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CLNICAL SIGNIFICANCE OF HYALURONAN IN PATIENTS WITH INTERSTITIAL PNEUMONIA

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Summary at a Glance

Although extracellular matrix proteins such as hyaluronan have been thought to be simple "glue" in interstitium of the lung, the results of this study showed that serum hyaluronan is a clinically useful biomarker of interstitial pneumonia, and hyaluronan is involved in the pathogenesis of interstitial pneumonia

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ABSTRACT

Backgroung: Hyaluronan is an important constituent of the extracellular matrix in lungs, and growing evidences have shown its important biological properties in lungs. However, the role of hyaluronan in interstitial pneumonia has not been fully clarified.

Objective: The goal of this study was to clarify the role of hyaluronan in interstitial pneumonia.

Methods: Hyaluronan in serum and bronchoalveolar lavage (BAL) fluid of chronic interstitial pneumonia (CIP) was measured and the correlation with clinical parameters was determined. In addition, the correlation between hyaluronan in serum and clinical parameters was analyzed in patients with acute exacerbation of interstitial pneumonia.

Results: When compared to healthy controls, serum hyaluronan was significantly greater in patients with CIP, and was positively correlated with serum biomarkers of inflammation and fibrosis, such as CRP and surfactant protein-D. In BAL fluid, the amount of hyaluronan was positively correlated with the percentage of inflammatory cells and the amount of CXCL8. When compared to CIP patients, patients with acute exacerbation of interstitial pneumonia had significantly greater amounts of serum hyaluronan and patients with the high serum hyaluronan had the worse 60 day-outcomes.

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Conclusions: This work shows serum hyaluronan is a clinically useful biomarker of interstitial pneumonia, and suggests hyaluronan is involved in the pathogenesis of interstitial pneumonia by recruiting inflammatory cells into the lungs.

Key words: Acute exacerbation, CXCL8, Hyaluronan, Interstitial pneumonia, idiopathic pulmonary fibrosis

Short title: Hyaluronan in interstitial pneumonia

ABBREVIATIONS

AE = acute exacerbation

BAL = bronchoalveolar lavage

CIP = chronic interstitial pneumonia

CVD-IP = collagen vascular disease-associated interstitial pneumonia

High-HA = a high serum hyaluronan group

HMW = high molecular weight

IPF = idiopathic pulmonary fibrosis

Low-HA = a low serum hyaluronan group

LMW = low molecular weight

TLR = Toll-like receptor

IP = interstitial pneumonia

IP-AE = acute exacerbation of interstitial pneumonia

IIP = idiopathic interstitial pneumonia

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INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a disease with progressive pulmonary fibrosis that causes chronic and progressive dyspnea and has a poor prognosis. Histopathologically, IPF presents usual interstitial pneumonia pattern, with deposition of collagen, proteoglycans, and other extracellular matrix proteins. It has been reported inflammatory cytokines, growth factors and Fas/FasL play critical roles in the pathogenesis of IPF [1, 2], although the precise mechanisms have not been clarified.

Hyaluronan, a component of the extracellular matrix is a nonsulfated glycosaminoglycan that accounts for about 70% of the glycosaminoglycans in the lungs. Under physiological conditions, it exists with a high molecular weight (HMW) of more than 10^6 Da and is involved in water regulation and angiogenesis. Unlike HMW hyaluronan, low molecular weight (LMW) hyaluronan of about 2×10^4 Da is a degradation product of HMW hyaluronan, which stimulates the production of inflammatory mediators such as CXCL8 through Toll-like receptor (TLR) 2 and 4 signalling pathways [3].

Although the reports in 1980s which have investigated the role of hyaluronan in diffuse lung diseases suggest hyaluronan is involved in the pathogenesis of interstitial pneumonia (IP) [4, 5], the role of hyaluronan has not been fully elucidated. Today,

with the clear re-classification of idiopathic interstitial pneumonia [6], wide international recognition of acute exacerbation of interstitial pneumonia (IP-AE) [7], and the significant elucidation of the molecular biological properties of hyaluronan, it is time to re-evaluate the role of hyaluronan in IP.

For this purpose, the levels of hyaluronan in serum and bronchoalveolar lavage (BAL) fluid were investigated in patients with IPF, as well as patients with idiopathic interstitial pneumonia other than IPF (IIP) and collagen vascular disease-associated interstitial pneumonia (CVP-IP). In addition, the level of hyaluronan in serum in patients with IP-AE was analyzed.

