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Prognostic significance of serum cytokines during acute exacerbation of idiopathic interstitial pneumonias treated with thrombomodulin

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ABSTRACT

Background Acute exacerbation (AE) has been reported to herald a poor prognosis in idiopathic pulmonary fibrosis and is now thought to do so in idiopathic interstitial pneumonias (IIPs). However, the pathophysiology of AE-IIPs is not sufficiently understood. In our previously reported SETUP trial, we found better survival in patients with AE-IIPs treated with corticosteroids and thrombomodulin than in those treated with corticosteroids alone. In that study, we collected serum samples to evaluate changes in cytokine levels and retrospectively examined the prognostic significance and pathophysiological role of serum cytokines in patients with AE-IIPs.

Methods This study included 28 patients from the SETUP trial for whom serial serum samples had been prospectively obtained. AE-IIPs were diagnosed using the Japanese Respiratory Society criteria. All patients were treated with intravenous thrombomodulin and corticosteroids from 2014 to 2016. Serum levels of 27 cytokines were measured using Bio-Plex. The high-resolution CT pattern at the time of diagnosis of AE was classified as diffuse or non-diffuse.

Results Univariate analysis revealed that higher serum levels of interleukin (IL)-2, IL-7, IL-9, IL-12, IL13, basic fibroblast growth factor, granulocyte-macrophage colony-stimulating factor, interferon-γ inducible protein-10, platelet-derived growth factor and regulated on activation, normal T cell expressed and secreted (RANTES) at AE were significant predictors of 90-day survival. The HRCT pattern was also a significant clinical predictor of 90-day survival. Multivariate analysis with stepwise selection identified a higher serum RANTES level at AE to be a significant predictor of 90-day survival, including after adjustment for HRCT pattern. Multivariate analysis with stepwise selection suggested that a marked increase in the serum IL-10 level on day 8 could predict 90-day mortality.

Conclusions A higher serum RANTES level at AE the time of diagnosis predicted a good survival outcome, and an elevated serum IL-10 level on day 8 predicted a poor survival outcome.

Trial registration number UMIN000014969.

Key messages

- What is the most important cytokine to predict survival of acute exacerbation (AE) in idiopathic interstitial pneumonias (IIPs)?
- We found that a higher serum regulated on activation, normal T cell expressed and secreted level at the time of diagnosis of AE predicted a good survival outcome, and an elevated serum interleukin-10 level on day 8 predicted a poor outcome.
- The result might suggest pathophysiological significance of these cytokines and lead to the new treatment of AE-IIPs.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a lung disease of unknown aetiology and has a poor prognosis.[1 2] Some patients with IPF experience rapid potentially fatal deterioration[1–4] known as acute exacerbation (AE) of IPF (AE-IPF). AE is the most common cause of death in IPF,[4] despite affected patients usually being treated with corticosteroids according to the 2011 American Thoracic Society (ATS)/European Respiratory Society (ERS)/Japanese Respiratory Society (JRS)/Latin American Thoracic Association guidelines for diagnosis and management of IPF.[1] Therefore, a better understanding of the pathophysiology of AE-IPF is required to improve its management. Furthermore, the prognosis of AE in unclassifiable idiopathic interstitial pneumonias (IIPs) or interstitial lung disease other than IIPs is as poor as that of IPF.[5 6]

Recombinant human soluble thrombomodulin (rhTM), which contains only the extracellular domain of thrombomodulin, has recently been approved for treatment of disseminated intravascular coagulation (DIC)[7] and has been reported to be effective

for AE-IPF[8] when coupled with conventional corticosteroid therapy. We have previously reported on the efficacy of rhTM in patients with AE-IIPs who participated in our prospective multicentre SETUP trial.[9] Thrombomodulin regulates coagulation[10] and inhibits inflammation by preventing adhesion of neutrophils to the endothelium, decreasing the expression of adhesion molecules and enhancing the barrier function of endothelium and degradation of high-mobility group box 1.

Although the pathophysiology of AE-IPF is unknown, disordered integrity of epithelial cells, cellular inflammation, cytokines, matrix metalloproteinases, coagulation and fibrinolysis are likely to be involved, and rapid changes in these phenomena are thought to contribute to AE-IPF.[4] Collard *et al*[11] found that serum Krebs von den Lungen (KL)-6, interleukin (IL)-6 and thrombomodulin levels were higher in patients with AE-IPF than in those with stable IPF. Moreover, using a small number of lung specimens from patients with AE-IPF or stable IPF, Konishi *et al*[12] found that gene expression of AE-IPF was associated with more severe epithelial injury and proliferation.

Although there have been a few reports on changes in serum levels of several cytokines in patients with AE-IPF,[13–15] their importance in the pathophysiology and prognosis of AE-IPF and AE-IIPs has not been examined in sufficient depth. Therefore, in this retrospective study, we evaluated the prognostic and pathophysiological significance of serum cytokine levels in patients with AE-IIPs who had participated in the SETUP trial and from whom serum samples had been collected.

METHODS

Subjects

The SETUP trial included 39 patients with a diagnosis of AE-IIPs based on the modified JRS criteria[16] who had been prospectively enrolled and treated with rhTM and conventional therapy, including corticosteroids and immunosuppressants, from 2014 to 2016. Patients with severe non-pulmonary disease, life-threatening bleeding, clinically significant infection, known hypersensitivity to rhTM, a cerebrovascular event within the past year or surgery within the previous month were excluded. Pregnancy or the possibility of pregnancy was an additional exclusion criterion. Serum samples had been collected from 28 of the 39 patients at the time of diagnosis of AE-IIPs (on day 1) and from 22 patients on day 8 (online supplemental figure 1). Patient characteristics of 28 patients with serum samples in the present study were similar to the rest of the subjects in SETUP trial (n=11) without serum samples (online supplemental table 1).

