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Polymyxin-B Hemoperfusion for Acute Exacerbation of Idiopathic Pulmonary Fibrosis: Serum IL-7 as a Prognostic Marker

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Abstract. *Background:* Acute exacerbation (AE) of idiopathic pulmonary fibrosis (IPF) has an extremely poor prognosis. Direct hemoperfusion with a polymyxin B-immobilized fiber column (PMX-DHP) has been used to improve oxygenation for acute respiratory distress syndrome. The study aim was to retrospectively determine the predictive factors affecting the prognosis of AE of IPF treated with PMX-DHP. *Methods:* We studied patients suffering from AE of IPF, treated with PMX-DHP combined with high-dose corticosteroid therapy. Stored serum taken before and after PMX-DHP therapy was analyzed for 27 cytokines and chemokines. *Results:* Nineteen patients with AE of IPF were studied. The median survival time after diagnosis of AE was 22 days. Survival rates after diagnosis of AE were 47.4% at 30 days, 31.6% at 60 days, and 26.3% at 90 days. Serum levels of Interleukin (IL)-7, an anti-fibrotic cytokine, in survivors at day 30 following PMX-DHP therapy ('Survivors') significantly increased after the treatment, compared to serum levels of non-survivors at day 30 after the therapy ('Nonsurvivors'), which did not demonstrate a significant change. Serum levels of IL-1β, interferon-γ and chemokine ligand (CCL) 2 levels were not significantly altered in 'Survivors,' but were significantly changed in 'Nonsurvivors.' Multivariate Cox proportional-hazards analysis showed that an increase in IL-7 levels after PMX-DHP therapy and treatment without intubation (other than invasive positive-pressure ventilation) were significantly better prognostic factors. *Conclusion:* The results suggest that serum IL-7 may be a useful prognostic factor for patients with AE of IPF treated with PMX-DHP, possibly reflecting underlying anti-fibrotic mechanisms. *(Sarcoidosis Vasc Diffuse Lung Dis 2011; 28: 113-122)*

Key words: acute exacerbation, cytokine, hemoperfusion, idiopathic pulmonary fibrosis, polymyxin

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrotic lung disease of unknown cause with a median survival of 3-5 years (1, 2). Some patients with IPF undergo sudden episode of rapid deterioration during the chronic course of the disease (2-5), termed acute exacerbation (AE) of IPF (6-8). Patients with AE of IPF are usually treated with a combination of high-dose corticosteroids and immunosuppressants. However, despite such treatment, the mortality rate has been reported to be high (3, 4, 9-12).

Direct hemoperfusion with a polymyxin B-immobilized fiber column (PMX-DHP) was developed as a new treatment for endotoxic shock. Endotoxins and other substances are absorbed by

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polymyxin B- immobilized polystylene fibers (13-15). Recent reports have suggested that PMX-DHP therapy may improve oxygenation in patients with acute respiratory distress syndrome (16, 17), a condition which is pathologically characterized by diffuse alveolar damage (DAD) (7). Studies have additionally indicated that PMX-DHP therapy might be potentially beneficial in AE of IPF (18-20). Recently, PMX-DHP therapy was shown to be effective in improving oxygenation and the systemic inflammatory response syndrome in patients with rapidly progressive interstitial pneumonias, including AE of IPF (21). However, numbers of patients with AE of IPF analyzed in these studies have been limited, and little is known about prognostic factors. In addition, few studies analyzing a broad range of cytokines and chemokines have been reported in patients with AE of IPF treated with PMX-DHP, while series of cytokines and chemokines have been analyzed as markers predicting outcomes in systemic sclerosis-related lung disease (22). We hypothesized that certain clinical markers and circulating biomarkers might serve as prognostic indicators in such patients. The aim of the present study was therefore to define prognostic factors by monitoring cytokine levels before and after the therapy in patients with AE of IPF. A portion of the present study has been previously reported only in the form of abstract at the 4th International WASOG Conference in October, 2007.

Methods

Subjects and diagnosis of AE of IPF

The present study was conducted retrospectively using the clinical data and stored serum samples under the informed patient consent from all patients. The study analyzed patients suffering from AE of IPF, treated with PMX-DHP and high-dose corticosteroid therapy in the National Hospital Organization Kinki-Chuo Chest Medical Center from January 2006 to March 2009.

