Original

Clinical Characteristics of Acute Exacerbations of Idiopathic Pulmonary Fibrosis and Involvement of Viral, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae* Infections

Makiko Anzai*, Yasutsugu Fukushima*, Kazuki Obara, Kazuhiko Matsuno,
Yasuko Kikkawa, Hirokuni Hirata, Kumiya Sugiyama, and Takeshi Fukuda

(*These authors contributed equally to this work.)

Department of Pulmonary Medicine and Clinical Immunology,
Dokkyo Medical University School of Medicine, Tochigi, Japan

SUMMARY

Background and Objective：To clarify the clinical characteristics of acute exacerbation of idiopathic pulmonary fibrosis (IPF) and the involvement of infections with pathogenic microorganisms and viruses in acute exacerbation.

Methods：During the 12 years from 2000 through 2011, we studied 50 patients who were admitted and received treatment for acute exacerbation of IPF in our department. Demographic characteristics, imaging findings, laboratory findings, changes in antibody titers against bacteria, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and known viruses, and outcomes were studied.

Results：Among the 50 patients with acute exacerbation of IPF (41 men and 9 women) 29 patients died (mortality rate, 58.0%). Computed tomography showed subpleural peripheral ground-glass opacities (GGO) in 5 patients, multiple patchy GGO in 19, and diffuse GGO in 26. Only the PaO_2/FiO_2 ratio was significantly lower in the non-survivors compared with survivors. Three patients had high titers of IgM antibodies against *C. pneumoniae*, but acute infection was ruled out by the changes in IgA and IgG antibodies in paired serum samples. Antibody titers against known viruses significantly increased in 2 patients (respiratory syncytial virus in 1 and adenovirus 11 in 1). In acute-phase serum samples, 7 patients had increased antibody titers against parainfluenza virus 3, resulted in no significant change in paired serum samples.

Conclusions：Our results suggest that known pathogens do not play a role in acute exacerbation of IPF. The outcomes of IPF remain poor, and the elucidation of the causes and pathological features of acute exacerbation of IPF, including the identification of unknown pathogens, is awaited.

Key Words：acute exacerbation, *Chlamydophila pneumoniae*, idiopathic pulmonary fibrosis, *Mycoplasma pneumoniae*, viral infection

Abbreviations：
BAL＝bronchoalveolar lavage
CF＝complement fixation
GGO＝ground-glass opacities
HI＝hemagglutination inhibition
HRCT＝high-resolution computed tomography

HSV = herpes simplex virus
IPF = idiopathic pulmonary fibrosis
NT = neutralization
PA = particle agglutination
RSV = respiratory syncytial virus
TTV = torque teno virus

INTRODUCTION

"Idiopathic pulmonary fibrosis（IPF）" is an advanced, life-threatening, interstitial lung disease of unknown origin[1,2]. In most cases, the clinical course is chronic and gradually progressive. The mean survival time has been reported to be 3 to 5 years[3,4]. New shadows（infiltrates）develop in both lung fields during the chronic phase in association with rapid disease progression, leading to respiratory failure. An "acute exacerbation of IPF" is diagnosed in the absence of distinct causes of disease exacerbation[5]. The annual incidence of acute exacerbations of IPF has been reported to range from 5% to 15%[6,7]. A decline in forced vital capacity and progressive hypoxemia are thought to increase the risk of acute exacerbation of IPF in association with disease progression[8]. Whether such acute exacerbations of IPF directly represent fibrous proliferation associated with the underlying disease or are caused by other known or unknown factors remains elusive[5,9]. Most acute exacerbations of IPF are accompanied by the presentation of symptoms suggesting airway infection, such as fever and cough, but the involvement of infection in such symptoms remains unclear[10,11]. Cytologic evaluation of bronchoalveolar lavage（BAL）fluid and histologic examination of lung specimens have yet to identify causative agents[12~15]. We therefore examined whether infections with bacteria, mycoplasma, chlamydophila, and known viruses are related to acute exacerbation of IPF.