The trial was registered with the University Hospital Medical Information Network Center (28 August 2014). All participants in SETUP provided written informed consent.

Diagnosis of underlying IIPs and AE-IIPs

The underlying IPF was diagnosed based on the ATS/ERS/JRS/Latin American Thoracic Association guidelines for diagnosis and management of IPF.[1] The diagnosis of IIPs was based on the ATS/ERS/JRS statement,[17] and IIPs that could not be diagnosed as IPF was classified as non-IPF IIPs. Patients with non-IPF IIPs did not undergo surgical lung biopsy and were diagnosed to have unclassifiable IIPs.

AE-IIPs were diagnosed based on the following modified JRS diagnostic criteria for AE-IPF.[16] (1) Within 1 month, the following three conditions should be satisfied: (A) progressively worsening dyspnoea; (B) new ground-glass opacities evident on high-resolution CT (HRCT) scans superimposed on background reticular opacity, traction bronchiectasis, traction bronchiolectasis or honeycombing; and (C) a reduction in resting partial pressure of oxygen in arterial blood (PaO_2) of >10 Torr compared with previous measurements. (2) Other causes of acute respiratory impairment, such as infection, pneumothorax, cancer, pulmonary embolism and congestive cardiac failure, should be excluded. However, the possibility that some patients had AE triggered by infection[4] could not be excluded because bronchoalveolar lavage could not be performed in all cases.

Treatment for AE-IIPs

Details of the treatment of the patients with AE-IIPs in the SETUP trial are provided in online supplemental table 2. Treatment included prednisolone with/without an immunosuppressant after intravenous administration of high-dose methylprednisolone for three consecutive days. Polymyxin B-immobilised fibre column direct hemoperfusion (PMX/DHP) therapy[13 15] was administered in one case using the Toraymyxin system (Toray Medical, Tokyo, Japan), which was attached for 4–6 hours at a flow rate of 80 mL/min and repeated once within 48 hours. Nafamostat mesilate (Torii Pharmaceuticals, Tokyo, Japan) was used to reduce the risk of clot formation in the catheter. Anticoagulant and antiplatelet drugs were permitted only to treat comorbidities. Invasive or non-invasive positive pressure ventilation or nasal high flow therapy was introduced to maintain oxygenation in some patients with severe respiratory failure. rhTM was started at a rate of 380 U/kg/day for 6 days, as approved for treatment of DIC,[7–9] on the day of AE-IIPs diagnosis.

Evaluation of AE-IIPs

AE-IIPs were evaluated before and at the time of diagnosis of AE as described in our previous report.[9] Briefly, the severity of IIPs (stages I–IV, mildest to most severe) in the stable state was evaluated in most cases within 6 months before the time of diagnosis of AE according to the JRS criteria.[16] Shortness of breath was evaluated based on the modified Medical Research Council score.[18] DIC was diagnosed according to the Japanese Association for Acute Medicine

scoring system.[19] The fibrin degradation product level was classified as higher (≥10 mg/L) or lower (<10 mg/L). The HRCT pattern at the time of diagnosis of AE-IIPs was classified as peripheral, multifocal or diffuse using the definition proposed by Akira *et al*[3] and subsequently according to whether it was diffuse or non-diffuse. Patients with a PaO_2/fraction of inspired oxygen (FiO_2) ratio ≤200[20] were deemed to have severely impaired oxygenation.

Evaluation of serum biomarkers

Serum KL-6 levels were measured using a commercial ELISA kit (Eizai, Tokyo, Japan). The KL-6 cut-off level was 500 U/mL.[9] Cytokine levels in the serum samples (pg/mL) were quantified using the Bio-Plex Suspension Array System with Bio-Plex Pro Human Cytokine Group 27-Plex Panel (Bio-Rad Laboratories, Hercules, California, USA) according to the manufacturer's instructions.[15] This panel was selected because we could simultaneously measure serum levels of chemokines, fibrotic cytokines and angiogenic cytokines, and it was used in our previous study.[15] The cytokines measured included IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7. IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, eotaxin/CC chemokine ligand (CCL) 11, basic fibroblast growth factor (b-FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor, interferon (IFN)-γ, IFN-γ inducible protein 10 (IP-10)/CXC chemokine ligand (CXCL) 10, monocyte chemotactic protein-1 (MCP-1)/CCL2, macrophage inflammatory protein (MIP)-1α/CCL3, MIP-1β/CCL4, platelet-derived growth factor (PDGF)-BB, regulated on activation, normal T cell expressed and secreted (RANTES)/CCL5, tumour necrosis factor (TNF)-α and vascular endothelial growth factor (VEGF)-A. The cytokine levels in each patient, out of the range below or above, were defined to the upper range or half of the lower range, respectively. Serum IL-1β, IL-15 and VEGF-A levels at the time of diagnosis of AE were out of range in more than half of all cases (n=26, n=22 and n=19, respectively). These three cytokines were excluded from further examination.

Statistical analysis

Continuous and categorical data are presented as the median (IQR) or the number (percentage) and were compared between the rhTM and control arms using the Wilcoxon rank-sum test and Fisher's exact test, respectively. Logistic regression analysis was performed to predict 90-day survival using clinical parameters, serum cytokine levels at the time of diagnosis of AE and the increase in serum cytokine levels on day 8. All statistical analyses were performed using JMP software V.11 (SAS Institute). All p values are two sided. A p value <0.05 was considered statistically significant.

