Online supplement

Prognostic Significance of Serum Cytokines during Acute Exacerbation of Idiopathic Interstitial Pneumonias treated with Thrombomodulin

Online supplemental Table S1. Patient characteristics of the subjects with serum sample in this study compared with AE-IIPs without serum sample in SETUP trial*

Parameters	Sample obtained (n=28)	Sample not obtained (n=11)	p-value
Stable state			
IPF/Non-IPF	8/20	4/7	0.7086
Sex, Male/Female	21/7	7/4	0.6940
Smoking. y/n	19/9	8/3	1.0000
mMRC, ≤ 1/ ≥ 2	10/18	5/6	0.7181
Stage, I - III / IV	17/11	7/4	1.0000
LTOT before AE, y/n	11/17	3/8	0.7129
Prednisolone before AE, y/n	9/19	6/5	0.2773
Antifibrotic drugs, y/n	5#/23	1§/10	0.6548
At the time of AE diagnosis			
Age, yrs	74.5 (69.75–79)	72 (64–80)	0.2170
HRCT pattern, diffuse/non-diffuse	12/16	3/8	0.4770
PaO_2/FiO_2 ratio, ≤200/>200	17/11	6/5	0.7338
WBC. /μL	10,150 (9,050–12,950)	10,700 (8,800–14,340)	0.9378
LDH, U/L	317.0 (245.5–404.5)	381.0 (290.0–451.0)	0.3030
KL-6, U/mL	1,196 (889–2,142)	859 (616–2,251)	0.2679
CRP, mg/dL	12.38 (5.07–14.28)	12.36 (5.05–14.24)	1.0000
FDP, <10 / ≥10 mg/L	21/7	7/4	0.6940
90-day survival, y/n	19/9	7/4	1.0000

AE, acute exacerbation; CRP, C-reactive protein; FDP, fibrin degradation product; FiO_2, fraction of inspired oxygen; HRCT, high-resolution computed tomography; IPF, idiopathic pulmonary fibrosis; IIPs, idiopathic interstitial pneumonias; 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.

*Each parameter was compared between the two groups using Fisher's exact test or the Wilcoxon rank-sum test.

#: Pirfenidone (n=5), nintedanib (n=1). In one case, pirfenidone was firstly prescribed and nintedanib was secondary administered. §: Pirfenidone (n=1).

Online supplementary Table S2. Therapy for AE-IIPs and management of respiratory failure

Therapy and Management	N=28
Therapy	
Steroid pulse, y/n	25/3
Prednisolone, y/n*	27/1
Dose of prednisolone**, mg/kg/day	0.980 (0.909–1.017)
Immunosuppressant, y/n	5/23
Pirfenidone, y/n	3/25
Nintedanib, y/n	0/28
Antifibrotic drugs#, y/n	3/25
Empiric antibiotics, y/n	28/0
Macrolide, y/n	5/23
Antacids, y/n	27/1
PMX-DHP therapy, y/n	1/27
Thrombomodulin, y/n	28/0
Anticoagulants#, y/n	6/22
Anti-platelet drugs#, y/n	5/23
Management of respiratory failure	
IPPV, y/n	4/24
NPPV, y/n	11/17
IPPV or NPPV, y/n	13/15
NHF, y/n	9/19
NHF without IPPV, NPPV, y/n	5/23
IPPV or NPPV or NHF, y/n	18/10

Abbreviations: AE, acute exacerbation; IIPs, idiopathic interstitial pneumonias; rhTM, recombinant human soluble thrombomodulin; AZP, azathioprine; CyA, cyclosporine A; CPA, cyclophosphamide; IPPV, invasive positive pressure ventilation; NPPV, non-invasive positive pressure ventilation; NHF, nasal high flow therapy.

*AE-IIPs patients with deceased and with no administration of prednisolone during a high-dose methylprednisolone therapy.

**Prednisolone dose is described as the median (IQR).

#: Anticoagulants and anti-platelet drugs were used for comorbidities.

Online supplementary Table S3. Clinical parameters according to the usage of antifibrotic drugs before the diagnosis of AE-IIPs (n=28)*

Clinical parameters	Usage of antifibrotic drugs (n=5)	No usage of antifibrotic drugs (n=23)	p-value
Stable state			
Gender, Male/Female	4/1	17/6	1.0000
IPF/Non-IPF	2/3	6/17	0.6056
Smoking, y/n	3/2	16/7	1.0000
mMRC, ≤ 1/ ≥ 2	1/4	9/14	0.6264
Stage, I - III / IV	0/5	17/6	0.0047
LTOT before AE, y/n	5/0	6/17	0.0047
Prednisolone before AE, y/n	1/4	8/15	1.0000
At the time of AE diagnosis			
PaO_2/FiO_2 ratio, ≤200/>200	3/2	14/9	1.0000
HRCT, diffuse/non-diffuse	3/2	9/14	0.6239
Outcome			
90-day survival, y/n	1/4	18/5	0.0256

Abbreviations; AE, acute exacerbation; FiO_2, fraction of inspired oxygen; HRCT, high-resolution computed tomography; IPF, idiopathic pulmonary fibrosis; IIPs, idiopathic interstitial pneumonias; LTOT, long-term oxygen therapy; mMRC, modified Medical Research Council score; PaO_2, arterial oxygen tension.