METHODS

Subjects and Study Design

The study group was comprised of 50 consecutive patients who were admitted to the hospital because of acute exacerbation of IPF from January 2000 through December 2011. The diagnosis of IPF was established histologically by thoracoscopic biopsy of the lung in 4 patients. In most of the other patients, the diagnosis was established on the basis of clinical findings and imaging studies of the chest on high-resolution computed tomography（HRCT）, referring to the international consensus statement[16]. Acute exacerbation of IPF was diagnosed on the basis of the following criteria[5,17]: a previous or concurrent diagnosis of IPF; aggravation of dyspnea within 1 month; new diffuse pulmonary infiltrates on chest radiography; worsening hypoxemia; and the absence of apparent infectious agents and heart failure. Testing of sputum cultures, blood cultures, or both was performed in all patients at the time of acute exacerbation of IPF to rule out distinct bacterial infection. On admission, general blood tests, blood chemical tests, arterial blood gas analysis, and serum separation（acute-phase serum samples）were performed, and serum levels of KL-6 and SP-D were measured. After 2 to 4 weeks of treatment, serum separation（convalescent serum samples）was performed. *Mycoplasma pneumoniae* particle agglutination（PA）tests were conducted, and antibody titers against *Chlamydophila pneumoniae* and various viruses were measured in paired serum samples. Clinical data were obtained from patient records. This study was approved by the Bioethics Committee of Dokkyo Medical University.

CT Examination

The chest HRCT protocol consisted of thin sections（1- to 2-mm collimation sections）obtained at 20-mm intervals through the chest in the supine position. Two observers（Y. F. and M. A.）, without knowledge of the patients' clinical information, examined the chest HRCT scans. Ground-glass opacity was defined as an area of slightly increased attenuation in which the bronchial walls and vessels remained visible. Consolidation was defined as an area of increased attenuation

with obscuring of the adjacent bronchial walls and vessels. Honeycombing was defined as an accumulation of cystic spaces with thickened walls.

Sample Collection and Serology

The blood samples obtained from the subjects were separated by centrifugation at 3,000 rpm for 15 min, and the separated serum was stored at −80℃ until use. *M. pneumoniae* PA tests were performed, and IgM, IgA, and IgG antibody titers against *C. pneumoniae* were measured. Positive results of *M. pneumoniae* PA tests were defined as an antibody titer of ≥1：640 times in single serum samples or a fourfold or greater increase in the titer in paired serum samples. IgM, IgA, and IgG antibody titers against *C. pneumoniae* were measured with the use of enzyme-linked immunosorbent assay kits (Hitazyme *C. pneumoniae*, Hitachi Chemical Company, Ltd., Tokyo, Japan). An index value of ≥1.60 was defined as positive for IgM antibodies[18], and an index value of ≥1.10 was defined as positive for IgA or IgG antibodies[19]. An increase of ≥1.00 in the index value for IgA antibodies and of ≥1.35 for IgG antibodies between paired serum samples was considered to indicate acute infection[19].

Antibody titers against various viruses were measured as follows：respiratory syncytial virus (RSV), complement fixation (CF) test；herpes simplex virus (HSV), CF；rubella virus, hemagglutination inhibition (HI) test；adenovirus 3, 4, 7, 11, 21, neutralization (NT) test；echovirus 1, 3, 4, and 5, NT；coxsackievirus A2, A3, A4, A5, and A6, NT；coxsackievirus B1, B2, B3, B4, B5, and B6, NT；parainfluenza virus 1, 2, and 3, HI；influenza virus A (H1N1) and influenza virus A (H3N2), HI；influenza virus B1 and B2. A fourfold or greater increase in antibody titers against viruses in paired serum samples was defined as positive. The procedures for the measurement of virus titers are described briefly below. For CF test, several virus antigen, and guinea-pig complement were purchased from Denka-Seiken Co. (Tokyo, Japan). The virus or normal antigen was added to the inactivated serum, which was sequentially diluted by twofold. The sample was then incubated with complement at 4℃ overnight. Sensitized liposome was added to the sample and incubated at 37℃ for 1 h. Release of carboxyfluorescein was assessed by measuring the optical density using a microplate reader. The maximum serum titer for negative lytic reaction was determined. For HI test, the inactivated serum was diluted by twofold and virus antigen (Denka-Seiken Co.) was mixed with the serum sample and incubated at room temperature for 1 h. Thereafter, 0.5% chicken or guinea-pig RBC was added to the sample and incubated at room temperature for 1 h. Inhibition of virus-induced haemagglutination was observed visually and the maximum serum titer causing HI was determined. For NT test, the inactivated serum was diluted by twofold. Mixed with adjusted virus ($100\,\mathrm{TCID}_{50}/25\,\mu\mathrm{L}$) purchased from Nissui-Seiyaku Co. (Tokyo, Japan) and incubated at room temperature for 30 min. Thereafter, the virus mixture was added to sensitive cell lines and cultured in Eagle MEM (Nissui-Seiyaku Co.) with 3% FBS at 35℃ for 5 days. Viral plaques were visualized by staining with 0.1% crystal violet in PBS containing 0.2% formaldehyde and counted.