METHODS

Subjects

The patients with chronic interstitial pneumonia (CIP) who had admitted to our hospital between 2006 and 2008 were analyzed. Patients with CIP presented no progression of symptoms such as dyspnea and radiological findings for at least three months. Serological tests, pulmonary function tests, high resolution CT, and BAL were performed for these patients. The diagnosis of idiopathic interstitial pneumonia was made in accordance with the ATS/ERS consensus conference statement [6, 8]. The diagnosis of IPF was made when the condition agreed with the diagnostic criteria for clinical IPF in cases without surgical biopsy. Collagen vascular disease associated-interstitial pneumonia (CVD-IP) was diagnosed when the patient with IP fulfilled the diagnostic criteria of collagen vascular disease, and CIP except IPF and CVD-IP was diagnosed as IIP. These three types of CIP were investigated retrospectively. Twelve healthy volunteers were used as controls. For analysis of IP-AE, the patients with IP-AE who had admitted to our hospital between 2000 and 2008 were analyzed. When fulfilled the following conditions, the diagnosis of IP-AE was made as previously reported [9]: progressive dyspnea within one month; bilateral infiltrates or ground glass opacities; decrease in PaO_2 of $\geq$10 Torr or $PaO_2/FiO_2 < 300$;

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and ruling out of pneumonia, heart failure, pulmonary thromboembolism, and pneumothorax. This study was conducted after receiving the approval of the Fukushima Medical University Ethics Committee.

Analysis of clinical parameters and hyaluronan

Hyaluronan levels in serum from the 12 healthy volunteers and patients with IP, and hyaluronan levels in BAL fluid from patients with IP were analyzed. Associations between hyaluronan in serum and blood parameters, pulmonary function tests, and inflammatory cell differentiation in BAL fluid were then analyzed. Measurements of hyaluronan levels were made using a commercially available kit (Fujirebio, Japan).

Analysis of CXCL8 in BALF

To clarify the mechanism for the relationship between hyaluronan and cell differentiation in BAL fluid, CXCL8 levels in BAL fluid was investigated. The concentration was measured using an ELISA kit (R&D Systems, Minneapolis, MN), as previously reported [10].

Analysis of hyaluronan in patients with acute exacerbation of interstitial

pneumonia

Hyaluronan levels in serum were measured in patients with IP-AE and compared to patients with clinically stable CIP. In addition, hyaluronan in serum was compared in identical individual during acute exacerbation (AE) with the stable phase. Furthermore, to investigate the association between hyaluronan in serum and prognosis, hyaluronan levels during AE and mortality at 30 and 60 days after the onset of AE were investigated.

Statistical analysis

Date are expressed as means ± SE, unless otherwise stated. For statistical analysis, Wilcoxon's signed rank test, Fisher's exact test and Pearson's correlation coefficient were used to analyze hyaluronan level in serum for identical individual during AE with the stable phase of IP, prognosis after AE with hyaluronan in serum and correlations between hyaluronan and clinical parameters, respectively. Unless otherwise indicated, Student's *t*-test and analysis of variance with Fisher's PLSD were used for compare two and groups, respectively. $P < 0.05$ was considered to be statistically significant.

RESULTS

Subjects and clinical parameters

Forty nine CIP patients including 24 IPF, 17 IIP, and 8 CVD-IP were analyzed. BAL was performed in 38 out of 49 patients. Surgical lung biopsy was performed in 14 patients (10 IPF, 3 IIP, and 1 CVD-IP). For CVD-IP, the number of patients with rheumatoid arthritis, scleroderma, dermatomyositis and Sjögren's syndrome is 1, 2, 1 and 4 patients, respectively.

In CVD-IP group, significantly more women were included (Table 1), and vital capacity and forced expiratory volume in one second were significantly lower among the three groups. In BAL fluid, the percentage of lymphocytes was significantly higher in IIP and CVD-IP than in IPF (Table 2).