*: Association between usage of antifibrotic drugs and clinical parameters at the stable state and at the time of diagnosis of AE-IIPs was evaluated by Fisher's exact test.

Online supplementary Table S4. Serum cytokines of AE-IIPs, Between IPF and non-IPF (n=28)*

Parameters	AE-IPF (n = 8)	AE-non-IPF (n = 20)	p-value
IL-ra	61.29 (26.06–179.92)	163.69 (8.45–426.18)	0.6274
IL-2	0.405 (0.307–1.072)	0.380 (0.240–0.685)	0.5579
IL-4	0.305 (0.197–0.415)	0.285 (0.197–0.405)	0.9797
IL-5	1.430 (1.010–2.960)	1.265 (0.930–2.097)	0.7794
IL-6	0.815 (0.250–2.132)	0.650 (0.225–1.822)	0.6654
IL-7	1.245 (0.660–2.137)	1.135 (0.815–1.767)	0.8786
IL-8	2.960 (1.895–46.822)	4.055 (3.030–6.877)	0.7993
IL-9	2.010 (1.787–3.077)	1.975 (1.810–2.860)	0.9594
IL-10	0.880 (0.497–2.060)	0.785 (0.537–1.677)	0.7026
IL-12	0.225 (0.120–0.432)	0.140 (0.067–0.390)	0.6441
IL-13	0.200 (0.082–0.447)	0.130 (0.062–0.307)	0.2728
IL-17	1.065 (0.727–1.722)	1.090 (0.870–1.375)	0.9189
Eotaxin/CCL11	16.890 (9.387–25.122)	18.36 (10.93–30.65)	0.6841
b-FGF	6.025 (4.912–7.772)	5.110 (4.720–6.720)	0.4263
G-CSF	1.560 (0.980–59.422)	4.550 (0.690–13.575)	0.6829
GM-CSF	0.050 (0.025–0.257)	0.040 (0.020–0.132)	0.5993
IFNγ	0.620 (0.400–2.037)	0.820 (0.542–2.047)	0.4006
IP-10/CXCL10	369.79 (154.25–1111.44)	546.2 (236.9–1143.0)	0.5085
MCP-1/CCL2	4.075 (2.360–18.022)	6.375 (3.555–17.785)	0.1860
MIP-1α/CCL3	0.540 (0.412–17.267)	0.685 (0.352–1.165)	0.5933
PDGF-BB	67.690 (36.397–100.035)	79.295 (49.235–112.792)	0.7219
MIP-1β/CCL4	246.25 (205.50–490.29)	230.64 (187.34–305.07)	0.4765
RANTES/CCL5	1863 (1672–4891)	1859 (1466–3061)	0.7602
TNF-α	4,855 (3.990–11.712)	4.385 (3.865–6.170)	0.5931

Abbreviations; AE, acute exacerbation; IIPs, idiopathic interstitial pneumonias; IPF, idiopathic pulmonary fibrosis; IL, interleukin; CCL, CC chemokine ligand; bFGF, basic fibroblast growth factor; G-CSF; granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IP-10, IFN-γ inducible protein; CXCL, CXC chemokine ligand; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T-cell expressed and secreted; TNF, tumor necrosis factor.

*: Serum cytokine levels of AE-IPF and AE-non-IPF (pg/mL) were compared with Wilcoxon rank sum test.

Online supplementary Table S5.

Association of serum RANTES/CCL5 levels and clinical parameters at the diagnosis of AE-IIPs (n=28)*

Parameters	rho	p-value
Stable state		
Gender, Male/Female	0.0204	0.9178
IPF/Non-IPF	0.0587	0.7666
Smoking, y/n	0.0189	0.9238
mMRC, ≤ 1/ ≥ 2	0.1015	0.6073
Stage, I - III / IV	-0.3622	0.0582
LTOT before AE, y/n	-0.3622	0.0582
Prednisolone before AE, y/n	0.0473	0.8109
Antifibrotic drugs, y/n	-0.5484**	0.0025
At the time of AE diagnosis		
WBC, /μL	0.0236	0.9053
CRP, mg/dL	0.2275	0.2444
LDH, U/L	-0.0616	0.7555
KL-6, U/mL	-0.2740	0.1583
PaO_2/FiO_2 ratio, ≤200/>200	0.2128	0.2770
HRCT, diffuse/non-diffuse	-0.1966	0.3160
FDP, <10/≥10 mg/dL	-0.0255	0.8974

