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Original article

Prevalence of viral infection in acute exacerbation of interstitial lung diseases in Japan

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ABSTRACT

Background: Fatal acute exacerbation of interstitial lung diseases is often accompanied by indicators of infection such as fever, cough, and sputum. Although viral infection can contribute to acute exacerbation of interstitial lung diseases, few studies have identified a relationship between acute exacerbations and viral infections. The present study aimed to prospectively clarify the role of viral infection in patients showing acute exacerbation of interstitial lung disease in Japan.

Methods: Nasopharyngeal swab specimens were collected from patients with acute exacerbation of interstitial lung disease between May 2017 and February 2019. Respiratory viruses were detected by the Luminex xTAG Respiratory Viral Panel FAST v2 RUO kit and the BioFire FilmArray Respiratory Panel assay.

Results: Three of 29 patients demonstrated respiratory viral infection during acute exacerbation of interstitial lung diseases. The infectious agents were identified as respiratory syncytial virus, respiratory syncytial virus and influenza A virus, and influenza A virus and rhino/enterovirus in the three patients, respectively.

Conclusions: These results suggest that viral infection did not frequently induce acute exacerbation of interstitial lung diseases in Japan.

Abbreviations: Acute exacerbation of interstitial lung disease, AE-ILD; idiopathic pulmonary fibrosis, IPF; American Thoracic Society, ATS; European Respiratory Society, ERS; Japanese Respiratory Society, JRS; Latin American Thoracic Society, ALAT; reverse transcription-polymerase chain reaction, RT-PCR; FilmArray respiratory panel, FA-RP; xTAG respiratory viral panel v2 RUO, xTAG-RVP; nasopharyngeal, NP; idiopathic interstitial pneumonia, IIP; combined pulmonary fibrosis and emphysema, CPFE; usual interstitial pneumonia, UIP; collagen vascular disease-interstitial lung disease, CVD-ILD; multidisciplinary discussions, MDD; parainfluenza virus, PIV; respiratory syncytial virus, RSV; pleuroparenchymal fibroelastosis, PPFE; residual volume, RV; total lung capacity, TLC; ratio of arterial oxygen partial pressure (PaO_2) to fractional inspired oxygen (F_IO_2), PaO_2/F_IO_2; lactate dehydrogenase, LDH; C-reactive protein, CRP; oral corticosteroid, OCS; bronchoalveolar lavage, BAL; human betaherpesvirus 7, HHV-7; chronic obstructive pulmonary disease, COPD; Torque teno virus, TTV; standard deviation, SD; body mass index, BMI; nonspecific interstitial pneumonia, NSIP; forced vital capacity, FVC; forced expiratory volume in 1 s, FEV1.0; diffusing capacity of carbon monoxide, DLco; DLco corrected for alveolar volume, DLco/VA; gender, age; physiology, GAP; Krebs von den Lungen-6, KL-6; not significant, N.S.

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1. Introduction

Interstitial lung diseases are a group of diseases based on chronic inflammation and fibrosis in the interstitium of the lung. These can be broadly divided into idiopathic interstitial pneumonia with unknown cause and secondary interstitial pneumonia associated with autoimmune disease, drugs, and dust inhalation. Interstitial lung disease shows a variety of disease progressions. Generally, the disease progresses gradually, but some patients experience rapid deterioration, termed as acute exacerbation. Acute exacerbation of interstitial lung disease (AE-ILD) is a condition in which a new infiltrative shadow appears in both lungs and rapidly progresses to respiratory failure. AE-ILD was originally described in the context of idiopathic pulmonary fibrosis (IPF). According to the official American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Society (ATS/ERS/JRS/ALAT) IPF guidelines, an acute exacerbation of IPF is defined as an acute clinical worsening of dyspnea that develops within 1 month without an alternative etiology [1]. Typically, AE-ILD has a poor prognosis, reaching 50% mortality, and is associated with high mortality within 6–12 months.