Association between hyaluronan levels and clinical parameters

Hyaluronan in serum was significantly higher in CIP patients than in healthy volunteers (Figure 1), but there was no difference in hyaluronan in serum among three groups. In CIP patients, hyaluronan in BAL fluid (59.5 ± 8.4 ng/ml) had no correlation with hyaluronan in serum, and no difference in serum hyaluronan was seen among the three groups. Hyaluronan in serum had positive correlations with LDH, CRP, ESR,

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and SP-D, and a positive trend with SP-A. However, no association was seen with pulmonary function or cell differentiation in BAL fluid (Table 3). Hyaluronan in BAL fluid showed negative correlations with total lung capacity and diffusion lung capacity, and positive correlations with percentage of neutrophils and lymphocytes in BAL fluid. In addition, significant positive correlations were seen with inflammatory markers such as CRP and ESR (Table 4).

Association between hyaluronan and CXCL8 in BAL Fluid

Since there was a positive correlation between hyaluronan and percentage of inflammatory cells such as neutrophils in BAL fluid, the level of CXCL8, a potent neutrophil chemokine previously known as IL-8, was measured in BAL fluid to identify potential mechanism whereby hyaluronan might increase neutrophil recruitment into the lungs. The amount of CXCL8 in BAL fluid was positively correlated with hyaluronan as well as percentage of neutrophils in BAL fluid (Figure 2).

Clinical characteristics of patients with acute exacerbation of interstitial pneumonia

IP-AE had occurred 24 times in 23 patients in our hospital between 2000 and 2008 (8

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with IPF, 10 with IIP, and 4 with CVD-IP). In IP-AE patients, PaO_2/FiO_2 decreased, and KL-6, SP-A and SP-D increased. However, no significant differences were seen among three groups (Table 5).

Analysis of hyaluronan in serum from patients with acute exacerbation of interstitial pneumonia

Hyaluronan in serum was much higher in IP-AE patients than stable CIP patients (Figure 3). We could analyze hyaluronan in serum in 12 patients with IP during AE and in the stable phase. In these patients, hyaluronan in serum was significantly higher during AE than in the stable phase (Figure 4). We next investigated the association between hyaluronan in serum and prognosis. After excluding one patient whose clinical outcome was unclear, 22 patients with IP-AE were divided into a high serum hyaluronan group (High-HA: n = 11) and a low serum hyaluronan group (Low-HA: n = 11), according to a median value of 76.2 ng/ml. Although no difference was seen in the mortality at 30 days after the onset of AE (4 deaths in High-HA vs. 1 death in Low-HA), the mortality at 60 days was significantly worse in High-HA compared with in Low-HA (7 deaths in High-HA vs. 1 death in Low-HA).

DISCUSSION

In this study, we have demonstrated 3 major findings; 1) in CIP patients, hyaluronan in serum was elevated and positively correlated with inflammatory and fibrosis markers; 2) hyaluronan in BAL fluid was negatively correlated with total lung capacity and diffusion lung capacity and positively correlated with the percentage of inflammatory cells and the CXCL8 in BAL fluid; 3) in IP-AE patients, hyaluronan in serum was higher than in the stable phase, and the prognosis was significantly worse in High-HA.

In IP, there is excess deposition of extracellular matrix including proteoglycans in the interstitium. Proteoglycan is glycoprotein with side chains of glycosaminoglycan and basic structure of repeating disaccharide units attached to core protein. For a long time, proteoglycan and glycosaminoglycan have been thought to be simple "glue" that existed in interstitium as extracellular matrix, but in recent years they have attracted attention after being shown to have diverse biological properties [11, 12]. We have recently demonstrated intratracheal instillation of mutant CXCL8 with weak binding affinity for glycosaminoglycan induced more neutrophil recruitment into murine lungs compared with the wild-type CXCL8 [10]. These facts show important roles of chemokines and glycosaminoglycan binding in inflammatory lung diseases.

Hyaluronan is nonsulfated glycosaminoglycan that binds to proteoglycans to form

high molecular weight complexes [13]. Because of its hydrophilic property, hyaluronan is considered to be important in water regulation and solute transport in the pulmonary interstitium [14], and to play pivotal roles in lung inflammation and injury [3, 15]. In patients with IPF, hyaluronan in BAL fluid was reported to be higher in progressive phases than in stable phases, and positively correlated with the number of inflammatory cells in BAL fluid, and expression of hyaluronan is seen in fibroblastic foci [5, 16]. In bleomycin-induced lung fibrosis, elevated hyaluronan in BAL fluid positively correlated with inflammatory cells in BAL fluid and associated with the degree of alveolitis in the fibrotic lungs [17], and overexpression of hyaluronan by myofibroblasts produced an aggressive phenotype leading to severe lung fibrosis [18]

In recent years, idiopathic interstitial pneumonia has been re-classified into seven distinct groups based on the classifications of the AECC statement [6], KL-6, SP-D, and other lung fibrotic markers have been used in regular clinical settings [19-21], and the occurrence of AE in the course of CIP has also come to be widely recognized [22, 23]. Furthermore, the molecular biological properties of hyaluronan have also become clearer. We therefore re-investigated the role of hyaluronan on the pathogenesis of IP.