Abbreviations: RANTES, regulated on activation, normal T-cell expressed and secreted; AE, acute exacerbation; IIPs, idiopathic interstitial pneumonias; IPF, idiopathic pulmonary fibrosis; mMRC, modified Medical Research Council score; LTOT, long-term oxygen therapy; WBC, white blood cell; CRP, C-reactive protein; LDH, lactate dehydrogenase; KL-6, Krebs von den Lungen-6; PaO_2, arterial oxygen tension; FiO_2, fraction of inspired oxygen; HRCT, high-resolution computed tomography; FDP, fibrin degradation product.

*: Correlation between serum levels of RANTES/CCL5 at the diagnosis of AE (pg/mL) and clinical parameters at AE and stable state was examined by Spearman rank correlation.

**: This result suggested serum levels of RANTES/CCL5 at the diagnosis of AE was significantly lower in patients with AE-IIPs treated with antifibrotic drugs before the diagnosis of AE than the other patients with AE-IIPs.

Online supplementary Table S6. Serum cytokines changes from baseline to day 8 (n=22)*

Parameters	AE-IIPs at baseline	At day 8	p-value
IL-ra	82.81 (11.83–392.45)	41.41 (7.60–77.64)	0.0025
IL-2	0.405 (0.240–0.745)	0.500 (0.347–0.745)	0.0551
IL-4	0.300 (0.212–0.417)	0.410 (0.290–0.552)	0.0010
IL-5	1.265 (0.920–2.215)	1.070 (0.607–1.645)	0.3790
IL-6	0.480 (0.212–1.425)	0.350 (0.265–0.500)	0.2189
IL-7	1.245 (0.860–1.845)	1.100 (0.630–1.710)	0.0530
IL-8	3.410 (2.412–6.397)	3.695 (2.225–6.475)	0.7417
IL-9	2.115 (1.870–3.060)	2.005 (1.650–2.797)	0.0358
IL-10	0.735 (0.492–1.670)	0.480 (0.185–0.820)	0.0284
IL-12	0.225 (0.120–0.405)	0.230 (0.142–0.460)	0.5332
IL-13	0.165 (0.085–0.350)	0.210 (0.115–0.602)	0.0064
IL-17	1.090 (0.870–1.465)	1.240 (0.950–1.702)	0.3334
Eotaxin/CCL11	18.36 (11.40–31.90)	32.91 (22.01–56.75)	<0.001
b-FGF	5.490 (4.720–7.850)	6.530 (5.800–8.577)	0.0029
G-CSF	2.725 (0.690–10.065)	3.685 (2.185–8.550)	0.7299
GM-CSF	0.050 (0.020–0.157)	0.012 (0.005–0.165)	0.7411
IFNγ	0.755 (0.545–2.002)	0.300 (0.242–0.602)	0.0003
IP-10/CXCL10	546.2 (205.9–1119.7)	281.7 (155.1–615.9)	0.0164
MCP-1/CCL2	5.460 (3.160–16.035)	5.535 (3.790–12.717)	0.8140
MIP-1α/CCL3	0.615 (0.352–1.035)	0.445 (0.292–1.090)	0.1575
PDGF-BB	85.45 (52.89–114.12)	61.72 (39.51–161.6)	0.1256
MIP-1β/CCL4	233.4 (191.7–301.0)	128.0 (111.6–161.6)	<0.0001
RANTES/CCL5	1883 (1750–3260)	1480 (1233–2058)	0.0010
TNF-α	4.385 (3.915–6.345)	3.855 (3.072–4.612)	<0.0001

Abbreviations; AE, acute exacerbation; IIPs, idiopathic interstitial pneumonias; IL, interleukin; CCL, CC chemokine ligand; bFGF, basic fibroblast growth factor; G-CSF; granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IP-10, IFN-γ inducible protein; CXCL, CXC chemokine ligand; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T-cell expressed and secreted; TNF, tumor necrosis factor.
*: Serum levels of each cytokine at AE-onset (pg/mL) were compared with those on day 8 by paired Wilcoxon rank sum test.

Online supplementary Table S7.

