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Clinical characteristics classified by the serum KL-6 level in patients with organizing pneumonia

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Abstract. *Background:* The serum Krebs von der Lungen-6 (KL-6) level is a useful marker correlated with the severity of various interstitial lung diseases. There have been few reports about the clinical characteristics of organizing pneumonia (OP) associated with the serum KL-6 levels. *Objective:* This study was performed to determine whether the serum KL-6 levels can help determine the optimal treatment for OP. *Designs:* Patients diagnosed with OP by clinical, radiological and histopathological findings were retrospectively reviewed. The OP patients were classified into two groups based on their serum KL-6 levels: normal KL-6 and high KL-6 groups. The two groups were compared with regard to their clinical and radiological data and therapeutic response one month after the start of treatment. *Results:* The clinical records of twenty-two patients diagnosed with OP were reviewed. The serum KL-6 level was elevated in 11 of the 22 patients. There were no obvious differences in the clinical data between the two groups, although patients in the normal KL-6 group tended to have a fever. There were no significant differences in the chest X-ray (CXR) score or computed tomography (CT) score between the two groups. The CXR scores were correlated with the serum KL-6 levels. At 1 month after the diagnosis, 11 patients who needed treatment with prednisolone were included in the high KL-6 group. *Conclusions:* Patients with normal KL-6 levels showed lower CXR and CT scores. The serum KL-6 level on admission is a useful marker to judge the need for corticosteroid treatment in OP patients. *(Sarcoidosis Vasc Diffuse Lung Dis 2013; 30: 43-51)*

Key words: organizing pneumonia, Krebs von der Lungen-6, chest X-ray score, computed tomography score

Introduction

Organizing pneumonia (OP) is a distinct clinical entity with features of an inflammatory pneumonia rather than a primary airway disorder. The clinical presentation is like community-acquired pneumonia because cough, fever, and dyspnea are common symptoms (1). In general, OP can be classified into cryptogenic organizing pneumonia (COP) or secondary OP (SOP) on the basis of the underlying diseases. COP is the idiopathic form of organizing pneumonia, as mentioned in The American Thoracic Society (ATS) and European Respiratory Society (ERS) statements regarding the classification of idiopathic interstitial pneumonias. SOP is associated with infections, drugs, connective tissue diseases, radiation, organ transplantation and other causes (2). The clinical and radiographic findings, treatment response, and prognosis in patients with both COP and SOP are similar and nonspecific (1).

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The diagnosis of OP is defined by the clinical, radiological and histopathological features (3-4). These radiological features show three patterns. The most common radiological feature of COP is peripheral, bilateral, and diffuse alveolar opacities in the lobes. The second classification is a solitary pulmonary nodule. The third type of radiological presentation is infiltration (2). The histopathological pattern is characterized by patchy filling of the lung alveoli and respiratory bronchioles by loose plugs of granulation tissue (5). Bronchoalveolar lavage (BAL) is one of diagnostic tools for OP. Although there are no major differences in the BAL findings between COP and SOP, the lymphocyte count in the BAL of patients with SOP is higher than that of COP patients (1). With regard to treatment, if the patients with OP have respiratory symptoms, they will need therapy with glucocorticoids, because spontaneous improvement is rare during the natural clinical course of OP (6). However, there have been reports of cases that improved with no treatment.

Krebs von der Lungen-6 (KL-6) is a mucin-like high molecular weight glycoprotein that is expressed in type II pneumocytes and respiratory bronchiolar epithelial cells in the normal lungs (7, 8). The serum KL-6 level is associated with the highest sensitivity, specificity and diagnostic accuracy for the presence of idiopathic pulmonary fibrosis (IPF). Surfactant protein-D (SP-D) is also a useful biomarker of interstitial lung disease (ILD) (9). SP-D is produced by two types of non-ciliated epithelial cells in the peripheral airway; alveolar type II cells and Clara cells (10). There was a report that KL-6 is superior to SP-D as a diagnostic marker of ILD when ROC curves were used to evaluate the diagnostic value of KL-6 and SP-D in ILD, and the level of SP-D was higher in patients with bacterial pneumonia, as well as those with ILD (11). There was one report of a comparison of the chest computed tomography (CT) findings and serum KL-6 levels in patients with COP (12). The findings of traction bronchiectasis and architectural distortion on chest CT scans in patients with COP were associated with increased serum KL-6 levels, which were suggested to be related to spontaneous improvement or relapse after steroid treatment. Therefore, we performed this study to retrospectively compare the clinical characteristics, treatment responses and prognosis between the high KL-6 and normal KL-6 groups of patients with OP, and to evaluate necessity of treatment for patients with OP.