Statistical Analysis

Data are expressed as mean±SD. Comparison between survivors and nonsurvivors were performed using nonparametric methods (Mann-Whitney *U*-test). Clinical data analysis was performed using JMP 10.0 (SAS Institute Inc., Cary, NC, USA). Statistical significance was defined as a *P* value less than 0.05.

RESULTS

From January 2000 through December 2011, a total of 50 patients (41 men and 9 women) were admitted to our hospital because of acute exacerbation of IPF. Twenty-nine patients (58%) did not respond to intensive therapy for acute exacerbation and died. Table 1 shows the clinical characteristics of the patients. Table 2 shows the laboratory data (on admission) and figure 1 shows the patterns on chest HRCT in the survivors and non-survivors. On chest HRCT, subpleural peripheral dominant GGO was found in 5 patients (1 death, 20%), multifocal GGO in 19 (11 deaths, 58%), and diffuse GGO in 26 (19 deaths, 73%) (Table 1, Fig. 1). Many non-survivors received methylprednisolone pulse therapy, immunosuppressant drugs, sivelestat sodium hydrate, and endotracheal intubation with artificial ventilation (Table 1). No patient received immunoglobulin preparations. On admission, white-cell

Table 1   Clinical Characteristics of Patients and Chest HRCT Patterns

Variable	Survivors (n = 22)	Non-survivors (n = 28)
Age, yr (range)	70 (54-82)	72 (59-87)
Gender (men/women)	17/5	24/4
Smoking (Current + Former), %	73	75
Fever (≥37.5℃), %	66	60
Methylprednisolone pulse, %	45	86
Immunosuppressive drugs, %	32	46
Sivelestat sodium hydrate, %	23	50
Mechanical ventilation, %	5	46
HRCT pattern of GGO		
Peripheral dominant	4	1
Multifocal	8	11
Diffuse	7	19

GGO denotes ground-glass opacities
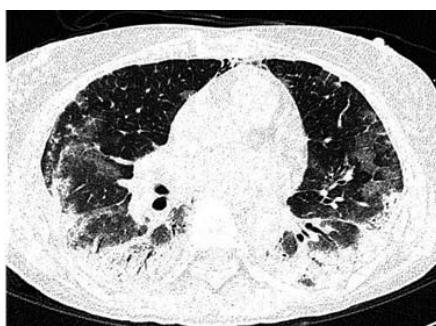
Peripheral dominant GGO (5 subjects)

Multifocal GGO (19 subjects)

Diffuse GGO (26 subjects)

Figure 1   Representative Chest HRCT Images on Acute Exacerbation of IPF
GGO denotes ground-glass opacities.

counts, C-reactive protein levels, serum lactate dehydrogenase levels, serum KL-6 levels, and serum surfactant protein D levels did not significantly differ between the survivors and non-survivors ; however, the PaO_2/FiO_2 ratio was significantly lower in the non-survivors (Table 2). The mean interval from disease onset to the start of therapy was similar in the survivors (7 days) and non-survivors (9 days).