RESULTS

Patient characteristics

The 28 patients with AE-IIPs comprised eight with IPF and 20 with non-IPF IIPs. Clinical parameters before and at the time of diagnosis of AE were similar between those with AE-IPF and those with AE-non-IPF. The 90-day survival rate was similar between these two groups (table 1). Underlying IIPs were treated with antifibrotic drugs (n=5; nintedanib for one case and pirfenidone for four cases) and prednisolone (n=9) before the time of diagnosis of AE (table 1). Univariate logistic regression analysis revealed that use of antifibrotic drugs in a stable state, a diffuse HRCT pattern and higher serum KL-6 and lactate dehydrogenase levels at the time of diagnosis of AE were significant clinical predictors of 90-day mortality (table 2). Use of antifibrotic drugs in a stable state and a diffuse HRCT pattern at the time of diagnosis of AE were significant predictors in multivariate analysis. Use of antifibrotic drugs at stable state was significantly associated with introduction of long-term oxygen therapy (p=0.0047) and disease severity stage IV (p=0.0047) before the diagnosis of AE (online supplemental table 3).

There was no significant difference in serum cytokine levels between the AE-IPF and AE-non-IPF groups (online supplemental table 4).

Survival and cytokines at the time of diagnosis of AE

Serum cytokine levels were compared between patients who were alive at 90 days and those who were not. Serum IL-2, IL-7, IL-9, IL-13, GM-CSF, IP-10, PDGF-BB and RANTES/CCL5 levels were higher in survivors than in non-survivors (table 3). Univariate logistic regression analysis revealed higher IL-2, IL-7, IL-9, IL-12, IL13, b-FGF, GM-CSF, IP-10, PDGF-BB and RANTES/CCL5 levels to be significant predictors of 90-day survival (table 4). Multivariate logistic analysis with stepwise selection revealed that IL-7 and RANTES/CCL5 should be included in the model; multivariate analysis using these two parameters identified RANTES/CCL5 to be a significant predictor of 90-survival (table 4). RANTES/CCL5 was a significant predictor after adjustment for HRCT pattern at the time of diagnosis of AE and antifibrotic drug use before AE (table 4). RANTES/CCL5 was a significant predictor of 90-day survival in AE-IIPs without use of antifibrotic drugs before AE (p=0.010, data not shown).

RANTES/CCL5 level and clinical parameters

The serum RANTES/CCL5 level was not significantly associated with any clinical parameter suggesting severe underlying IIPs in a stable state or during AE, including use of long-term oxygen therapy in a stable state and the PaO_2/FiO_2 and HRCT pattern at the time of diagnosis of AE. There was a significant correlation between the serum RANTES/CCL5 level and administration of antifibrotic drugs before AE (online supplemental table 5); however, only five patients were treated with these agents (table 1).

Table 1 Patient characteristics in this study*

Parameters	AE-IIPs (n=28)	AE-IPF (n=8)	AE-non-IPF (n=20)	P value
Stable state				
Sex, male/female	21/7	8/0	13/7	0.0749
Smoking, yes/no	19/9	7/1	12/8	0.2144
mMRC, ≤1/≥2	10/18	3/5	7/13	1.0000
Stage, I–III/IV	17/11	4/4	13/7	0.6715
LTOT before AE, yes/no	11/17	4/4	7/13	0.6175
Prednisolone before AE, yes/no	9/19	2/6	7/13	1.0000
Antifibrotic drugs, yes/no	5†/23	2‡/6	3§/17	0.6056
At the time of diagnosis of AE				
Age, years	74.5 (69.75–79.0)	74.5 (69.5–78.25)	74.5 (69.75–84.0)	0.7405
HRCT pattern, diffuse/non-diffuse	12/16	4/4	8/12	0.6908
PaO_2/FiO_2 ratio, ≤200/>200	17/11	6/2	11/9	0.4188
WCC, /μL	10 150 (9050–12 950)	10 300 (9000–14 400)	10 150 (9250–12 950)	0.9594
LDH, U/L	317.0 (245.5–404.5)	296.5 (246.5–414.25)	341.0 (245.5–403.75)	0.9797
KL-6, U/mL	1196 (889–2,142)	1024.5 (889.5–1394)	1346 (849.75–2359)	0.3091
CRP, mg/dL	12.38 (5.07–14.28)	5.455 (0.38–12.56)	2.982 (0.871–9.815)	0.8787
FDP, 10</≥10 mg/L	21/7	4/4	17/3	0.1423
90-day survival, yes/no	19/9	6/2	13/7	1.0000

*Each parameter was compared between the AE-IPF and AE-non-IPF groups using Fisher's exact test or the Wilcoxon rank-sum test.
†Pirfenidone (n=4) and nintedanib (n=1).
‡Pirfenidone (n=1) and nintedanib (n=1).
§Pirfenidone (n=3) and nintedanib (n=0).
AE, acute exacerbation; CRP, C reactive protein; FDP, fibrin degradation product; FiO_2, fraction of inspired oxygen; HRCT, high-resolution CT; IIPs, idiopathic interstitial pneumonias; IPF, idiopathic pulmonary fibrosis; KL-6, Krebs von den Lungen-6; LDH, lactate dehydrogenase; LTOT, long-term oxygen therapy; mMRC, modified medical research council score; PaO_2, arterial oxygen tension; WCC, white blood cell count.

Cytokine levels from days 1–8

By day 8, there was a significant increase in serum IL-4, IL-13, eotaxin and b-FGF levels and a significant decrease in serum IL1-ra, IL-9, IL-10, IFN-γ, IP-10, MIP-1β, RANTES/CCL5 and TNF-α levels (online supplemental table 6).