The diagnosis of IPF was based on the criteria from ATS/ERS 2000 and JRS (1, 23). Patients were diagnosed with AE of IPF based on the following criteria slightly modified from the criterion of AE on IPF (7, 23): (1) Within one month, the following three conditions are all satisfied in the chronic course of IPF disease progression: (i) progressive worsening dyspnea (ii) new ground-glass opacities evident on HRCT superimposed on a background reticular or honeycomb pattern, and (iii) a reduction in PaO_2 at rest is lower of more than 10 Torr compared to previous measurements; (2) The exclusion of obvious causes of acutely impaired respiratory function, such as infection, pneumothorax, cancer, pulmonary embolism or congestive cardiac failure. Apparent infections were carefully excluded by measuring antibodies for Mycoplasma pneumoniae and /or Chlamydia pneumoniae in paired sera, β-D glucan and Cytomegalovirus antigen, as well as plasma endotoxin levels (Endospecy test; Seikagaku Biobusiness, Tokyo, Japan). Bacterial cultures of blood, sputum (n = 19), bronchoalveolar lavage (BAL) fluid (n = 5), and endotracheal aspirates (n =7) were performed. This retrospective study was approved by the Ethics Committee of the National Hospital Organization Kinki-Chuo Chest Medical Center, and written informed consent was obtained from each patient or guardian.

Radiological and pathological diagnosis of idiopathic pulmonary fibrosis

CT scanners (HiSpeed Advantage and LightSpeed 16; GE Healthcare, Milwaukee, WI, USA) were used for the present study. Thin-section CT examinations were performed at 120 kVp, 160 mAs, and 1.5-mm collimation at 15-mm intervals. Three experts of diffuse lung diseases (KT, TA and YI) and one chest radiologist (MA) retrospectively reviewed HRCT films without patient identification. The reviewers also classified each case as definite IPF, probable IPF or other IPF based on the criteria (24) originally used in a phase 3 trial of IFN-γ 1b (25). The 12 definite IPF and the three probable IPF were grouped into the 'typical IPF' cohort; while all other IPF cases were classified as 'atypical IPF.' Surgical biopsy (n = 4) and autopsy (n = 1) specimens were available in five cases: all were diagnosed as usual interstitial pneumonia by a lung pathologist (MK). Of these, four cases were classified as 'typical IPF' by both radiological and pathological diagnosis. One case initially diagnosed as 'atypical IPF' by radiological evaluation was subsequently re-classified as 'typical IPF' based on pathological findings.

Combination treatment of AE of IPF with PMX-DHP and corticosteroids

Immediately after the diagnosis of AE of IPF, patients were treated with high-dose corticosteroids (methylprednisolone, 1,000 mg/day) for three consecutive days followed by a tapered dosing of prednisolone. PMX-DHP therapy (Toraymyxin, Toray Medical, Tokyo, Japan) was started within seven days of commencing the high-dose corticosteroids. A double-lumen catheter was inserted into the femoral or jugular vein. A PMX-DHP column was administered for 4-6 hours at a flow rate of 80 mL/min, and repeated once within 48 hours. Nafamostat mesilate (Torii Pharma, Tokyo, Japan) was used as an anticoagulant.

Measurement of cytokines, chemokines, KL-6, Surfactant Protein (SP)-A and SP-D

Blood samples were obtained just before and 48 to 72 hours after PMX-DHP therapy and sera were stored at -70°C. Serum levels of 27 cytokines and chemokines were measured using the Bio-Plex Cytokine Suspension Array System (BIORAD, Tokyo, Japan) (26), and included Interleukin (IL)-1β, IL-1 receptor antagonist (RA), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, chemokine ligand (CCL)2, CCL3, CCL4, CCL5, CCL11, CXC chemokine ligand (CXCL)10, basic fibroblast growth factor (FGF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon (IFN)-γ, platelet-derived growth factor (PDGF)-BB, tumor necrosis factor (TNF)-α and vascular endothelial growth factor (VEGF). Each measurement was done in duplicate by two investigators (AM, MH). Serum KL-6, SP-A, and SP-D were measured with ELISA using commercial kits (27).