Bacteriologic examinations on admission revealed no clinically significant pathogens in any patient. Convalescent-phase serum samples could not be obtained from some patients who died during admission. Consequently, paired serum samples could be evaluated in 31 patients, including 10 non-survivors. The results of *M. pneumoniae* PA tests of acute-phase serum samples were negative in all patients, and there was no in-

Table 2　Comparison of Laboratory Data on Admission Between Survivors and Non-survivors

	Survivors (n = 22)	Non-survivors (n = 28)	P-Value
WBC (X10^9/l)	9.54 ± 3.46	10.7 ± 3.36	NS
CRP (mg/dl)	4.25 ± 7.48	5.40 ± 6.98	NS
LDH (U/l)	316 ± 120	367 ± 190	NS
KL-6 (U/ml)	1428 ± 675	1900 ± 1095	NS
SPD (ng/ml)	404 ± 255	429 ± 368	NS
PaO$_2$/FiO$_2$ (mmHg)	290 ± 54.9	159 ± 72.5	<0.001

Values are mean ± SD.
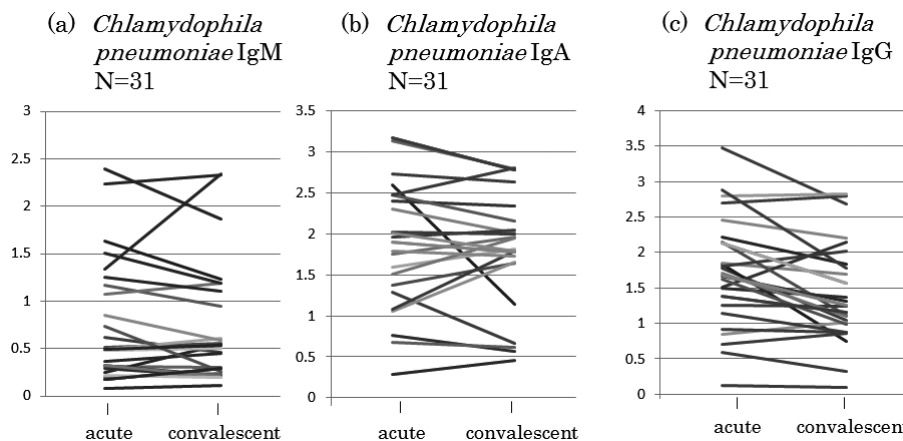
Figure 2　Changes in Index Values for IgM Antibodies (a), IgA Antibodies (b), and IgG
　　　　　　Antibodies (c) against *C. pneumoniae* in Acute- and Convalescent-phase
　　　　　　Serum Samples (Paired Serum Samples)
　　　　In acute-phase serum samples, 3 patients had an IgM index of ≥1.60, but the IgA and
　　　　IgG indexes did not satisfy the criteria for positivity. These patients were considered false
　　　　positive. None of the 31 patients satisfied the criteria for a positive IgA or IgG index.

crease in antibody titers between paired serum samples. The *C. pneumoniae* IgM antibody index exceeded 1.60 in 3 patients. However, none of these patients had significant increases in IgA or IgG antibody titers in paired serum samples. The *C. pneumoniae* IgA antibody index in acute-phase serum samples exceeded 1.10 in 21 patients, but the index did not increase by ≥1.00 in paired serum samples in any of these patients, ruling out the possibility of acute infection. The *C. pneumoniae* IgG antibody index in acute-phase serum samples exceeded 1.10 in 24 patients. However, none of these patients showed an increase of ≥1.35 in paired serum samples. Acute infection was therefore ruled out (Fig. 2).

On virus antibody tests, the RSV antibody titer significantly increased from 1：4 to 1：32 in 1 patient and the adenovirus 11 antibody titer significantly increased from 1：4 to 1：32 in 1 patient. In acute-phase

serum samples, antibody titers against parainfluenza virus 3 were elevated (≥1：320) in 7 patients, but none of these patients showed a significant increase in paired serum samples. In summary, changes in antibody titers against known viruses examined in this study did not distinctly suggest viral infection in any patient, with the exception of 2 patients who had a significant increase in virus antibody titer (RSV in 1, and adenovirus 11 in 1) (Fig. 3).