Methods

Our study was approved by the ethics committee of Tokyo Medical University, and all patients gave written informed consent on admission for bronchoscopy.

Subjects and diagnostic criteria

The study population consisted of 22 patients (12 males and 10 females) with OP, who were admitted to the Department of Pulmonary Medicine, Tokyo Medical University, between January 1, 2005 and December 31, 2011. OP was histopathologically diagnosed and included the data regarding the clinical and radiological diagnosis prior to the patient referral for transbronchial lung biopsy (TBLB) under bronchoscopy. OP was histologically diagnosed by the presence of loose plugs of granulation tissue in the respiratory bronchioles and/or exudative infiltration and alveolitis in the alveoli. OP was classified into COP and SOP on the basis of the existences of underlying diseases.

The enrolled patients were retrospectively reviewed for their clinical information and laboratory data on admission; the white blood cell count (WBC), hemoglobin, platelet count, lactate dehydrogenase (LDH; normal range: 106-211U/l), KL-6 (normal range: <430U/ml), C-reactive protein (CRP; normal range: 0.3mg/dl), anti-nuclear antibody (ANA; normal range: <40), creatinine (normal range; 0.6-1.1mg/dl) and respiratory condition (oxygen saturation (SpO_2)/fraction of oxygen concentration (F_IO_2) ratio (SpO_2/ F_IO_2) were all assessed.

Chest X-ray and chest CT scans

All patients underwent chest X-ray (CXR) and chest CT scans before bronchoscopy. The CXR scans were re-evaluated one month after treatment or at the follow-up examination. The CXR score (range; 0-4) was measured by the modified Murray lung injury score (12). We used a helical CT scanner (Light speed VCT, GE healthcare, Milwaukee, USA) for the analysis. After standard 10 -mm thick

contiguous scanning to screen for chest abnormalities, scanning with 1 -mm collimation was performed for the affected lesions. The severity of the interstitial changes, such as ground-glass opacity and fibrosis, was scored with reference to the modified scoring method proposed by Kazerooni for six slices obtained at full inspiration (13). The percentage of the lung with interstitial changes was determined using the following five-point scale: CT score 1, 0 < interstitial changes <5% involvement; 2, 5% ≤ interstitial changes <25%; 3, 25% ≤ interstitial changes <50%; 4, 50% ≤ interstitial changes <75%; and 5, ≥75% interstitial changes. The maximum CT score was 30.Two pulmonologists independently interpreted the findings of CT and high-resolution CT scans without information about the clinical history of the patient.

Bronchoscopic procedures

BAL and TBLB were performed for all patients. BAL was obtained by the instillation of 50 mL of 0.9% saline in three aliquots, either into the segment involved when the shadowing was localized, or into the middle lobe when shadowing was diffuse on chest CT scans. The total cell counts of unfractionated BAL fluid were counted using a hemocytometer. Differential counts were performed using a smear of May-Giemsa stain. The pellet in the BAL fluid was analyzed for lymphocyte subsets by flow cytometry using clusters of differentiation (CD) 3, CD4, CD8, CD29 and CD56 monoclonal antibodies (Becton Dickinson Co, Mountain View, CA). TBLB specimens were obtained from infiltration shadows on chest CT scans. The specimens obtained by TBLB were placed on glass slides, fixed with 10% formalin and processed for Hematoxylin-Eosin staining. One pathologist, who had no knowledge of the clinical data of the patients diagnosed with OP, performed the analyses.

Treatment

Patients who were symptomatic or exhibited respiratory failure were started on treatment within a week after diagnosing OP. The patients were first orally administered 0.5 mg/kg/day of prednisolones for two weeks, and tapered to 5 mg after these two weeks of initial administration. If the patient had no complaints and was not experiencing respiratory failure, they were followed as an outpatient every two weeks.