Statistical analysis

Kaplan-Meier analysis was used for the analysis of survival. Since the data were not normally distributed, they are presented as the median score with the interquartile range (IQR) in parenthesis. Differences in baseline clinical data between 'Survivors' and 'Nonsurvivors' were compared using the nonparametric Man-Whitney U test for continuous variables, and χ^2 test for categorical variables. Changes in cytokines, chemokines, and PaO_2/FiO_2 (P/F) ratio before and after PMX-DHP therapy were assessed using the Wilcoxon signed rank sum test. Adjustment for multiple comparisons was not applied due to the limited number of study subjects and the relatively rare disorder investigated in this study (22). Cox proportional hazards regression models were used to calculate hazard ratios for mortality. Univariate analyses were performed using clinical data and laboratory data, including levels of the 27 cytokines before PMX-DHP therapy and those changes after the therapy. All variables identified as significant were included in the multivariate model. The JMP Statistics Program (SAS Institute Inc., NC, USA) was used for all analyses. Values of P less than 0.05 were considered statistically significant.

Results

Baseline clinical data of the patient

The clinical characteristics of the 19 patients are summarized in Table 1. Sixteen patients were classified as having 'typical IPF,' while the remaining three patients were classified as having 'atypical IPF.' Before their episode of AE, seven patients had previously received corticosteroids, four had received immunosuppressants, and two had been receiving long-term supplemental oxygen. Plasma endotoxin levels were within normal limits (<1.0 pg/ml) in all 19 patients.

Combination treatment of PMX-DHP and high dose corticosteroids, and the safety of PMX-DHP therapy

PMX-DHP therapy was started 1 (0-2) day after initial high-dose corticosteroid therapy. The P/F ratio at the time of PMX-DHP therapy was less than 200 mmHg in 14 patients. Intubated invasive positive-pressure ventilation (IPPV) had been performed on 7 patients, and non-invasive positive-pressure ventilation (NPPV) on a further seven patients at the time of PMX-DHP therapy. No adverse events, such as bleeding, hypotension, or thrombocytopenia, were observed.

The median observation period (updated on Feb. 21, 2011) was 850 days (range 480-1,660 days).

Table 1. Clinical characteristics of subjects at initial presentation (baseline data)

	Total (n = 19)	'Survivors' (n = 9)	'Nonsurvivors' (n = 10)	*p* value
Gender, male/female	12/7	7/2	5/5	0.204
Age (years)	73 (67-76)	73 (65-76)	74 (66-76)	0.902
Smoking history, N/Ex/C	4/14/0 (n = 18)	1/8/0	3/6/0 (n = 9)	0.248
Smoking index, pack-years	45 (6-59)	45 (17-58)	45 (0-61)	0.755
Typical IPF/atypical IPF	16/3	8/1	8/2	0.592
Baseline therapy				
Corticosteroid	7	3	4	0.763
Immunosuppressant	4	2	2	0.906
Long term oxygen therapy	2	1	1	0.937
%VC	61.5 (56.9-86.4) (n = 9)	61.5 (58.5-87.2) (n = 5)	67.5 (47.3-86.5) (n = 4)	0.747
P/F ratio (mmHg)	106 (86-135)	135 (89-231)	108 (85-130)	0.391
WBC (x10^3/L)	12800 (9100-16900)	11400 (6500-13950)	15100 (11550-18500)	0.037*
CRP (mg/dL)	9.3 (2.5-15.0)	9.8 (1.7-22.0)	9.1 (2.8-16.4)	0.903
LDH (IU/L)	347 (277-411)	325 (268-385)	350 (316-584)	0.427
KL-6 (U/mL)	1250 (900-2250)	1290 (955-2060)	1595 (615-3198)	0.930
SP-D (ng/mL)	227 (179-545)	188 (130-489)	293 (188-662)	0.206
SP-A (ng/mL)	103 (79-140) (n = 17)	100 (73-103) (n = 7)	127 (81-149)	0.223

Enrolled patients were classified as 'Survivors' or 'Nonsurvivors' 30 days after PMX-DHP therapy
Values are given as number or median (IQR). N/Ex/C = non-smoker/ex-smoker/current smoker; IPF= idiopathic pulmonary fibrosis; P/F ratio = PaO_2/FiO_2 ratio; WBC = white blood cell count; CRP = C-reactive protein; LDH = lactate dehydrogenase; KL-6 = Krebs von den Lungen-6; SP-D = surfactant protein-D; SP-A = surfactant protein-A.
* Statistically significant value (p<0.05)