Survival and increased cytokines on day 8

The increase in serum IL-2, IL-5, IL-7, IL-9, IL-10, G-CSF, IFN-γ and MCP-1 levels on day 8 was significantly smaller in survivors than in non-survivors at 90 days (table 5). The median increase in the levels of these cytokines except for IL-2 was less than zero, suggesting a decrease on day 8 in 90-day survivors. Univariate logistic regression analysis revealed that an increase in IL-7, IL-10, G-CSF, IFN-γ and MCP-1 levels suggested mortality at 90 days, and multivariate analysis with stepwise selection suggested that an increase in IL-10 on day 8 could predict 90-day mortality (table 6).

Increased serum IL-10 and clinical parameters

There was no association between an increased IL-10 level on day 8 and any other clinical parameter at the time of diagnosis of AE or in a stable state (online supplemental table 7).

DISCUSSION

We have examined the levels of multiple cytokines in serum samples that were prospectively collected from patients with AE-IIPs enrolled in the SETUP trial.[9] This type of evaluation is rare. We found that a higher serum RANTES/CCL5 level at the time of diagnosis of AE-IIPs suggested a good prognosis after treatment with corticosteroids and thrombomodulin. Furthermore, a greater increase in serum IL-10 at 1 week after the time of diagnosis of AE was a significant predictor of a poor prognosis.

RANTES/CCL5 is reportedly a chemotactic factor for lymphocytes, eosinophils, monocytes and mast cells and is produced by lymphocytes, fibroblasts, platelets, endothelial cells and epithelial cells.[21] RANTES/CCL5 has been found to be elevated in the bronchoalveolar lavage fluid of patients with nonspecific interstitial pneumonia[22] and in those with IPF.[23] Pierce *et al*[24] showed that RANTES/CCL5 works with CCL21 to affect pulmonary fibrosis. RANTES/CCL5 is reported to increase inflammation and fibrosis in the presence of renal or hepatic disease.[25] Hence, the good prognosis suggested by a higher RANTES/CCL5 level at the time of diagnosis of AE in our study indicates that it has an important pathophysiological role in AE-IIPs.

Table 2 Prognostic significance of clinical parameters in patients with AE-IIPs determined by logistic regression analysis

Parameter	OR*	95% CI	P value
Univariate			
Stable state			
Sex, male/female	1.2499	0.2060 to 10.2093	0.8140
Smoking, yes/no	2.2400	0.4135 to 12.3435	0.7376
Diagnosis, IPF/non-IPF	0.6190	0.0765 to 3.5887	0.6037
mMRC, ≤1/≥2	1.1666	0.2251 to 6.9237	0.8560
Stage, I–III/IV	2.7083	0.5354 to 14.8724	0.2275
LTOT before AE, yes/no	2.7083	0.5354 to 14.8724	0.2275
Prednisolone before AE, yes/no	2.2400	0.4135 to 12.3435	0.3431
Antifibrotic drugs before AE, yes/no	14.400	1.6803 to 319.0268	0.0137
At the time of diagnosis of AE			
Age, years	0.9929	0.8995 to 1.0986	0.8863
HRCT pattern, diffuse/non-diffuse	9.8000	1.7196 to 83.0325	0.0091
PaO_2/FiO_2 ratio, ≤200/>200	0.7291	0.1425 to 3.8110	0.7014
WCC, /μL	1.0001	0.9999 to 1.0004	0.1607
LDH, U/L	1.0065	1.0007 to 1.0145	0.0244
KL-6, U/mL	1.0009	1.0001 to 1.0020	0.0146
CRP, mg/dL	0.9678	0.8582 to 1.0777	0.6268
FDP, ≥10/<10 mg/L	4.2666	0.7166 to 28.9076	0.1102
Multivariate†			
HRCT pattern, diffuse/non-diffuse	13.3634	1.7053 to 285.2057	0.0118
Antifibrotic drugs before AE, yes/no	21.3180	1.6243 to 860.5019	0.0179

*An OR >1 means each categorical parameter or increase in each continuous parameter indicates a high risk of 90-day mortality.
†Multivariate analysis with a stepwise selection procedure was performed to predict 90-day survival using significant parameters with a p value <0.05.
AE, acute exacerbation; CRP, C reactive protein; FDP, fibrin degradation product; FiO_2, fraction of inspired oxygen; HRCT, high-resolution CT; IIPs, idiopathic interstitial pneumonias; IPF, idiopathic pulmonary fibrosis; KL-6, Krebs von den Lungen-6; LDH, lactate dehydrogenase; LTOT, long-term oxygen therapy; mMRC, modified Medical Research Council score; PaO_2, arterial oxygen tension; WCC, white blood cell count.

We also found that serum IL-7, IL-9, IL-13, GM-CSF, IP-10, PDGF-BB and RANTES/CCL5 levels were significantly higher in survivors than in non-survivors. PDGF[26] and IL-13[27] are profibrotic cytokines, similar to RANTES/CCL5,[21–25] while IL-7,[28 29] GM-CSF[30] and IP-10/CXCL10[31] are antifibrotic cytokines. Whether IL-9 is profibrotic[32] or antifibrotic[33] is controversial; however, IL-9 is associated with regulation of pulmonary fibrosis. The results of our study suggest that an early increase in fibrosis-associated profibrotic and antifibrotic cytokines might affect the prognosis of AE-IIPs.

The pathophysiology of AE-IIPs is not yet fully clarified; however, it is thought to involve acute epithelial injury superimposed on chronic progressive interstitial lung disease. If the epithelial cells regenerate rapidly, the intact alveolar epithelial cell layer can suppress proliferation of fibroblasts and pulmonary fibrosis may be inhibited.[26 34 35] Although overgrowth of fibroblasts and overproduction of collagen are not favourable for pulmonary fibrosis, keratinocyte growth factor secreted by fibroblasts helps recovery from injury-stimulated proliferation of alveolar epithelial cells.[36] Hence, rapid recruitment of fibroblasts at the site of injury by fibrosis-related cytokines and increased production of keratinocyte growth factor might lead to rapid repair of alveolar epithelial cells. Rapid reduction in fibrosis-related cytokine levels and proliferating fibroblasts might be necessary to prevent development of fibrosis.