Although AE-ILD is often accompanied by indicators of infection such as fever, cough, and sputum, the precise etiology is usually unknown. Viral infection could contribute to AE-ILD. However, only few studies have suggested the involvement of viral infections in acute exacerbations. Recent studies reported the presence of some respiratory viruses in BAL fluid in 9% of patients with acute exacerbation of IPF [2]. However, the clinical features of viral infection, the viruses involved, and the frequency of these infections have not been clarified in Japan. Traditionally, pathogen diagnosis in cases of respiratory virus infection is performed by virus isolation/identification using cultured cells. However, this method can be performed only in limited facilities, and it requires technicians who can handle viruses and cells. Genetic tests such as reverse transcription-polymerase chain reaction (RT-PCR) and real-time RT-PCR have been introduced recently. The BioFire FilmArray Respiratory Panel (FA-RP) assay and the Luminex xTAG Respiratory Viral Panel v2 RUO (xTAG-RVP) kit are multiplex real-time PCR systems for simultaneous detection of 22 and 19 respiratory pathogens, respectively, in a single specimen. The present study aimed to clarify the role of viral infection in patients with AE-ILD in Japan by using these systems.

2. Materials and methods

2.1. Patient enrollment

Nasopharyngeal (NP) swab specimens were prospectively collected from 29 patients with AE-ILD between May 2017 and February 2019 after obtaining patient consent. The definition of acute exacerbation was based on the criteria for acute exacerbation of IPF by the Japanese Respiratory Society in 2004 and the International Working Group report in 2016. In particular, we gave greater weight to the diagnostic criteria proposed by the International Working Group report in 2016. Specifically, idiopathic interstitial pneumonia (IIP) was diagnosed on the basis of the 2018 ATS/ERS/JRS/ALAT international consensus guideline. Although currently there is no consistent definition of combined pulmonary fibrosis and emphysema (CPFE), on the basis of past reports, we diagnosed CPFE by the presence of lower lobe-dominant fibrosis (usual interstitial pneumonia [UIP] pattern) and more than 10% emphysema in the upper lobe [3]. For collagen vascular disease-interstitial lung disease (CVD-ILD), one case showed rheumatoid arthritis, and the other showed systemic lupus erythematosus, and both cases were accompanied by obvious fibrosis with predominantly lower lobe involvement. Rheumatoid arthritis was diagnosed on the basis of the diagnostic criteria proposed by the American College of Rheumatology/European League Against Rheumatism in 2010 [4]. Systemic lupus erythematosus was diagnosed on the basis of the diagnostic criteria proposed by The Systemic Lupus International Collaborating Clinics (SLICC) group in 2012 [5]. Multidisciplinary discussions (MDDs) with pulmonologists, radiologists, and, occasionally, pathologists were performed in all cases. MDD was performed by direct discussion while viewing clinical, image, and pathological information. The criteria for AE-IPF were based on the diagnostic criteria proposed by the International Working Group report in 2016, i.e., (i) acute worsening or development of dyspnea of typically <1 month duration; (ii) computed tomography with new bilateral ground-glass opacity and/or consolidation superimposed on a background pattern consistent with UIP pattern; and (iii) deterioration not fully explained by cardiac failure or fluid overload. If a patient previously or concurrently diagnosed with IPF fulfilled the international consensus guideline, the patient was diagnosed with an exacerbation of IPF. Apparent bacterial pneumonia cases were omitted. This study was approved by the Institutional Review Board of Fukuoka University (No 16-3-04, March 31, 2016), and all participants or proxies gave written informed consent. This study was registered as UMIN000023251.

2.2. Laboratory methods

All NP swab specimens were collected within 3 days of admission, suspended in viral transport medium, and stored at −80 °C until testing. Nucleic acids were extracted from 200 μL of the viral transport medium using QIAGEN according to the manufacturer's protocol. Ten microliters of the extracted nucleic acid were tested using Luminex xTAG-RVP and analyzed on a MAGPX® system using the manufacturer's protocol. Specimens in which the virus was detected by xTAG-RVP were also tested by Biofire FA-RP assay using manufacturer's protocol [6].

The Luminex xTAG-RVP assay detects nucleic acids of 19 viruses that cause upper respiratory tract infections, including adenovirus, coronavirus (229E, HKU1, OC43, NL63), human metapneumovirus, human rhinovirus/enterovirus, influenza A virus (H1/2009, H1, and H3), influenza B virus, parainfluenza virus (PIV) types 1–4, respiratory syncytial virus (RSV), and human bocavirus. In addition to these viruses, BioFire FA-RP can also detect bacteria such as *Bordetella pertussis*,

Chlamydophila pneumoniae, and *Mycoplasma pneumoniae*. However, FA-RP cannot detect human bocavirus.