Survival rates from the time of AE onset were 47.4% at 30 days, 31.6% at 60 days, and 26.3% at 90 days, with a median of 22 days (n = 19) (Figure 1). At 30 days after PMX-DHP therapy, patients were classified as 'Survivors' and 'Nonsurvivors' with nine surviving patients and ten deaths accordingly. White blood cell count was noted to be significantly higher among the 'Nonsurvivors' compared to the 'Survivors' (p = 0.037) (Table 1). Otherwise, there were no significant differences in the other baseline clinical characteristics, including %VC, P/F ratio, and the blood examination data of CRP, LDH, KL-6, SP-D, and SP-A between the two groups (Table 1). Representative chest HRCT scans of a patient with IPF (usual interstitial pneumonia) before and after the treatment of PMX-DHP are shown in Figure 2.

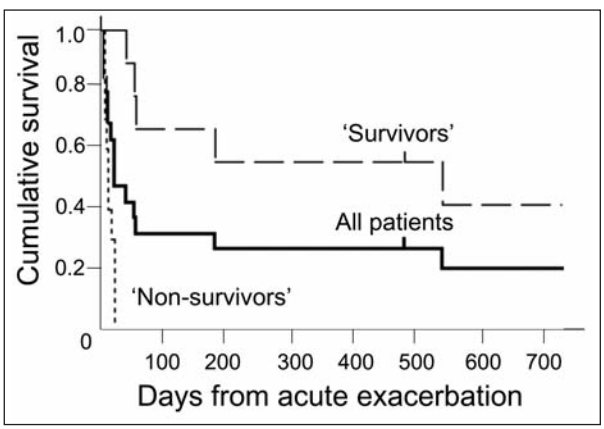

Fig. 1. Overall survival of all patients (n = 19). Survival was calculated from the day of diagnosis of acute exacerbation. The solid line indicates all patients; the dash-dotted line, survivors at day 30 following PMX-DHP therapy ('Survivors') (n = 9); the broken line, non-survivors at day 30 following PMX-DHP therapy ('Nonsurvivors') (n = 10). 'Survivors' demonstrated significantly better survival rates than 'Nonsurvivors' (p < 0.0001) in the log-rank test

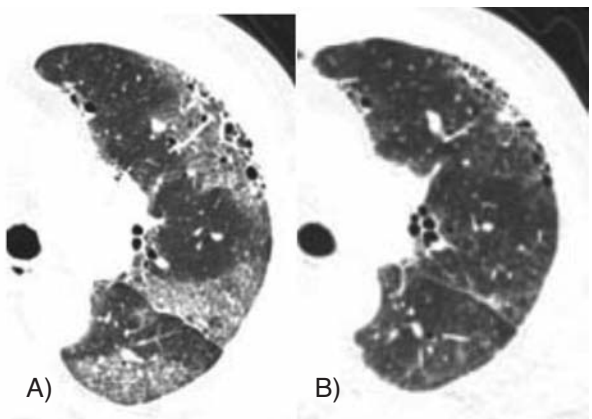

Fig. 2. Chest HRCT of a middle-aged male with usual interstitial pneumonia diagnosed by surgical lung biopsy, before (A) and 60 days after (B) treatment with PMX-DHP. Before treatment, the patient's chest HRCT revealed new diffuse ground glass opacities (A), which improved after treatment (B).

Change in oxygenation, serum cytokines and chemokines in the two groups

There was a significant increase in P/F ratio at seven days after therapy in 'Survivors,' increasing from a median of 135 mmHg (89-231 mmHg) before therapy, to 188 mmHg after therapy (162-263 mmHg) (p = 0.036; n = 9). The P/F ratio did not change significantly among 'Nonsurvivors' which was 108 mmHg (85-130 mmHg) before therapy and 88 mmHg (41-133 mmHg) seven days post-therapy (p = 0.778; n = 6). The P/F ratio among the entire patient cohort was not significantly altered being 110 mmHg (87-139 mmHg) before therapy and 164 mmHg (95-225 mmHg) seven days post-therapy (p = 0.175; n = 15).