The pathophysiology of AE-IPF has some similarity to that of the COVID-19 caused by SARS-CoV-2.[37] The virus infects the epithelium of the upper and lower airways via ACE 2 receptors, and diffuse alveolar damage occurs after extensive apoptosis of alveolar epithelial cells. Recently, Sugiyama *et al*[37] reported a low CCL17 level at the time of diagnosis of SARS-CoV-2 infection to be a significant predictor of severe COVID-19. CCL17 is reported to be a profibrotic cytokine in mice with bleomycin-induced lung injury[38] and to be elevated in bronchioalveolar lavage fluid from patients with IPF.[39] The disadvantage of lower levels of profibrotic cytokines in early lung injury might be similar between COVID-19 infection and AE-IPF or AE-IIPs.

Fibrocytes are circulating cells with fibroblast-like properties that are involved in both normal tissue wound

Table 3 Serum cytokines at the time of diagnosis of AE-IIPs: comparison between 90-day survivors and non-survivors*

Parameters	Survivors (n=19)	Non-survivors (n=9)	P value
IL-ra	95.48 (25.88 to 248.77)	161.6 (6.76 to 766.6)	0.8240
IL-2	0.430 (0.340 to 0.950)	0.240 (0.155 to 0.405)	0.0128
IL-4	0.300 (0.190 to 0.440)	0.270 (0.205 to 0.335)	0.4163
IL-5	1.380 (1.010 to 2.320)	1.010 (0.780 to 1.615)	0.0847
IL-6	0.480 (0.24 to 1.390)	1.100 (0.190 to 2.150)	0.7490
IL-7	1.410 (1.080 to 1.970)	0.800 (0.445 to 1.340)	0.0138
IL-8	3.510 (2.410 to 7.140)	3.970 (2.110 to 27.220)	0.9021
IL-9	2.150 (1.940 to 3.160)	1.790 (1.600 to 2.045)	0.0058
IL-10	0.900 (0.590 to 1.610)	0.590 (0.375 to 1.775)	0.3370
IL-12	0.260 (0.120 to 0.490)	0.120 (0.050 to 0.175)	0.0738
IL-13	0.210 (0.120 to 0.380)	0.070 (0.040 to 0.105)	0.0083
IL-17	1.090 (0.087 to 1.720)	1.090 (0.700 to 1.335)	0.5059
Eotaxin/CCL11	17.55 (10.17 to 32.92)	18.79 (10.98 to 20.76)	0.6055
b-FGF	5.860 (4.720 to 7.850)	4.720 (4.410 to 6.025)	0.0823
G-CSF	5.210 (0.980 to 25.140)	1.560 (0.34 to 22.688)	0.4151
GM-CSF	0.070 (0.040 to 0.320)	0.020 (0.020 to 0.040)	0.0119
IFNγ	0.800 (0.530 to 2.220)	0.620 (0.510 to 1.410)	0.5218
IP-10/CXCL10	605.6 (307.6 to 1293.8)	215.4 (154.8 to 538.7)	0.0195
MCP-1/CCL2	5.650 (3.280 to 22.530)	4.500 (2.640 to 13.145)	0.4029
MIP-1α/CCL3	0.690 (0.400–1.230)	0.510 (0.280 to 8.205)	0.3758
PDGF-BB	87.81 (68.72 to 144.2)	47.88 (21.35 to 91.49)	0.0366
MIP-1β/CCL4	248.2 (186.1 to 307.1)	200.3 (193.6 to 355.3)	0.6759
RANTES/CCL5	2370 (1803 to 5610)	1568 (1333 to 1859)	0.0085
TNF-α	4.680 (4.040 to 6.420)	3.840 (3.205 to 5.130)	0.0518

*Serum cytokine levels at the time of diagnosis of AE (pg/mL) were compared between 90-day survivors and non-survivors using the Wilcoxon rank-sum test.
G-CSF; granulocyte colony-stimulating factor; AE, acute exacerbation; b-FGF, basic fibroblast growth factor; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IIPs, idiopathic interstitial pneumonias; IL, interleukin; IP-10, IFN-γ inducible protein; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T cell expressed and secreted; TNF, tumour necrosis factor.

repair and fibrosis. Fibrocytes secrete a variety of cytokines to augment fibrotic lesions by activating resident fibroblasts.[40] Fibrocytes are an important source of fibroblasts and myofibroblasts. In one study, circulating fibrocytes indicated a poor prognosis in IPF,[41] with circulating fibrocyte counts in the blood of patients with AE-IPF found to be elevated by up to 10-fold in comparison with the values in stable IPF.

Human fibrocytes express several chemokine receptors, including CCR2, CCR5, CCR7 and CXCR4.[42] RANTES/CCL5 is known to induce migration of circulating fibrocytes in patients with severe asthma[43] and might be associated with pulmonary fibrosis. Although this hypothesis supports the notion that RANTES/CCL5 worsens pulmonary fibrosis, Tai *et al*[44] demonstrated in a mouse model that intravenous infusion of fibrocytes ameliorated lipopolysaccharide-induced acute lung injury by decreasing inflammatory cytokine and chemokine levels and reducing accumulation of neutrophils in the lung. Early recruitment of an appropriate number of fibrocytes might lead to normal repair instead of pulmonary fibrosis; this hypothesis is consistent with our finding that higher levels of serum RANTES/CCL5 at the time of diagnosis of AE are associated with a good prognosis in patients with AE-IIPs.