2.3. Statistical analysis

Clinical data are expressed as means or percentages. The primary comparison was between the virus-positive and virus-negative groups in AE-ILD patients. Intergroup comparisons were performed conservatively using nonparametric methods (Mann–Whitney *U* test) and chi-squared analyses as appropriate. A P value less than 0.05 was considered significant. Data were recorded and analyzed using Stat Mate V.

3. Results

Twenty-nine patients with AE-ILD were enrolled in the study, and NP swab specimens were collected from each patient. The median age of the patients was 71 years (range, 49–86 years). Eight patients were female, and twenty-one were male, yielding a female-to-male ratio of 1:2.6 (Table 1). Twenty patients (68.9%) had acute exacerbations during the winter season. Of the 29 patients, four underwent surgical lung biopsy and all were diagnosed with IPF. Based on the 2018 ATS/ERS/JRS/ALAT international guideline, of the 16 IPF patients, 15 had a UIP pattern and one had a probable UIP pattern. We diagnosed CPFE by lower lobe-dominant fibrosis (UIP pattern) and more than 25% emphysema in the upper lobe. No biopsy was performed on pleuroparenchymal fibroelastosis (PPFE) patients. However, because these patients showed superior fibrosis in the upper lobe and their respiratory function test results were available [(residual volume (RV)/total lung capacity (TLC)% predicted.) $\geq$ 115%)], we diagnosed these as probable PPFE [7]. The assessment of seasonal distribution showed five patients each in January and November, four in December, three each in October and February, two each in May and September, and one patient in March, April, June, and August. From October to February, 20 patients (68.9%) presented with acute exacerbations. In all cases, a minimum dose of 1 mg/kg of steroid was given. Furthermore, broad-spectrum antimicrobials such as carbapenems, fluoroquinolones, and fourth-generation cephems were used in all cases. Sputum culture, blood culture, and urinary pneumococcal and legionella antigen tests were performed to exclude bacterial infection. Bronchoalveolar lavage was performed in four patients. In all cases, no significant bacteria indicating bacterial infection were found. Of the 29 patients, two were intubated (both died), eight were treated with non-invasive positive pressure ventilation (six died), and the remainder were given oxygen via a non-rebreather mask or nasal cannula (two died).

Among the specimens, at least one respiratory pathogen was found in three specimens (Table 2), with the NP swab from one patient containing a single virus (RSV), while specimens from the remaining two patients demonstrated two viruses (RSV and influenza A virus, influenza A virus and rhino/enterovirus). Luminex xTAG-RVP assay identified one case of RSV, one of RSV and influenza virus, and one of influenza virus. BioFire FA-RP identified only one case of RSV, which the Luminex study found to be RSV and influenza virus (Case 2 in Table 2). This patient was deceased.

Table 1 – Patient characteristics.

	n = 29
Age (±SD)	73.9 (±8.8)
Male (%)	21 (72.4)
BMI (±SD)	21.9 (±4.5)
Disease type (%)	
IPF	16 (55.2)
CPFE	6 (20.7)
NSIP	4 (13.8)
CVD-ILD	2 (6.9)
PPFE	1 (3.4)
Smoking status (%)	
Ex	14 (48.3)
Never	13 (44.8)
Current	2 (6.9)
Respiratory function test(±SD)	
FVC	2.26 (±1.08)
% FVC	73.8 (±22.7)
FEV1.0	1.78 (±0.79)
FEV1.0%	80.6 (±9.5)
% FEV1.0	74.2 (±21.1)
DL_{CO}	7.00 (±3.23)
DL_{CO}/VA	2.78 (±0.77)
% DL_{CO}	45.1 (±18.2)
% DL_{CO}/VA	64.7 (±19.2)
GAP score (±SD)	3.5 (±1.2)
Pre-treatment (%)	
OCS	11 (37.9)
Pirfenidone	4 (13.8)
Nintedanib	1 (3.4)
Home oxygen therapy	3 (13.8)