The serum profile of 27 cytokines and chemokines before and after (48-72 hours) PMX-DHP therapy are shown in Table 2 and 3. There were no significant differences in the baseline levels between the two groups (data not shown). IL-7 levels increased significantly after therapy (Δ IL-7) among the 'Survivors' (p = 0.039), but were not significantly altered among 'Nonsurvivors.' Conversely, IFN-γ levels were significantly reduced among 'Nonsurvivors' (p = 0.020) but not in 'Survivors.' CCL2 levels also increased significantly after therapy in 'Nonsurvivors' (p = 0.020) as was CCL4 levels in both 'Survivors' and 'Nonsurvivors' (p = 0.039 and 0.014, respectively). IL-1β levels remained unchanged in 'Survivors,' but were significantly reduced among 'Nonsurvivors' (p = 0.027). Serum KL-6, SP-D, and SP-A did not change significantly following therapy in either group (data not shown).

Prognostic factors for PMX-DHP therapy for AE of IPF

To determine the prognostic factors for AE of IPF when treated with PMX-DHP therapy com-

Table 2. Baseline and changes in serum cytokine levels among 'Survivors'

Variables	Baseline * (n = 9)	Δ cytokines† (n = 8)	p value
Il-1β	2.5 (1.2-47.8)	-0.52 (-2.7-0.93)	0.461
IL-1 RA	205 (93-7073)	160 (-15-511)	0.250
IL-2	3.0 (0.0-110)	0 (-133-3.75)	0.438
IL-4	4.2 (0.88-7.4)	-1.1 (-9.8-0.21)	0.383
IL-5	3.5 (1.4-10.5)	-1.1 (-9.5-0.94)	0.313
IL-6	25.2 (7.4-3057)	-6.7 (-121-11.6)	0.383
IL-7	13.3 (3.3-19.3)	5.2 (0.4-8.4)	0.039‡
IL-8	17.7 (4.1-139)	-6.5 (-29.0-11.6)	0.516
IL-9	70.3 (13.5-459)	38.5 (-148.2-86.8)	0.641
IL-10	10.4 (4.7-74.3)	10.6 (-6.4-33.3)	0.383
IL-12	41.5 (10.1-79.5)	26.8 (1.9-35.1)	0.055
IL-13	4.5 (2.8-56.9)	9.7 (-21.7-17.3)	0.641
IL-15	0.0 (0.0-76.7)	0.0 (-29-2.9)	0.625
IL-17	0.0 (0.0-63.8)	18.0 (-0.2-41.6)	0.297
CCL2	33.7 (11.5-618)	0.8 (-33.4-88.8)	0.844
CCL3	4.3 (0.0-14.7)	0.0 (-6.3-219)	0.813
CCL4	91.3 (52.0-134)	121 (11-240)	0.039‡
CCL5	3427 (1333-4646)	1231 (-1271-2720)	0.547
CCL11	16.0 (1.74-153)	0.9 (-137-15)	0.938
CXCL10	361 (147-1705)	-71 (-808-143)	0.250
basic FGF	0.0 (0.0-22.2)	0.0 (-2.5-4.7)	1.000
G-CSF	47.0 (20.0-5306)	14.0 (-594-56)	0.742
GM-CSF	49.8 (0.0-124)	1.5 (-54-24)	0.813
IFN-γ	84.6 (25.9-266)	6.3 (-103-80)	0.945
PDGF-BB	1653 (218-11934)	2049 (-531-7195)	0.148
TNF-α	38.6 (18.3-367)	3.78 (-10-85)	1.000
VEGF	58.2 (0.00-336)	89.3 (-142.5-116.7)	0.844

IL, interleukin; RA, receptor antagonist; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; FGF, fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; IFN, interferon; PDGF, platelet derived growth factor; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor

* Baseline, serum levels of cytokines or chemokines before PMX-DHP

† Δ cytokines, (serum level at 48-72 h after PMX-DHP therapy) – (serum level before therapy), one group of post-PMX data is lacking

Data are presented as median (interquartile range) (pg/mL)

‡ Statistically significant value (p<0.05)

Table 3. Baseline and changes in serum cytokine levels among 'Nonsurvivors'