IL-7 inhibits production of transforming growth factor (TGF)-β and fibroblast signalling.[28] Moreover, IL-7 and TGF-β play counter-regulatory roles in synthesis of collagen by fibroblasts in pulmonary fibrosis.[29] We have shown that a higher IL-7 level at the time of diagnosis of AE can predict a good prognosis and that the IL-7 level was significantly lower on day 8 than at the time of diagnosis. We have previously reported that an increase in IL-7 24–48 hours after the start of PMX/DHP therapy predicts a good survival outcome.[15] These results may seem inconsistent; however, the exact time course of the serum IL-7 level from the time of diagnosis of AE-IIPs (day 1) to day 8 is not known, and the peak level may be on day 3.

Table 4 Prognostic significance of serum cytokines for AE-IIPs determined by logistic regression analysis for 90-day mortality

Parameters	OR*	95% CI	P value
Univariate analysis			
IL-ra	1.0002	0.9986 to 1.0015	0.7622
IL-2	0.0180	$5.151\times e^{-5}$ to 0.5882	0.0164
IL-4	0.0121	$2.495\times e^{-6}$ to 3.698	0.1477
IL-5	0.4017	0.0787 to 1.1196	0.0873
IL-6	1.0282	0.5270 to 1.8497	0.9261
IL-7	0.1333	0.0133 to 0.6706	0.0089
IL-8	0.9962	0.9652 to 1.0098	0.6278
IL-9	0.1535	0.0070 to 0.8868	0.0180
IL-10	0.9250	0.3274 to 2.2574	0.8672
IL-12	0.0067	$3.545\times e^{-6}$ to 0.7440	0.0326
IL-13	$7.762\times e^{-5}$	$4.94\times e^{-10}$ to 0.1873	0.0092
IL-17	0.4369	0.0572 to 2.2218	0.3331
Eotaxin/CCL11	0.9626	0.8830 to 1.0327	0.3008
b-FGF	0.5378	0.2200 to 0.9869	0.0444
G-CSF	0.9968	0.9590 to 1.0276	0.8467
GM-CSF	$1.128\times e^{-7}$	$1.91\times e^{-21}$ to 0.1393	0.0141
IFNγ	0.7547	0.2793 to 1.4865	0.4491
IP-10/CXCL10	0.9976	0.9943 to 0.9997	0.0166
MCP-1/CCL2	0.9773	0.8973 to 1.0293	0.4307
MIP-1α/CCL3	1.0107	0.8953 to 1.1226	0.8424
PDGF-BB	0.9764	0.9483 to 0.9968	0.0184
MIP-1β/CCL4	0.9859	0.9564 to 1.0068	0.2011
RANTES/CCL5	0.9979	0.9950 to 0.9996	0.0020
TNF-α	0.6796	0.3111 to 1.0278	0.0744
Multivariate analysis†			
IL-7	0.2270	0.0177 to 1.5548	0.1384
RANTES/CCL5	0.9980	0.9953 to 0.9998	0.0263
Adjusted by clinical parameters‡			
RANTES/CCL5	0.9970	0.9934 to 0.9993	0.0018
HRCT pattern, diffuse/non-diffuse	19.2129	1.9938 to 438.0654	0.0084

*OR >1 means an increase in each continuous parameter (pg/mL) indicating high risk of 90-day mortality.
†Multivariate logistic regression analysis with stepwise selection was performed using 10 significant cytokines by univariate analysis with a p value <0.05: IL-2, IL-7, IL-9, IL-12, IL13, b-FGF, GM-CSF, IP-10, PDGF and RANTES.
‡Multivariate logistic regression analysis with stepwise selection was performed using RANTES and HRCT pattern at the time of diagnosis AE and use of antifibrotic drugs before AE.
AE, acute exacerbation; b-FGF, basic fibroblast growth factor; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HRCT, high-resolution CT; IFN, interferon; IIPs, idiopathic interstitial pneumonias; IL, interleukin; IP-10, IFN-γ inducible protein; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T cell expressed and secreted; TNF, tumour necrosis factor.

In this study, we demonstrated that a greater increase in serum IL-10 1 week after the time of diagnosis of AE was a significant predictor of a poor prognosis. IL-10 is a well-known anti-inflammatory cytokine that inhibits production of proinflammatory cytokines and chemokines by monocytes and macrophages.[45] The profibrotic role of IL-10 is controversial. Silica-induced pulmonary fibrosis was markedly less severe in IL-10 deficient mice than in wild-type mice because of a reduction in lung levels of TGF-β, which is a potent inducer of pulmonary fibrosis.[45] However, transient expression of IL-10 by gene transfer for less than 1 week reduced the severity of bleomycin-induced pulmonary fibrosis by reducing production and activation of TGF-β in bronchioalveolar lavage fluid

Table 5 Increase in serum cytokines in patients with AE-IIPs who were alive at 90 days and those who were not*