In this study, hyaluronan in BAL fluid was positively correlated with the percentage of inflammatory cells in BAL fluid, and hyaluronan in serum was significantly higher in

CIP patients and positively correlated with inflammatory and fibrosis markers. These facts suggest hyaluronan could be useful as a clinical marker of CIP.

Hyaluronan is synthesized mainly by fibroblasts in the lungs, and growth factors such as TGF-β make a major contribution to its synthesis [24]. Hyaluronan synthase was recently identified, and in humans it exists in three isoforms [25]. In healthy lungs, hyaluronan moves from the lungs to the systemic circulation via lymphatics, and it is finally metabolised and excreted in the liver [26]. The mechanism of increased level of hyaluronan in IP is not clear, but increased production or decreased metabolism of hyaluronan in the lungs may be involved. In addition, because hyaluronan is degraded by reactive oxygen species from HMW into LMW molecules [27], HMW hyaluronan is degraded into LMW hyaluronan in the lungs of IP in which reactive oxygen species are increased [28]. In fact, recent reports have shown LMW hyaluronan exists as well as HMW hyaluronan in BAL fluid of patients with IPF [29]. Among hyaluronidases, enzymes which degrade HMW hyaluronan, hyaluronidase-2 is shown to be mainly involved in the production of LMW hyaluronan [30], and increased expression of hyaluronan synthases by TGF-β in lung fibroblasts may also be related to the increased production of hyaluronan in IP lungs. In addition, in bleomycin-induced lung fibrosis, LMW hyaluronan inhibits apoptosis of lung epithelial cells via activation of

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TLR-dependent NF-κB activation, while it initiates inflammatory responses via TLR2 and 4. Furthermore, it has been reported overexpression of HMW hyaluronan in the lungs suppressed acute lung injury [3]. These recent reports also show the importance of hyaluronan in lung inflammation and repair, while precise elucidation of the hyaluronan metabolic pathway in the body is complicated.

To further investigate the role of hyaluronan in IP, we also analyzed the level of CXCL8. It has recently been demonstrated increased percentage of neutrophil in BAL fluid is a predictor of early mortality in patients with IPF [31] as well as IP associated with systemic sclerosis [32, 33], and neutrophils are considered to be involved in the pathogenesis of IP. CXCL8 in BAL fluid showed a positive correlation with the percentage of neutrophils as well as hyaluronan in BAL fluid, showing, in IP, hyaluronan is involved in neutrophil migration into the lungs via CXCL8. In fact, LMW hyaluronan promotes expression of inflammatory cytokines and chemokines in alveolar macrophages via TLR activation. From previous findings and the results of the present study, neutrophil migration into IP lungs is at least in part, dependent of the increased production of CXCL8 from alveolar macrophages by stimulation of TLRs with hyaluronan which is produced from fibroblasts and other cells [3].

In recent years, IP-AE, in which respiratory failure progresses rapidly together with

the appearance of new opacities in the bilateral lungs during the course of CIP, has widely recognised. Initially, AE was reported only in patients with IPF, but recent reports have shown AE also occur in patients with non-IPF [1, 34]. The cause of AE is unknown, but once it occurs, the prognosis is poor, with a mortality rate of more than 80%, making it important as a prognostic factor of IP [35]. In the present study, hyaluronan in serum was higher during AE than in the stable phase, and the prognosis of patients in High-HA was significant worse than patients in Low-HA, showing hyaluronan in serum could be a useful predictive biomarker of prognosis in patients with IP-AE.