Variables	Baseline * (n = 9)	Δ cytokines† (n = 8)	p value
Il-1β	2.5 (1.2-47.8)	-0.52 (-2.7-0.93)	0.461
Il-1β	2.0 (1.4-7.7)	-0.7 (-3.4- -0.2)	0.027‡
IL-1 RA	162 (79.3-1297)	54 (-283-186)	0.922
IL-2	0.0 (0.0-18.1)	0.0 (-0.7-13.5)	0.563
IL-4	2.7 (1.5-4.8)	0.0 (-3.2-1.2)	0.193
IL-5	1.8 (0.7-7.7)	0.3 (-1.6-0.9)	0.770
IL-6	22.2 (6.7-122)	-0.5 (-13.9-29.8)	1.000
IL-7	13.8 (4.0-37.5)	-0.7 (-12.7-2.7)	0.375
IL-8	24.6 (13.4-55.3)	5.0 (-11.2-50.7)	0.557
IL-9	66.5 (12.5-175)	10 (-55-34)	0.846
IL-10	12.5 (6.9-27.7)	5.6 (-13.2-16.0)	0.695
IL-12	11.7 (1.8-57.1)	7.0 (-8.2-16.9)	0.625
IL-13	10.5 (2.0-57.1)	3.8 (-18.7-10.2)	0.625
IL-15	0.0 (0.0-25.2)	0.0 (-6.0-3.1)	0.625
IL-17	0.0 (0.0-54.6)	0.0 (-6.7-19.8)	0.844
CCL2	35.5 (13.3-67.9)	32.7 (1.8-96.2)	0.020‡
CCL3	5.8 (0.0-15.5)	-0.4 (-7.2-0.3)	0.470
CCL4	89.4 (78.4-110.1)	54.1 (9.9-143.4)	0.014‡
CCL5	976 (0-12512)	-60 (-8296-1228)	0.383
CCL11	18.4 (0.0-44.1)	-4.4 (-13.7-1.4)	0.148
CXCL10	195 (121-1409)	-64 (-515-67)	0.275
basic FGF	0.0 (0.0-40.1)	0.0 (-33.3-0.0)	0.250
G-CSF	44.9 (29.3-78.8)	-1.8 (-19.7-28.7)	0.922
GM-CSF	11.4 (0.0-53.6)	10.3 (-1.2-12.6)	0.195
IFN-β	132 (55-193)	-71.3 (-135.2- -10.3)	0.020‡
PDGF-BB	6794 (800-14104)	-106 (-7797-3189)	0.432
TNF-α	18.9 (11.9-57.2)	-7.3 (-23.4-23.0)	1.000
VEGF	34.3 (0.0-227)	-21.7 (-80.5-36.8)	0.322

IL, interleukin; RA, receptor antagonist; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; FGF, fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; IFN, interferon; PDGF, platelet derived growth factor; TNF-α, tumour necrosis factor-α; VEGF, vascular endothelial growth factor
* Baseline, serum levels of cytokines or chemokines before PMX-DHP
† Δ cytokines, (serum level at 48-72 h after PMX-DHP therapy) – (serum level before therapy)
Data are presented as median (interquartile range) (pg/mL)
‡ Statistically significant value ($p<0.05$)

bined with high dose corticosteroid therapy, we analyzed the data using univariate Cox's proportional hazards regression models. Treatment without intubation (other than IPPV) at the time of PMX-DHP therapy and changes in serum IL-7 levels before and after PMX-DHP therapy (Δ IL-7) were both associated with better prognosis (hazard ratio = 0.309, and 0.946, p = 0.0436 and 0.0420, respectively) (Table 4). Multivariate Cox's proportional hazards regression models, including those two values, showed that treatment without intubation (other than IPPV) at the time of PMX-DHP therapy and Δ IL-7 were significant factors for prognosis (hazard ratio 0.114 and 0.908, p = 0.0044 and 0.0066, respectively) (Table 5).

Discussion

This is the first report to describe the treatment of AE of IPF using PMX-DHP in up to 19 patients. Our results suggest that PMX-DHP therapy is a safe and tolerable procedure, and that prognosis in treated patients can be predicted using clinical (treatment without intubation) and laboratory data (serum IL-7 levels).