DISCUSSION

A diagnosis of acute exacerbation of IPF requires ruling out various factors, including pneumonia, pulmonary infarction, heart failure, steroid dose reduction, injury caused by general anesthesia, surgery, or bronchoscopy[20,21], and adverse effects of drugs[22]. In the present series of 50 patients, we ruled out potential effects of these exacerbating factors as extensively as
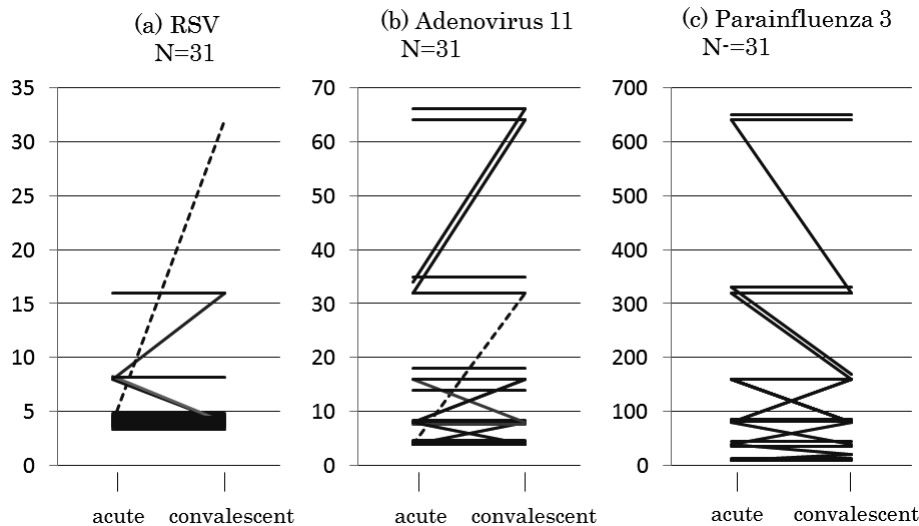
Figure 3 Changes in Respiratory Syncytial Virus（a）, Adenovirus 11（b）, and Parainfluenza
Virus 3（c）in Acute- and Convalescent-phase Serum Samples（Paired Serum
Samples）
（a）The antibody titer significantly increased in 1 patient（by 4 fold）（dotted line）.
（b）The antibody titer significantly increased in 1 patient（by 4 fold）（dotted line）.
（c）Seven patients had antibody titers higher than 1 : 320 in acute-phase serum samples,
but none of these patients showed a significant increase in paired serum samples.
RSV denotes Respiratory Syncytial Virus.

possible. The mortality rate among the 50 patients with acute exacerbation of IPF who were treated in our department was 58%. The main predictor of poor outcomes in patients with acute exacerbation of IPF was a decreased PaO_2/FiO_2 ratio on admission（290± 54.9 mmHg in survivors vs. 159±72.5 mmHg in non-survivors）. Consistent with the results of a study by Akira et al. that assessed changes on chest HRCT[23], the mortality rate was highest（73%）among the 26 patients with diffuse GGO in our study.

The results of *M. pneumoniae* PA tests were negative in acute-phase serum samples, and there was no increase in antibody titers in paired serum samples in the 31 patients in whom these tests were performed. It is therefore unlikely that *M. pneumoniae* infection participated in acute exacerbation of IPF. As for *C. pneumoniae* infection, 3 patients had an IgM antibody index of ≥1.60 in acute-phase serum samples, but none of these patients significant increases in IgA or IgG antibody titers. They were therefore considered false-positive cases. Miyashita et al. reported that 16.5 % of patients with chronic pulmonary disease and 8.6 % of healthy subjects had a *C. pneumoniae* IgM antibody index of ≥1.60, which is considered positive for *C.*

pneumoniae infection. However, the results of both microimmunofluorescence tests and culture tests were negative. Their findings suggested that false-positive results of *C. pneumoniae* IgM antibody assay occur at these probabilities[24]. There was no significant increase in IgA or IgG antibody titers meeting the criteria for positivity in the 31 patients for whom paired serum samples were available. These results suggested that *C. pneumoniae* infection did not have a role in acute exacerbation of IPF. Tomioka et al. studied 27 patients with acute exacerbation of IPF to assess the role of *C. pneumoniae* infection. Among the 15 patients in whom antibody titers were confirmed in paired serum samples, 2 had increased titers of *C. pneumoniae* IgA and IgG antibodies. But they concluded that it was uncommon for *C. pneumoniae* infection to participate in acute exacerbation of IPF[25].