Parameters	Survivors (n=16)	Non-survivors (n=6)	P value
ΔIL-ra	−53.0175 (−297.7100 to −4.1050)	−132.3275 (−371.7150 to 32.3925)	0.8537
ΔIL-2	0.0050 (−0.0850 to 0.2150)	0.2600 (0.0925 to 0.3300)	0.0356
ΔIL-4	0.1050 (0.0625 to 0.1875)	0.0600 (−0.0300 to 0.1800)	0.4835
ΔIL-5	−0.6200 (−1.1050 to 0.0425)	0.5700 (−0.0550 to 1.0225)	0.0183
ΔIL-6	−0.1950 (−0.3725 to 0.0625)	0.1175 (−0.5975 to 0.6150)	0.3379
ΔIL-7	−0.3200 (−0.7425 to 0.0675)	0.1100 (−0.2525 to 0.7400)	0.0389
ΔIL-8	−0.4350 (−2.7925 to 0.9325)	0.5400 (−0.4375 to 6.9350)	0.3020
ΔIL-9	−0.4300 (−0.9275 to −0.0200)	0.0700 (−0.1800 to 0.5450)	0.0296
ΔIL-10	−0.5875 (−0.9575 to −0.2275)	0.2200 (0.0100 to 1.3975)	0.0015
ΔIL-12	−0.0100 (−0.1525 to 0.1400)	0.1200 (−0.0250 to 0.2125)	0.2530
ΔIL-13	0.1000 (0.0025 to 0.4475)	0.0950 (−0.0150 to 0.2750)	0.7399
ΔIL-17	0.0450 (−0.1725 to 0.3575)	0.0200 (−0.3625 to 0.6225)	0.7963
ΔEotaxin/CCL11	17.7050 (13.0075 to 37.4450)	10.3600 (−4.7950 to 26.0525)	0.1048
Δb-FGF	0.3450 (0.1500 to 1.4000)	1.2900 (0.2025 to 3.0475)	0.2381
ΔG-CSF	−0.0350 (−12.2625 to 1.4288)	2.6325 (0.7013 to 3.1888)	0.0325
ΔGM-CSF	−0.0175 (−0.0800 to 0.0775)	0.0075 (−0.0150 to 0.0850)	0.1798
ΔIFNγ	−0.5350 (−1.7175 to −0.2850)	−0.0900 (−0.2525 to 0.2575)	0.0183
ΔIP-10/CXCL10	−214.3300 (−570.4550 to 32.1200)	−23.8450 (−393.8175 to 22.3575)	0.4610
ΔMCP-1/CCL2	−1.5100 (−9.5725 to −0.3175)	5.5650 (2.3700 to 27.1600)	0.0032
ΔMIP-1α/CCL3	−0.2050 (−0.3600 to −0.0025)	0.0400 (−0.0700 to 0.3200)	0.0900
ΔPDGF-BB	−11.0900 (−52.3975 to 12.8250)	−5.800 (−35.8575 to 44.4400)	0.6583
ΔMIP-1β/CCL4	−109.4150 (−167.2625 to −58.6475)	−88.8850 (−161.6425 to −58.8800)	0.7124
ΔRANTES/CCL5	−837.7300 (−2753.1075 to −447.1125)	−194.8350 (−1785.9525 to 243.8575)	0.1845
ΔTNF-α	−1.200 (−2.3025 to −0.5375)	−0.4850 (−1.6625 to 0.6525)	0.1404

*Increase in serum cytokines on day 8 (pg/mL) was compared between survivors and non-survivors on day 91 by the Wilcoxon rank-sum test.
AE, acute exacerbation; b-FGF, basic fibroblast growth factor; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IIPs, idiopathic interstitial pneumonias; IL, interleukin; IP-10, IFN-γ inducible protein; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T cell expressed and secreted; TNF, tumour necrosis factor.

when compared with mice that underwent control gene transfer.[46 47] These inconsistent reports concerning the effect of IL-10 on fibrosis might reflect a difference in duration of IL-10 expression.

Long-term, lung-specific overexpression of IL-10 in mature mice for 1 and 3 months resulted in infiltration of mononuclear cells into the lung and increased expression of MCP-1/CCL2 mRNA[48]; moreover, pulmonary fibrosis in IL-10 transgenic mice was found to be inhibited by anti-MCP1/CCL2 antibody.[48] Induction of MCP-1/CCL2 by long-term elevation of serum IL-10 in transgenic mice may be consistent with the significant predictive value of ΔMCP-1 with regard to 90-day survival noted in our present study. Association between persistent elevation of IL-10 and pulmonary fibrosis in the transgenic mice[48] might explain our finding of a poor prognosis in patients with a marked increase in serum IL-10 levels by day 8. This hypothesis is consistent with the poor survival documented in patients with interstitial lung disease associated with clinically amyopathic dermatomyositis who are antimelanoma differentiation-associated gene 5 antibody positive and show an increase in serum IL-10 during their clinical course.[49]

The results of this study could alter the treatment strategy for AE-IIPs. Nintedanib[50] is known to suppress the effects of profibrotic cytokines, including VEGF,[51] b-FGF[52] and PDGF.[25] The ability of nintedanib to inhibit AE of IPF was demonstrated in the INPULSYS trial[50]; however, there was no improvement in survival after the time of diagnosis of AE in nintedanib-treated cases.[53] If our findings are correct, use of nintedanib soon after the time of diagnosis of AE might result in a poor survival outcome in patients with AE-IIPs. Higher serum levels of profibrotic cytokines, including b-FGF and PDGF, at the time of diagnosis of AE were significantly good prognostic factors in our patients with AE-IIPs. Hence, administration of nintedanib for more than 7 days after the time of diagnosis of AE rather than early administration

Table 6 Prognostic significance of an increase in serum cytokine levels at day 8 from baseline levels for AE-IIPs: logistic regression analysis of 90-day mortality

