of immunosuppressive therapy on the progression of the disease. Pathologic studies of CHP by Akashi et al. showed that characteristic findings, such as centrilobular and perilobular fibrosis with bridging fibrosis, remained in autopsies of CHP patients treated with corticosteroid therapy (5), and the same form of fibrosis was seen in our case. This finding suggests the progression of centrilobuar and perilobular fibrosis with bridging fibrosis remained, despite the administration of immunosuppressive therapy. Now that the number of lung transplantations procedures is increasing in Japan, we believe that a review of explants can further our understanding of the progression of interstitial pneumonias (13).

We herein described a case in which CHP was treated with lung transplantation. Pathological studies of SLB and explanted lung specimens helped us trace the fibrotic progression of CHP. We conclude that the explanted lung revealed the characteristic development of broad-bridging fibrosis and a marked decrease in the number of normal alveoli.

The authors state that they have no Conflict of Interest (COI).

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