Several studies have reported acute exacerbations of IPF caused by viral infection, but the relation remains uncertain[26~28]. In one study of 43 patients, multiplex polymerase chain reaction（PCR）assay of BAL-fluid samples detected rhinovirus in 2 patients, human coronavirus OC43 in 1, and parainfluenza virus 1 in 1[29]. In our study, among the 31 patients in whom changes in

antibody levels could be confirmed in paired serum samples, 7 showed increased parainfluenza virus 3 antibody levels（≧320 fold）in acute-phase serum samples, with had no significant increase between paired serum samples, suggesting the risk of prior infection. Two patients had significant increases in antibody levels（RSV in 1 and adenovirus 11 in 1）. The RSV-positive patient was a 69-year-old man who had multifocal GGO on chest HRCT and died 21 days after disease onset. The adenovirus 11-positive patient was a 77-year-old man with multifocal GGO on chest HRCT；this patient survived. Thus, the potential involvement of virus infection could not be ruled out in only 2 patients. Consistent with the results of Wootton et al.,[29] infection with known viruses was not detected in most patients with acute exacerbation of IPF.

Our study had several important limitations. First, our major objective was to investigate the involvement of microorganisms in acute exacerbation of IPF. However, we indirectly evaluated potential involvement by measuring antibody titers in the sera of patients and did not assess microorganisms in local lung lesions. Second, 29 of the 50 patients died during treatment, and paired serum samples could not evaluated in all patients. The possibility remains that patients with viral infection or infection with *M. pneumoniae* or *C. pneumoniae* may have been included among the non-survivors. Third, all patients received high-dose steroids, and 20 received immunosuppressants. It remains unclear whether these drugs negatively affected the production of various types of antibodies. To avoid these problems and to identify causative microorganisms in acute-phase serum samples, culture tests to identify pathogens in specimens of local lung lesions or techniques for genetic diagnosis are required. Interestingly, a recent study reported that torque teno virus （TTV）was detected on genetic analysis in BAL fluid samples obtained from 12 of 43 patients with acute exacerbation of IPF[29]. In the present study, unfortunately, we did not evaluate TTV.

In summary, the mortality rate among 50 patients with acute exacerbation of IPF was 58%. The presence of diffuse GGO on chest HRCT and a decreased PaO_2/FiO_2 ratio were associated with poor outcomes. In 31 patients, including non-survivors, for whom paired serum samples were available, *M. pneumoniae*

and *C. pneumoniae* infection was apparently unrelated to acute exacerbation of IPF. Two（6%）of the 31 patients had infection（RSV in 1 and adenovirus in 1）. However, no changes in antibody levels suggestive of viral infection were found in the other 29 patients. Therefore, infection with the known viruses studied apparently had a negligible role in acute exacerbation of IPF. Further larger studies are needed to determine whether infection with known TTV and other known viruses, unknown viruses, or other microorganisms contribute to acute exacerbation of IPF and to identify appropriate treatment for this condition, currently associated with a poor prognosis.

REFERENCES

1) Panos RJ, Mortenson RL, Nicolli SA, et al：Clinical deterioration in patients with idiopathic pulmonary fibrosis：Cause and assessment. Am J Med **88**：396-404, 1990.

2) Wells AU, du Bois RM：Prediction of disease progression in idiopathic pulmonary fibrosis. Eur Respir **7**：637-639, 1994.

3) Turner-Warwick M, Burrows B, Johnson A：Cryptogenic fibrosing alveolitis：Clinical features and their influence on survival. Thorax **35**：171-180, 1980.

4) Hubbard R, Johnston I, Britton J：Survival in patients with cryptogenic fibrosing alveolitis：A population-based cohort study. Chest **113**：396-400, 1998.

5) Collard HR, Moore BB, Flaherty KR, et al：Idiopathic Pulmonary Fibrosis Clinical Research Network Investigators. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med **176**：636-643, 2007.

6) Azuma A, Nukiwa T, Tsuboi E, et al：Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med **171**：1040-1047, 2005.

7) Taniguchi H, Ebina M, Kondoh Y, et al：Pirfenidone in idiopathic pulmonary fibrosis. Eur Respir J **35**：821-829, 2010.

8) Kurosu K, Takiguchi Y, Okada O, et al：Identification of annexin 1 as a novel autoantigen in acute exacerbation of idiopathic pulmonary fibrosis. J Immunol **181**：756-767, 2008.