Our study has several limitations. First, the classification of IP may not be sufficient, because the pathological evaluation of lung tissues obtained by surgical lung biopsy was performed only in the limited patients. There is a possibility that some patients with IPF who had atypical clinical and/or radiological findings are include in the IIP group, because we can not distinguish such IPF patients with IIP other than IPF by the diagnostic criteria of ATS/ERS consensus conference statement. Second, for the analysis of some data, we put all patients in the three groups together. Because the pathogenesis of IPF is considered to be different from other types of IP especially in terms of fibrotic process, it may be better to get the results and analyze the data in each

group separately. However, the role of HA, particularly on pulmonary inflammation (e. g., neutrophil migration) could not be different between IPF and other types of IP. Furthermore, recent evidence suggests that inflammation is involved in the pathogenesis of not only IP other than IPF but also IPF. For example, increased percentage of neutrophil in BALF was shown as a predictor of mortality in patients with IPF [31], and Reilkoff RA *et al.* have recently shown that regulatory T cells are associated with progressive IPF [36]. However, the limited number of patients and difficulty of accurate diagnosis for some patients without surgical lung biopsy made the separate analysis of each group difficult in the present study, further analysis for more patients with interstitial pneumonia with a precise definition is necessary to resolve these limitations. The diagnostic criteria of the recent official ATS/ERS/JRS/ALAT statement for IPF could be helpful [37]. Third, we had not analyzed the molecular size of hyaluronan in the present study. However, LMW hyaluronan is supposed to be increased in biological samples from our patients, because El-Chemaly S *et al.* have recently shown that LMW hyaluronan is increased in BALF from patients with IPF [29].

In summary, hyaluronan in serum is a clinically useful biomarker in IP including AE, and hyaluronan is involved in the pathogenesis of IP by recruiting inflammatory cells

into the lungs.

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FIGURE LEGENDS

Figure 1: The Serum Level of Hyaluronan in healthy volunteers (HV) and chronic interstitial pneumonia (CIP). The level of hyaluronan in serum was significantly higher in patients with CIP than in HV. * $p < 0.05$ vs. HV.

Figure 2: Correlation of CXCL8 with Neutrophil Percent and Hyaluronan in bronchoalveolar lavage fluid (BALF). The concentration of CXCL8 in BALF from patients with interstitial pneumonia had positive correlation with neutrophil percent (A) and hyaluronan (B) in BALF.

Figure 3: The Serum Levels of Hyaluronan in Patients with Stable and Acute Exacerbation of Interstitial Pneumonia. The level of hyaluronan in serum was significantly higher in patients with acute exacerbation of IP than in IP-stable. IP-stable = patients with stable IP; IP-AE = patients with acute exacerbation of IP. Values are the mean + SEM. * $p < 0.05$ vs. IP-stable.

Figure 4: Evolution of Serum hyaluronan (HA) levels in Patients with Interstitial Pneumonia before and upon Acute Exacerbation (n = 12). Serum HA upon acute

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exacerbation is significantly higher than before acute exacerbation.

Table 1. Clinical Characteristics of Patients with CIP

	Total	IPF	IIP	CVD-IP	P value
Subjects n	49	24	17	8	
Age yrs	64.0 ± 1.3	64.6 ± 1.7	63.5 ± 2.5	63.1 ± 3.0	NS
Gender M/F	36/13	22/2	12/5	2/6*	P < 0.05
PaO_2 mmHg	79.1 ± 2.3	81.5 ± 3.5	74.3 ± 4.1	81.4 ± 2.9	NS
WBC /μL	7821 ± 376	7804 ± 426	8281 ± 650	6950 ± 1385	NS
CRP mg/dl	0.8 ± 0.2	0.5 ± 0.2	1.1 ± 0.6	1.3 ± 0.6	NS
ESR mm/hr	35 ± 4	31 ± 4	34 ± 6	51 ± 13	NS
LDH U/ml	243 ± 12	238 ± 10	256 ± 29	234 ± 37	NS
KL-6 U/ml	1438 ± 142	1529 ± 228	1444 ± 241	1153 ± 176	NS
SP-A ng/ml	113.9 ± 12.4	126.5 ± 19.1	109.4 ± 22.4	83.8 ± 15.2	NS
SP-D ng/ml	232.0 ± 21.1	249.3 ± 31.2	226.7 ± 39.5	190.1 ± 36.8	NS

CIP = chronic interstitial pneumonia, CRP = C reactive protein, CVD-IP = collagen vascular disease associated with interstitial pneumonia, ESR = erythrocyte sedimentation rate, IPF: idiopathic pulmonary fibrosis, IIP: idiopathic interstitial pneumonia other than IPF, KL-6 = Krebs von den lungen-6, SP-A = surfactant protein-A, SP-D = surfactant protein-D. Values are the mean ± SEM. * P < 0.05 vs IPF group
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Table 2. PFT and BAL Analysis of Patients with CIP