SD, standard deviation; IPF, idiopathic pulmonary fibrosis; CPFE, combined pulmonary fibrosis and emphysema; NSIP, nonspecific interstitial pneumonia; CVD-ILD, collagen vascular disease-interstitial lung disease; PPFE, pleuroparenchymal fibroelastosis; FVC, forced vital capacity; FEV1.0, forced expiratory volume in 1 s; DLCO, diffusing capacity of carbon monoxide; DL_{CO}/VA, DL_{CO} corrected for alveolar volume; OCS, oral corticosteroid; GAP, gender, age, physiology.

Table 3 shows the clinical background and clinical data of the 29 patients with and without respiratory viral infection. Respiratory function test was performed 3 months previously on average (0.25–48 months). No significant intergroup differences were found for age, sex smoking status, respiratory function test, hospitalization period, or laboratory data. In the virus-positive group, duration of antibiotic use was high. Furthermore, based on the data for the PaO_2/F_IO_2 ratio and hospitalization, the condition of the virus-positive group tended to be worse than that of the virus-negative group. In addition, we compared the clinical course between virus-positive and virus-negative groups. In the virus-positive group, lactate dehydrogenase (LDH) levels at 1 week post-onset tended to be high in absolute value, but not significantly different (virus-positive, 372.3 ± 36.7 vs virus-negative, 295.7 ± 22.5; p = 0.162). C reactive protein (CRP) level also demonstrated the tendency to be high in the virus-positive group at 1 week post-onset (virus-positive, 4.19 ± 2.06 vs virus-negative, 2.26 ± 0.69; p = 0.297). Oral corticosteroid (OCS) use before acute exacerbation appeared to be more frequent in the virus-positive group but was not significant.

Table 2 – Summary of virus detection cases.

Case	Age	Sex	Disease type	Luminex xTAG-RVP	BioFire FA-RP	BMI	Smoking status	Brinkman index	OCS	% FVC	FEV1.0	% FEV1.0%	% FEV 1.0%	DL_{CO}/VA	% DL_{CO}/VA
1	73	M	CPFE	RSV	–	19.7	Ex	430	Prednisolone 10 mg/day	102.8	3.22	97.2	125.3	1.58	35.9
2	79	F	NSIP	influenza A RSV	RSV	30.6	Never	0	Prednisolone 10 mg/day	44.6	0.70	80.4	47	3.59	86.2
3	77	M	PPFE	influenza A rhino/enterovirus	–	21.2	Ex	400	–	76.9	2.72	88.8	85	3.86	90.5

M, male; F, female; CPFE, combined pulmonary fibrosis and emphysema; NSIP, nonspecific interstitial pneumonia; PPFE, pleuroparenchymal fibroelastosis; xTAG-RVP, xTAG respiratory viral panel v2 RUO; FA-RP, FilmArray respiratory panel; RSV, respiratory syncytial virus; OCS, oral corticosteroid; FVC, forced vital capacity; FEV1.0, forced expiratory volume in 1 s; DL_{CO}, diffusing capacity of carbon monoxide; DL_{CO}/VA, DL_{CO} corrected for alveolar volume.

Table 3 – Comparison of virus-positive and -negative groups.