9) Collard HR, Calfee CS, Wolters PJ, et al：Plasma biomarker profiles in acute exacerbation of idiopathic

pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol **299**：L3-7, 2010.

10) Huie TJ, Olson AL, Cosgrove GP, et al：A detailed evaluation of acute respiratory decline in patients with fibrotic lung disease：aetiology and outcomes. Respirology **15**：873-875, 2010.

11) Konishi K, Gibson KF, Lindell KO, et al：Gene expression profiles of acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med **180**：167-175, 2009.

12) Saydain G, Islam A, Afessa B, et al：Outcome of patients with idiopathic pulmonary fibrosis admitted to the intensive care unit. Am J Respir Crit Care Med **166**：839-842, 2002.

13) Ambrosini V, Cancellieri A, Chilosi M, et al：Acute exacerbation of idiopathic pulmonary fibrosis：report of a series. Eur Respir J **22**：821-826, 2003.

14) Rice AJ, Wells AU, Bouros D, et al：Terminal diffuse alveolar damage in relation to interstitial pneumonias. Am J Clin Pathol **119**：709-714, 2003.

15) Kim DS, Park JH, Park BK, et al：Acute exacerbation of idiopathic pulmonary fibrosis：frequency and clinical features. Eur Respir J **27**：143-150, 2006.

16) American Thoracic Society. Idiopathic pulmonary fibrosis：diagnosis and treatment. International consensus statement. American Thoracic Society（ATS）, and the European Respiratory Society（ERS）. Am J Respir Crit Care Med **161**：646-664, 2000.

17) Kondoh Y, Taniguchi H, Kawabata Y, et al：Acute exacerbation of idiopathic pulmonary fibrosis：analysis of clinical and pathologic findings in three cases. Chest **103**：1808-1812, 1993.

18) Kishimoto T, Ogawa M, Shiga S, et al：Setting of serological criteria for specific IgM antibody to C. pneumoniae by Hitazyme C. pneumoniae in adults. Kansenshogaku Zasshi **75**：762, 2001.

19) Kishimoto T, Matsushima T, Morikawa T, et al：Assay of specific anti-Chlamydia pneumoniae antibodies by ELISA method. 3. Setting of serological criteria.

JPN Assoc Infect Dis **73**：457-466, 1999.

20) Azuma A, Hagiwara K, Kudoh S, et al：Basis of acute exacerbation of idiopathic pulmonary fibrosis in Japanese patients. Am J Respir Crit Care Med **177**：1397-1398, 2008.

21) Hiwatari N, Shimura S, Takishima T, et al：Bronchoalveolar lavage as a possible cause of acute exacerbation in idiopathic pulmonary fibrosis patients. Tohoku J Exp Med **174**：379-386, 1994.

22) Honore I, Nunes H, Groussard O, et al：Acute respiratory failure after interferon-γ therapy of end-stage pulmonary fibrosis. Am J Clin Pathol **167**：953-957, 2003.

23) Akira M, Kozuka T, Yamamoto S, et al：Computed tomography findings in acute exacerbation of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med **178**：372-378, 2008.

24) Miyashita N, Obase Y, Fukuda M, et al：Evaluation of serological tests detecting Chlamydophila pneumoniae-specific immunoglobulin M antibody. Intern Med **45**：1127-1131, 2006.

25) Tomioka H, Sakurai T, Hashimoto K, et al：Acute exacerbation of idiopathic pulmonary fibrosis：role of Chlamydophila pneumoniae infection. Respirology **12**：700-706, 2007.

26) Jakab GJ：Sequential virus infection, bacterial super-infections, and fibrogenesis. Am Rev Respir Dis **142**：374-379, 1990.

27) Egan JJ, Woodcock AA, Stewart JP：Viruses and idiopathic pulmonary fibrosis. Eur Respir J **10**：1433-1437, 1997.

28) Konishi K, Gibson KF, Lindell KO, et al：Gene expression profiles of acute exacerbation of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med **180**：167-175, 2009.

29) Wootton SC, Kim DS, Kondoh Y, et al：Viral infection in acute exacerbation of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med **183**：1698-1702, 2011.