	Total (n = 49)	IPF (n = 24)	IIP (n = 17)	CVD-IP (n = 8)	P value
VC L	2.3 ± 0.1	2.5 ± 0.2	2.4 ± 0.2	1.7 ± 0.1*†	P < 0.05
VC % pred	76.1 ± 2.7	77.0 ± 4.4	80.5 ± 1.7	66.1 ± 6.4	NS
FEV_1 L	1.93 ± 0.09	2.07 ± 0.13	1.93 ± 0.12	1.35 ± 0.11*†	P < 0.05
FEV_1/FVC %	85.8 ± 1.3	86.6 ± 1.6	87.7 ± 2.3	78.1 ± 3.4	NS
TLC % pred	79.3 ± 2.7	80.4 ± 3.8	84.8 ± 4.3	69.4 ± 6.4	NS
DL_{CO} % pred	55.8 ± 3.5	54.7 ± 5.2	58.6 ± 6.7	50.4 ± 6.5	NS
DL/V_A % pred	77.7 ± 3.7	72.9 ± 4.6	80.4 ± 8.5	83.0 ± 7.8	NS
Total Cell Count (x 10^4/ml BALF)	33.2 ± 9.1	27.5 ± 4.5	47.7 ± 21.4	15.2 ± 3.6	NS
AM %	67.7 ± 3.2	77.4 ± 2.5	61.6 ± 6.0	63.5 ± 10.3	NS
Lym %	17.9 ± 2.1	10.6 ± 1.6	19.8 ± 3.7*	24.9 ± 4.8*	P < 0.05
Neu %	11.2 ± 2.6	8.1 ± 1.7	15.9 ± 6.5	9.5 ± 5.0	NS
Eos %	3.7 ± 0.8	5.1 ± 1.6	3.2 ± 1.0	2.2 ± 0.7	NS

Patients with CVD-IP include rheumatoid arthritis (n = 1), scleroderma (n = 2), dermatomyositis (n = 1) and primary Sjögren syndrome (n = 4). AM = alveolar macrophages, BALF = bronchoalveolar lavage fluid, CIP = chronic interstitial penumonia, CVD-IP = collagen cascular disease associated interstitial pneumonia, DLCO = diffusing capacity for carbon monoxide, Eos = eosinophils, FEV1 = Forced expiratory volume in one second, FVC = forced vital capacity, IIP = idiopathic interstitial pneumonia other than IPF, IPF = idiopathic pulmonary fibrosis, Lym = lymphocytes; Neu = neutrophils, PFT = pulmonary function test, TLC = total lung capacity. Values are the mean ± SEM. * P < 0.05 vs IIP group, † P < 0.05 vs IPF group
81x60mm (300 x 300 DPI)

Table 3. Correlation Analysis between Serum Hyaluronan and Clinical Parameters in Patients with CIP

	Correlation coefficients	P value
KL-6 U/ml	0.099	0.905
SP-A ng/ml	0.314	0.055
SP-D ng/ml	0.405	0.011
LDH U/ml	0.658	<0.001
CRP mg/dl	0.502	0.001
ESR mm/hr	0.406	0.013
PaO_2 mmHg	0.007	0.969
VC % pred	0.273	0.102
FEV_1 L	0.212	0.208
TLC % pred	0.224	0.235
DL_{CO} % pred	0.102	0.580
Total Cell Count $x10^4$/ml BALF	0.103	0.625
Lym in BALF %	0.091	0.619
Neu in BALF %	0.211	0.245

CIP = chronic interstitial pneumonia, BALF = bronchoalveolar lavage fluid, CRP = C reactive protein, DLCO = diffusing capacity for carbon monoxide, ESR = erythrocyte sedimentation rate, Lym = lymphocytes; Neu = neutrophils, KL-6 = Krebs von den lungen-6, SP-A = surfactant protein-A, SP-D = surfactant protein-D, VC = vital capacity.