	Positive (n = 3)	Negative (n = 26)	p value
Age (±SD)	76 (±3.0)	73 (±9.2)	N.S.
Male (%)	2 (66)	19 (73)	N.S.
BMI (±SD)	23.8 (±5.9)	21.7 (±4.4)	N.S.
WBC (±SD)	12700 (±3935)	10465 (±3124)	N.S.
CRP (±SD)	11.3 (±6.8)	9.7 (±6.3)	N.S.
LDH (±SD)	314 (±97)	320 (±84)	N.S.
KL-6 (±SD)	646 (±192)	1019 (±550)	N.S.
FVC (±SD)	2.41 (±1.34)	2.24 (±1.08)	N.S.
% FVC (±SD)	74.7 (±29.1)	73.7 (±22.5)	N.S.
$FEV_{1.0}$ (±SD)	2.21 (±1.33)	1.71 (±0.71)	N.S.
$FEV_{1.0}$% (±SD)	88.8 (±8.4)	79.4 (±9.2)	N.S.
% $FEV_{1.0}$ (±SD)	85.7 (±39.1)	72.5 (±18.2)	N.S.
DL_{CO}/VA (±SD)	3.01 (±1.24)	2.72 (±0.68)	N.S.
% DL_{CO}/VA (±SD)	70.8 (±30.3)	63.3 (±17.2)	N.S.
Brinkman index (±SD)	276 (±240)	560 (±743)	N.S.
Prednisolone use before acute exacerbation (%)	2 (66)	9 (34)	N.S
PaO_2/F_IO_2 ratio (±SD)	148 (±124)	222 (±103)	0.26
Days of antibiotic use (±SD)	12.6 (±0.5)	8.4 (±2.7)	0.01
Hospitalization days (±SD)	43 (±17.4)	27 (±15.4)	0.10
Death (%)	1 (33)	9 (34)	N.S.

SD, standard deviation; BMI, body mass index; WBC, white blood cell; CRP, C-reactive protein; LDH, lactate dehydrogenase; KL-6, Krebs von den Lungen-6; FVC, forced vital capacity; FEV1.0, forced expiratory volume in 1 s; DLCO, diffusing capacity of carbon monoxide; DLCO/VA, DLCO corrected for alveolar volume; OCS, oral corticosteroid; PaO_2/F_IO_2 ratio, the ratio of arterial oxygen partial pressure (PaO_2) to fractional inspired oxygen (F_IO_2); N.S., not significant.

4. Discussion

Only a few previous studies have investigated viral infections in AE-ILD. Among adults in the USA hospitalized with community-acquired pneumonia, patients demonstrated respiratory infection with one or more viruses (23%) and also combined bacterial and viral pathogens (3%) [8]. With regard to interstitial lung diseases, Wootton and colleagues reported using bronchoalveolar lavage (BAL) and serum from patients with AE-IPF [2]. Four of 43 patients with AE-IPF showed evidence of common respiratory viral infection. PIV, rhinovirus, and seasonal coronavirus were detected, but no viruses were detected in the BAL fluids from stable patients [2]. Konishi and colleagues reported no viral gene expression in AE-IPF patients by using gene expression microarrays [9]. In another study, the presence of persistent or chronic, but not acute, viral infections, including those of Epstein–Barr virus, cytomegalovirus, human betaherpesvirus 7 (HHV-7), and HHV8, significantly increased the risk of developing IPF, but not exacerbation of IPF [10]. Previously, Ushiki and colleagues reported detection of one RSV infection in 14 AE-ILD patients using the Cycleave PCR kit [11]. To the best of our knowledge, this is the first prospective report using these globally available RT-PCR methods for detection of respiratory viruses in

AE-ILD in Japan. In the present study, three of 29 patients with AE-ILD demonstrated respiratory virus infection. Taken together, these results suggest that respiratory viral infection does not frequently induce acute exacerbation of interstitial lung diseases.

In this study, three of 29 cases were positive for respiratory virus infection in assessments by the Luminex xTAG-RVP assay, while Biofire FA-RP assay demonstrated only one patient to be virus-positive with RSV only. RSV is the most common single cause of respiratory hospitalization of infants and is the second largest cause of lower respiratory infection mortality worldwide [12]. In adults, there are 8482 deaths per year attributable to RSV in the UK, with 93% of those occurring in individuals aged more than 65 years. Deaths due to RSV respiratory disease increase after the age of 49, rising from 4.2% of all respiratory disease deaths in adults aged 18–49 years to 5.9% in adults aged 50–64 years, with a mortality rate of 38% compared with 3% in patients admitted from the community [12]. In contrast to infants, diagnosis of RSV infections in adults is difficult due to low levels of virus shedding [13]. However, treatments against RSV are now being developed [14]. Ushiki and colleagues also reported the relationship between AE-ILD and RSV and suggested the possibility that RSV produces proinflammatory cytokines to induce AE-ILD [11]. Although the precise role of RSV in AE-ILD remains unknown, we should consider the pathogenesis of RSV in AE-ILD.