We found that a significant increase was observed in the P/F ratio at seven days after the first PMX-DHP session in 'Survivors,' while this did not differ significantly among 'Nonsurvivors.' Hara et al. described improved oxygenation at seven days after the first PMX-DHP session in patients with rapidly progressive interstitial pneumonias (21). This discrepancy may be partly explained by different underlying each disease: our study focused exclusively on

Table 4. Results of univariate analysis of prognostic factors influencing survival with PMX-DHP and high-dose corticosteroid therapy

Variables	Hazard ratio	95%CI		*p* value
		Lower	Upper	
Age, years*	0.993	0.921	1.073	0.8649
Gender, male†	0.172	0.699	6.369	0.1720
Smoking, pack-years*	0.990	0.968	1.010	0.3035
Atypical IPF†	1.180	0.181	4.431	0.8338
Treatment before AE†	1.158	0.353	3.382	0.7962
Duration from the time of IPF diagnosis*	0.998	0.977	1.016	0.8031
Duration of acute symptoms before PMX*	1.036	0.976	1.097	0.2366
Duration between steroid and PMX*	1.295	0.996	1.641	0.0530
Treatment without intubation (other than IPPV §)†	0.309	0.095	0.966	0.0436‡
P/F ratio*	1.003	0.997	1.007	0.3289
%VC, %* (n = 9)	0.989	0.949	1.019	0.5064
WBC, X 10^9 /L*	1.000	1.000	1.002	0.0771
LDH, IU/L*	1.004	0.999	1.008	0.1097
CRP, mg/mL*	0.972	0.912	1.027	0.3232
KL-6, U/mL*	1.000	1.000	1.001	0.4089
SP-D, ng/mL*	1.001	0.999	1.002	0.4037
SP-A , ng/mL* (n = 17)	1.012	0.999	1.025	0.0623
Δ IL-7‡, pg/mL*¶ (n = 18)	0.946	0.900	0.998	0.0420‡

* Continuous scale. † Dichotomous scale. ‡ Statistically significant value. § IPPV, invasive positive-pressure ventilation. ¶ Δ IL-7 denotes change in IL-7 level before and after PMX-DHP
KL-6 = Krebs von den Lungen-6; SP-D = surfactant protein-D; SP-A = surfactant protein-A

Table 5. Results of multivariate analysis of prognostic factors influencing survival with PMX-DHP and intravenous high dose corticosteroid therapy

Variables	Hazard ratio	95%CI		*p* value
		Lower	Upper	
Treatment without intubation (other than IPPV §)†	0.114	0.018	0.517	0.0044‡
Δ IL-7 *¶	0.908	0.840	0.972	0.0060‡

* Continuous scale. † Dichotomous scale. ‡ Statistically significant value
§ IPPV, invasive positive-pressure ventilation
¶ Δ IL-7 denotes change in IL-7 level before and after PMX-DHP

AE of IPF while the abovementioned report featured a range of interstitial pneumonias, such as those associated with collagen vascular diseases, drug-induced forms and acute interstitial pneumonia.

In the current PMX-DHP study, nine (47.4%) and five (26.3%) patients survived more than 30 days, 90 days, respectively. The survival rate of these patients is relatively higher, as compared with the corresponding survival rates of approximately 20% survival rate at 30 days and 4% survival rate at 90 days in patients with AE of IPF treated with high-dose corticosteroid and immunosuppressants (3, 4, 9, 28). Recently, Song et al has reported better outcomes (90-day survival rate treated with conventional therapy 40.0%) (12); however their subjects might include milder cases compared to ours (the P/F ratio at the time of AE of IPF 253 mmHg versus 106 mmHg) (12). Further prospective multicenter randomized controlled trials are required to determine the efficacy of PMX-DHP therapy in patients with AE of IPF.

Our findings indicate that IL-7 may be a potentially useful biomarker for prognosticating patients with AE of IPF after PMX-DHP therapy. This is the first report to analyze a series of 27 cytokines in sera from patients with AE of IPF treated with PMX-DHP therapy, although previous reports have analyzed a smaller number of cytokines (18-21).

Serum IL-7 levels significantly increased after therapy (Δ IL-7) among 'Survivors.' IL-7 inhibits transforming growth factor-beta (TGF-β) production and fibroblast signaling (29). In addition, IL-7

and TGF-β play counter-regulatory roles in fibroblast collagen synthesis in pulmonary fibrosis (30). BAL fluid analyses of patients with idiopathic interstitial lung disease have demonstrated lower levels of IL-7 compared with systemic sclerosis patients with interstitial lung disease (22). Our findings appear to reflect this idea, given that increased levels of IL-7 have an anti-fibrotic effect on fibrotic lung tissue.