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Table 4. Correlation Analysis between Hyaluronan in BALF and Clinical Parameters in Patients with CIP

	Correlation coefficients	P value
KL-6 U/ml	0.128	0.457
SP-A ng/ml	0.002	0.993
SP-D ng/ml	0.016	0.924
LDH U/ml	-0.172	0.316
CRP mg/dl	0.424	0.011
ESR mm/hr	0.366	0.033
PaO_2 mmHg	0.175	0.339
VC % pred	-0.328	0.055
FEV_1 L	-0.255	0.140
TLC % pred	-0.448	0.019
DL_{CO} % pred	-0.048	0.026
Total Cell Count $x10^4$/ml BALF	0.099	0.602
Lym in BALF %	0.347	0.044
Neu in BALF %	0.483	0.004

CIP = chronic interstitial pneumonia, BALF = bronchoalveolar lavage fluid, CRP = C reactive protein, DLCO = diffusing capacity for carbon monoxide, FEV1 = forced expiratory volume in one second, ESR = erythrocyte sedimentation rate, Lym = lymphocytes; Neu = neutrophils, KL-6 = Krebs von den lungen-6, SP-A = surfactant protein-A, SP-D = surfactant protein-D, YLC = total lung capacity, VC = vital capacity.
81x60mm (300 x 300 DPI)

Table 5. Clinical Data of Patients with Acute Exacerbation of Interstitial Pneumonia

	Total	IPF	IIP	CVD-IP	P value
Subjects n	22	8	10	4	
Cases n	23	9 (39%)	10 (43%)	4 (17%)	
Age yrs	65.3 ± 2.0	64.2 ± 4.0	70.2 ± 1.6	55.8 ± 2.6*	P < 0.05
Gender M/F	14/8	6/2	7/3	1/3†	P < 0.05
PaO_2/FIO_2	218.7 ± 19.5	176.9 ± 27.7	257.4 ± 28.8	205.3 ± 48.5	NS
WBC /μL	10977 ± 898	9863 ± 890	10970 ± 1541	13225 ± 2641	NS
CRP mg/dl	5.8 ± 1.2	5.6 ± 2.0	7.3 ± 2.1	2.5 ± 1.7	NS
ESR mm/hr	28 ± 8	20 ± 13	31 ± 12	33 ± 25	NS
LDH U/ml	416 ± 28	391 ± 47	388 ± 27	536 ± 89	NS
KL-6 U/ml	1668 ± 251	1907 ± 334	1548 ± 447	1554 ± 519	NS
SP-A ng/ml	208.4 ± 102.2	131.3 ± 33.4	72.6 ± 4.8	421.5 ± 234.5	NS
SP-D ng/ml	327.2 ± 54.9	471.8 ± 111.0	279.4 ± 70.9	193.8 ± 68.3	NS

Patients with CVD-IP include dermatomyositis (n = 2), polymyositis (n = 1), Sjögren syndrome (n = 2), scleroderma (n = 1). One dermatomyositis patient is complicated with Sjögren syndrome. CVD-IP = collagen cascular disease associated interstitial pneumonia, ESR = erythrocyte sedimentation rate, IIP = idiopathic interstitial pneumonia other than IPF, IPF = idiopathic pulmonary fibrosis, KL-6 = Krebs von den lungen-6, SP-A = surfactant protein-A, SP-D = surfactant protein-D. Values are the mean ± SEM. * P < 0.05 vs IIP group, † P < 0.05 vs IPF group
81x60mm (300 x 300 DPI)

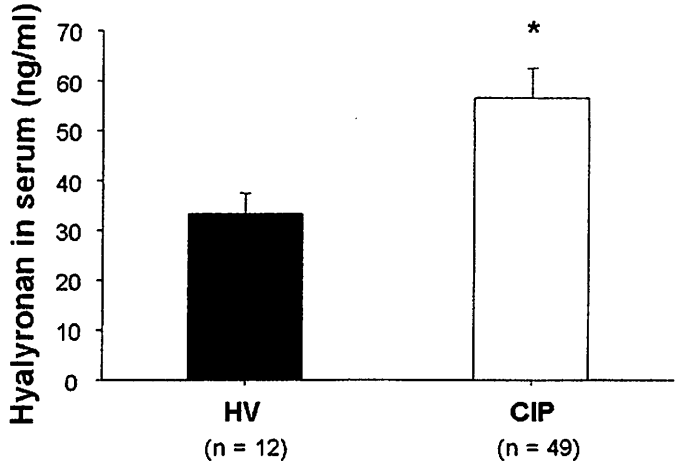

Figure 1: The Serum Level of Hyaluronan in healthy volunteers (HV) and chronic interstitial pneumonia (CIP). The level of hyaluronan in serum was significantly higher in patients with CIP than in HV. * $p < 0.05$ vs. HV.
81x60mm (300 x 300 DPI)