In addition, influenza virus was detected along with other viruses in two cases by Luminex xTAG-RVP. Multiple viral infection is not rare, and co-infection rates of 4.8%–42.5% have been reported [15–17]. Influenza virus also induces acute exacerbation of chronic obstructive pulmonary disease (COPD) [18]. Interestingly, a case of AE-IPF after pandemic influenza (H1N1) vaccination was reported [19]. However, the association with AE-ILD was not confirmed. Further study and accumulation of evidence are warranted to address this question.

The Biofire FA-RP and the Luminex xTAG-RVP assays show cross-reactivity between rhinovirus and enterovirus [20]. One case demonstrated a combination of influenza virus infection and rhinovirus/enterovirus. Rhinovirus is one of the most frequently detected pathogens in the common cold. Rhinovirus is also known to induce AE-COPD [18], but there is little evidence that rhinovirus induces AE-ILD. However, Wootton and colleagues reported two cases in which rhinoviruses were detected in AE-ILD [2]. Thus, rhinovirus infection should be carefully monitored in ILD patients. The authors also revealed additional evidence of viral infection (herpes simplex virus, Epstein–Barr virus, and Torque teno virus [TTV]) in patients with acute exacerbation by pan-viral microarrays, suggesting a relationship between TTV and acute exacerbation. Unfortunately, we could not assess TTV in this study.

In this study, we used two RT-PCR systems for detection of respiratory viruses. The BioFire FA-RP was the easiest to use and had the shortest time to result. The Luminex xTAG-RVP was the first large multiplex panel cleared by the FDA. With multiple steps, it had the longest hands-on time and longest time to result. Studies comparing these molecular multiplex platforms to in-house molecular methods have shown overall sensitivities and specificities, respectively, of 89.4% and 99.6% for the BioFire FA-RP [21], and 91.2% and 99.7% for the Luminex xTAG-RVP [22]. The sensitivity of each assay fluctuated by viral target, with the greatest discrepancies noted for adenovirus and influenza virus B detection. There was no statistical difference between the xTAG-RVP and Biofire FA-RP [15–17]. In the present study, the Luminex xTAG-RVP assay demonstrated three cases of respiratory virus infection, whereas the BioFire FA-RP identified only one case. The Luminex xTAG-RVP assay might be more sensitive, but setting of an appropriate threshold may represent a problem. Additionally, it has been reported that the Luminex xTAG-RVP assay has a high false-positive rate [20] and a longer procedure time than BioFire FA-RP and may detect minute contamination. For these reasons, we speculate that there were differences in the results between the two assays.

We compared the clinical background and clinical course between the respiratory virus-positive and virus-negative groups. There were no differences in clinical setting between the two groups. The death rate was also similar. White blood cell count and CRP and LDH levels were serum markers of acute exacerbation, with CRP and LDH tending to be high. Thus, the potential for worsening in AE-ILD was increased by virus infection. Another possibility was that subsequent bacterial infection after viral infection played a certain role. Although prednisolone use before acute exacerbation seemed to be more frequently observed, it was not significant and the precise role of PSL in AE-ILD remains unknown. Bronchial asthma exacerbated by virus infection was reported to be more severe than that in normal subjects [23]. Studies covering more cases of AE-ILD are necessary to clarify the role of virus infection in this condition.

There may be some possible limitations in this study. First, because the number of cases was small, we could not detect distinguishing clinical characteristics in the respiratory virus infection group. Second, this study was performed at a single center, leading to the possibility of bias. Third, although the sensitivity and specificity of multiplex screening is high, we did not try to isolate the virus. Fourth, in this study, pharyngeal swab was used as a specimen. Nasal aspirate and lower airway specimens such as BAL fluids, which are considered to have a higher virus concentration, were not used. Although colonization of respiratory virus was denied in a previous study [2], we did not directly exclude colonization using stable patients. However, the present study is the first Japanese prospective study of respiratory virus detection in AE-ILD using globally available methods. In future, we shall try to overcome these limitations. Further multi-center prospective studies will be needed to clarify the role of viral infection in AE-ILD.

Authors' contributions to the study

RO and MF conceived and carried out the experiments, and prepared the manuscript. TM, RH, and HK cooperated with accumulation of clinical data. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare there are no conflicts of interests.

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