Association of serum ΔIL-10* levels and clinical parameters at the onset of AE-IIPs (n=22)**

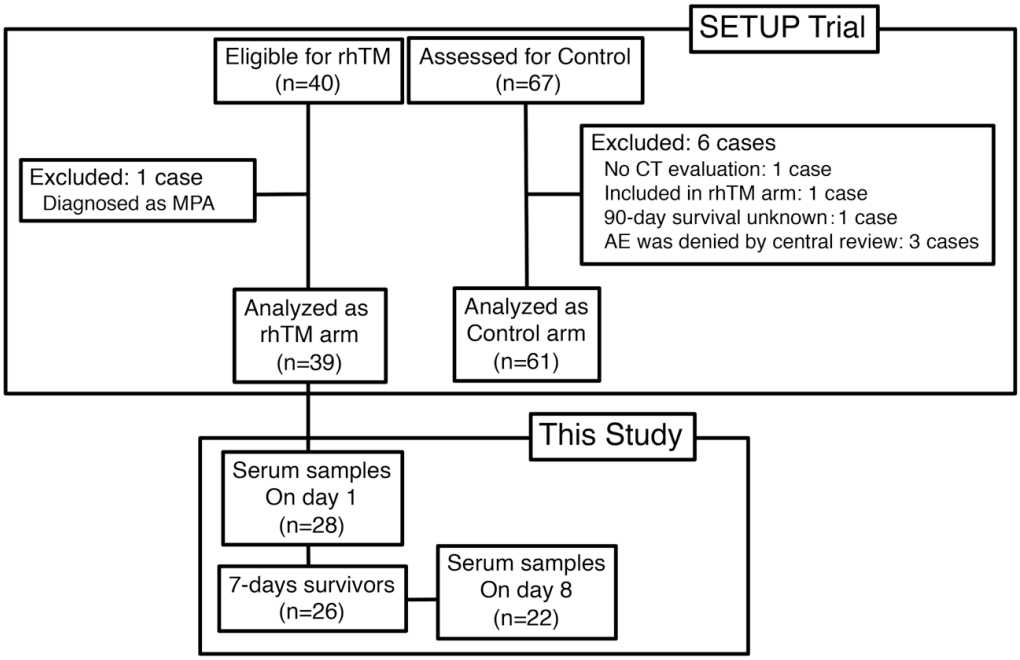
Parameters	rho	p-value
Stable state		
Gender, Male/Female	-0.0483	0.8311
IPF/Non-IPF	-0.3591	0.1007
Smoking, y/n	-0.1126	0.6117
mMRC, ≤ 1/ ≥ 2	-0.0805	0.7219
Stage, I -III / IV	0.0223	0.9214
LTOT before AE, y/n	0.0223	0.9214
Prednisolone before AE	0.0154	0.9458
Antifibrotic drugs, y/n	-0.3241	0.1412
At the time of AE diagnosis		
WBC, /μL	-0.0385	0.8650
CRP, mg/dL	-0.1226	0.5869
LDH, U/L	0.2576	0.2471
KL-6, U/mL	0.2299	0.3034
PaO_2/FiO_2 ratio, ≤200/>200	-0.0573	0.7999
HRCT, diffuse/non-diffuse	0.1341	0.5519
FDP, <10/≥10 mg/dL	0.2309	0.3013

Abbreviations: AE, acute exacerbation; IIPs, idiopathic interstitial pneumonias; RANTES, regulated on activation, normal T-cell expressed and secreted; AE, acute exacerbation; IIP, idiopathic interstitial pneumonia; IPF, idiopathic pulmonary fibrosis; mMRC, modified Medical Research Council score; LTOT, long-term oxygen therapy; WBC, white blood cell; CRP, C-reactive protein; LDH, lactate dehydrogenase; KL-6, Krebs von den Lungen-6; PaO_2, arterial oxygen tension; FiO_2, fraction of inspired oxygen; HRCT, high-resolution computed tomography; FDP, fibrin degradation product.

*: ΔIL-10 means increase in serum levels of IL-10 on day 8 compared with those at AE onset.

**: Correlation between serum levels of ΔIL-10 on day 8 (pg/mL) and clinical parameters at AE and stable state was examined by Spearman rank correlation.

Online supplementary Figure S1



Subjects of this study were selected from those in the SETUP trial. From October 2014 to March 2016, 40 cases of AE-IIPs were prospectively enrolled and treated with rhTM and conventional therapy. One case was excluded from the trial, with 39 cases included in the rhTM arm. As the control arm, 67 consecutive AE-IIPs cases were diagnosed and treated with conventional therapy without rhTM between 2011 and 2013 and 6 cases were excluded by central assessment. Sixty-one cases were included in the control arm. Serum samples on day 1 were obtained from 28 AE-IIPs patients out of 39 AE-IIPs patients in rhTM arm. Twenty-six AE-IIPs patients survived for more than 7 days and serum samples on day 8 were obtained from 22 patients out of the 26 patients.

Abbreviations: MPA, microscopic polyangiitis; AE, acute exacerbation; rhTM, recombinant human soluble thrombomodulin; IIPs, idiopathic interstitial pneumonias; FDP, fibrin degradation product.