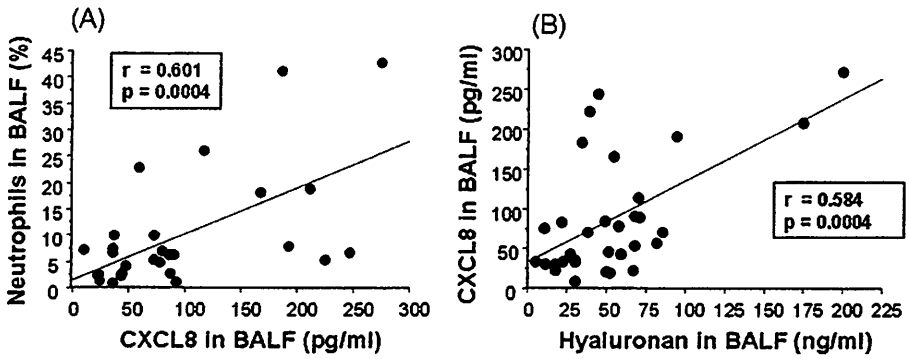

Figure 2: Correlation of CXCL8 with Neutrophil Percent and Hyaluronan in bronchoalveolar lavage fluid (BALF). The concentration of CXCL8 in BALF from patients with interstitial pneumonia had positive correlation with neutrophil percent (A) and hyaluronan (B) in BALF.
81x60mm (300 x 300 DPI)

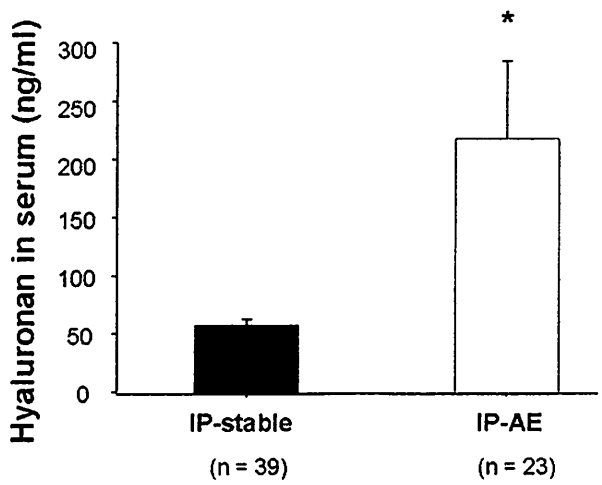

Figure 3: The Serum Levels of Hyaluronan in Patients with Stable and Acute Exacerbation of Interstitial Pneumonia. The level of hyaluronan in serum was significantly higher in patients with acute exacerbation of IP than in IP-stable. IP-stable = patients with stable IP; IP-AE = patients with acute exacerbation of IP. Values are the mean + SEM. * $p < 0.05$ vs. IP-stable.
81x60mm (300 x 300 DPI)

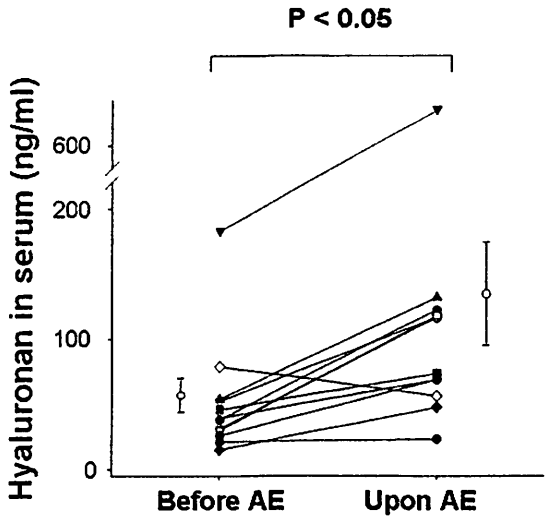

Figure 4: Evolution of Serum hyaluronan (HA) levels in Patients with Interstitial Pneumonia before and upon Acute Exacerbation (n = 12). Serum HA upon acute exacerbation is significantly higher than before acute exacerbation.
81x60mm (300 x 300 DPI)