Parameters	OR*	95% CI	P value
Univariate analysis			
ΔIL-ra	1.0003	0.9987 to 1.0038	0.7276
ΔIL-2	6.5971	0.2441 to 453.9501	0.2575
ΔIL-4	0.1854	0.000223 to 68.6763	0.5734
ΔIL-5	1.1894	0.7046 to 2.1647	0.4765
ΔIL-6	2.1743	0.8055 to 14.1524	0.1614
ΔIL-7	12.6586	1.5148 to 407.2435	0.0130
ΔIL-8	1.0164	0.9928 to 1.0842	0.3159
ΔIL-9	3.7582	0.9318 to 34.8311	0.0692
ΔIL-10	489.7742	5.4452 to 3 967 492	0.0002
ΔIL-12	0.9146	0.0764 to 4.1373	0.9122
ΔIL-13	0.3314	0.0034 to 4.5552	0.4703
ΔIL-17	0.7084	0.0691 to 4.7883	0.7327
ΔEotaxin/CCL11	0.9306	0.8249 to 1.0037	0.0657
Δb-FGF	1.4213	0.7410 to 2.9857	0.2883
ΔG-CSF	1.2878	1.0029 to 2.1824	0.0418
ΔGM-CSF	2.6346	0.0085 to 996.8476	0.7260
ΔIFNγ	24.2498	1.7469 to 4292.726	0.0063
ΔIP-10/CXCL10	1.0002	0.9986 to 1.0018	0.7270
ΔMCP-1/CCL2	1.4248	1.0812 to 2.2406	0.0009
ΔMIP-1α/CCL3	2.4528	0.9378 to 28.5121	0.1541
ΔPDGF-BB	1.0104	0.9931 to 1.0346	0.2433
ΔMIP-1β/CCL4	1.0048	0.9970 to 1.0211	0.2765
ΔRANTES/CCL5	1.0001	0.9999 to 1.0007	0.2813
ΔTNF-α	2.1615	0.9022 to 7.3186	0.0918
Multivariate analysis†			
ΔIL-10	489.7	5.445 to 3 967 492	0.0002

*OR >1 means an increase in each parameter (pg/mL) indicating a high risk of 90-day mortality.
†Multivariate logistic regression analysis with stepwise selection was performed using five significant (p<0.05) cytokines by univariate analysis: ΔIL-7, ΔIL10, ΔG-CSF, ΔIFN-γ and ΔMCP-1.
b-FGF, basic fibroblast growth factor; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IIPs, idiopathic interstitial pneumonias; IL, interleukin; IP-10, IFN-γ inducible protein; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T cell expressed and secreted; TNF, tumour necrosis factor.

might confer a survival advantage in AE-IPF. In addition, we have shown AE-IIPs with use of antifibrotic drugs before AE showed worse survival than those without their use (table 2 and online supplemental table 3). This can be explained by difference in severity of patients of IIPs before AE with or without use of antifibrotic drugs in our study (online supplemental table 3); however, cessation of antifibrotic drugs temporally during the 7 days after the AE diagnosis might be better to improve prognosis of AE-IIPs from our hypothesis. How to use antifibrotic drugs for AE-IPF patients is important problem to be solved by future studies.

Effects of thrombomodulin on cytokine production have not been clarified sufficiently; however, they have been investigated for other diseases except for AE-IIPs. Kudo *et al*[54] reported IL-10 and TGF-β in the lung homogenate of lipopolysaccharide induced acute respiratory distress syndrome (ARDS) model mice treated by rhTM was significantly elevated 24 hours after the onset of ARDS as compared with control mice without rhTM treatment. Temporal elevation of these two cytokines might help recovery from the epithelial injury and inflammation at AE as we have shown from our data. In addition, rhTM might inhibit endothelial cell injury and help reduction of RANTES/CCL5 as shown in patients treated with haematopoietic stem cell transplantaion.[55 56] When and how long to administer rhTM might be important to improve the prognosis of AE-IIP.

This study has several limitations. First, the numbers of patients with AE-IPF and AE-non-IPF were too small to draw definitive conclusion. We cannot divide subjects into original and validation cohorts. We cannot separately analyse AE-IPF and AE-non-IPF to reduce bias, either. Second, serum samples were obtained from patients with AE-IIPs treated with rhTM and corticosteroids of SETUP trial; however, serum samples could not be obtained from all the subjects of the trial. Third, a randomised controlled trial of rhTM could not clarify its efficacy in AE-IPF,[57] and we cannot recommend it as a treatment for AE-IPF or AE-IIPs based on the present evidence. Therefore, our present findings need to be confirmed in a study of patients with AE-IIPs who are treated with corticosteroids only. Fourth, the levels of several cytokines could not be measured simultaneously using the Bio-Plex system. Fifth, PMX/DHP therapy is known to reduce various serum cytokine levels[13] and might have affected the serum cytokine profile on day 8; however, only one case was treated with the therapy.

CONCLUSIONS

An elevated serum RANTES/CCL5 level at the time of diagnosis of AE predicted a good survival outcome in patients with AE-IIPs and a marked increase in serum IL-10 on day 8 predicted a poor survival outcome. Therefore, early elevation of serum RANTES/CCL5 and a rapid reduction of IL-10 could predict 90-day survival in patients with AE-IIPs treated with corticosteroids and rhTM.

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Contributors TA contributed to study conception and design, radiological findings, data analysis and interpretation and manuscript preparation. TA had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. HM, SY, YO, CS, KT and HK contributed to study conception and design, data acquisition, data analysis and interpretation and manuscript preparation. YI and MM contributed to study conception and design, data analysis and interpretation and manuscript preparation. MH and KH contributed to data acquisition, data analysis and interpretation and manuscript preparation. MA contributed to the radiological findings and manuscript preparation. All authors approved the final version of the manuscript.

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Patient consent for publication Not required.

Ethics approval This study was approved by the Institutional Review Board of the Kinki-Chuo Chest Medical Center (KCCMC; approval number: 447, 24 February 2014), as well as the review boards of the other institutions. Written informed consent was obtained from all participants in the rhTM arm.

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