Our findings also showed that serum IFN-γ levels were reduced in 'Nonsurvivors.' IFN-γ decreases collagen production by fibroblasts and is considered to have an anti-fibrotic effect (31). Moreover, it has been shown that IPF patients with higher levels of serum IFN-γ respond better to corticosteroids (32). Reduced levels of serum IFN-γ in 'Nonsurvivors' may therefore reflect anti-fibrotic effects, although effect of IFN-γ-1b has been controversial in a clinical trial of IPF (33).

Serum CCL2 levels were not significantly changed after the institution of therapy among 'Survivors,' but significantly increased among 'Nonsurvivors.' CCL2 is produced by a variety of cells, including monocytes. Recent reports have revealed that PMX selectively binds to monocytes and neutrophils (34, 35). The removal of monocytes may reduce the interaction between monocytes and endothelial cells, thus potentially inhibiting CCL2 production (35). Hence, an elevated level of CCL2 in 'Nonsurvivors' may indicate worsening pulmonary fibrosis. These findings seem to contradict that of Hara et al. (21), although this discrepancy might be attributed to the timing of blood sampling, and the range of diseases studied.

Serum IL-1β levels were significantly reduced after therapy in 'Nonsurvivors'. IL-1 has been reported to play a critical role in some wound healing processes (36). Moreover, Type 2 pneumocyte proliferation, possibly a wound healing process in the lung, has been observed histologically alongside DAD in patients with AE of IPF (7, 37). This suggests that IL-1β might be associated with the wound healing processes in patients with AE of IPF.

Multivariate Cox's proportional hazards regression models showed that Δ IL-7 and treatment without intubation (other than IPPV) at the time of PMX-DHP therapy were better prognostic factors for AE post-therapy. However, IPPV treatment may be an indicator of worse pulmonary function at baseline. Changes in serum IL-7 levels at 48 to 72 hours after PMX-DHP therapy may instead be a more useful prognostic factor. A recent study of a neonate with myeloproliferative disorder and hepatic fibrosis that demonstrated a decrease of IL-7 after exchange transfusion and a poor outcome (38) may support our findings.

It is unclear as to how PMX-DHP therapy improves oxygenation in AE of IPF. In previous reports, improvements in oxygenation were not attributed to the adsorption of endotoxin by the PMX column (18-20). Recently, Abe et al. demonstrated that neutrophils and some monocytes were adsorbed by the PMX column in AE of interstitial pneumonias (34). In addition, pulmonary recruitment of neutrophils has been reported to be important in AE of IPF (3, 39). However, the number of peripheral neutrophils was not significantly altered after PMX-DHP therapy in our study being 111900 $x10^9$/L (8100-15600 $x10^9$/L) before therapy and 111600 $x10^9$/L (9130-15100 $x10^9$/L) after therapy ($p = 0.715$; $n = 18$) or in the previous report (34). One possibility that remains is that the activated neutrophils may be specifically adsorbed by PMX column. In fact, Abe et al. demonstrated that cells adsorbed by PMX highly expressed HLA-DR, CD14, CD62L and CD114, suggesting active and inflammatory neutrophils (34). Further studies are required to elucidate the precise mechanism behind this treatment.

Our study had limitations. First, not all patients underwent BAL or endotracheal aspiration due to progressive hypoxemia. Therefore, an infectious etiology might not have been completely ruled out in our cohort. However, this is unlikely since other possible comprehensive investigations were performed for the diagnosis. Second, in our analyses of a large number of serum cytokines and chemokines, adjustment for multiple comparisons was not applied due to the limited number of study subjects and the fact that AE of IPF is a relatively rare disorder. Future studies will need to recruit a large number of patients to confirm our observations. Third, changes in cytokines and chemokines might be associated with the PMX-DHP therapy either directly and/or indirectly.

In summary, the present study demonstrates that serum IL-7 may be a useful prognostic factor for patients with AE of IPF treated with PMX-DHP, possibly reflecting underlying anti-fibrotic mechanisms. Our findings provide new insights into the

molecular basis of this disease. We advocate further studies to explore other clinical measures and biomarkers in clinical trials that feature PMX-DHP therapy.

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