All patients were followed regarding their laboratory data (serum KL-6 level), radiological data (CXR score) and respiratory condition (SpO_2/ F_IO_2) a month after the diagnosis and/or initiation of treatment.

Statistical analyses

The data are shown as the means ± SE. The Mann-Whitney U test was used to assess the differences between each group. Fisher's exact test was performed to test for differences in the distribution of these categorical variables between the groups. P values < 0.05 were considered to be significant.

Results

Characteristics of the patients reviewed in the study

We diagnosed 22 patients (12 male and 10 female) as having OP during the period of the study. Their mean age was 60.8±0.67 years. Fourteen patients (63.6%) were former or current smokers. Their mean pack-years was 34.3±15. Ten patients (45.5%) were diagnosed with COP and 12 patients (54.5%) with SOP. Table 1 shows that the

Table 1. Enrolled patients

	Number (%)
Age (years)	60.8±0.67
Sex, M/F	11/11
Smoking history	11 (50.0)
Underlying disease	
Cryptogenic OP	11 (50.0)
Secondary OP	11 (50.0)
CRF	3 (13.6)
RA	2 (9.1)
MDS	3 (13.6)
SLE	1 (4.5)
Drug	2 (9.1)
Radiation exposure	1 (4.5)

M=Male; F=female; OP=organizing pneumonia; CRF=chronic renal failure; RA=rheumatic arthritis; MDS=myelodysplastic syndrome; SLE=systemic lupus erythematosus

underlying diseases in patients with SOP were: chronic renal failure (CRF) in three patients, myelodysplastic syndrome (MDS) in three, rheumatoid arthritis (RA) in two, drug-induced in two, systemic lupus erythematosus (SLE) in one, and radiation exposure in one patient.

Relationship between the serum KL-6 levels and the clinical data

Patients were classified into normal KL-6 and high KL-6 groups as shown in Table 2 on the basis of their serum KL-6 levels on admission. The serum KL-6 level was increased in 11 of the 22 patients. SOP was tended to be more common in the normal KL-6 group (p=0.099). There were no significant differences in the sex, age, smoking history or SpO_2/F_IO_2 between the two groups. There were significant differences in the LDH, hemoglobin and serum KL-6 level between the two groups (p=0.022, 0.014, and 0.0020, respectively). With regard to symptoms, the patients in the normal KL-6 group tended to have a fever. Three patients with a high KL-6 level and four with a normal level were positive for ANA.

Evaluation of BAL fluid in OP patients

As shown in Table 3, both groups showed increased numbers of lymphocytes in the BAL. The percentages of the differentiated cells in the BAL were not significantly different between the groups. A CD4/CD8 ratio of lymphocytes in the BAL was normal range in both groups. There were small numbers of pulmonary natural killer (NK) and NKT cells in the BAL in both of the groups. The

Table 2. Characteristics of high KL-6 and normal KL-6

	High KL-6 (n=11)	Normal KL-6 (n=11)	P value
Age (years)	65. 3±5.9	56.3±1.5	0.16
Sex, M/F	6/5	6/5	0.33
Smoking history	7	4	0.197
Pack-year	31.0±12.9	40.0±18.6	0.37
Cryptogenic/Secondary	7/4	3/8	0.099
Symptoms			
Fever	1	5	0.068
Cough	5	6	0.50
Sputum	2	2	0.41
Chest pain	1	2	0.39
Shortness of breath	6	5	0.50
Laboratory data			
WBC	5200±197	5982±254	0.47
Neutrophils %	59.3±0.87	54.2±2.3	0.54
Eosinophils %	3.5±0.16	5.0±0.41	0.30
Lymphocytes %	26.4±0.73	22.9±2.1	0.65
Hemoglobin	12.6±0.19	10.1±0.21	0.014
Platelet	22.2±1.0	21.7±1.4	0.93
LDH	298±12.6	183±6.3	0.022
Creatinine	1.46±0.20	2.60±0.32	0.37
CRP	1.24±0.12	6.91±0.71	0.028
Serum KL-6	1838±142	215±6.6	0.0020
Positive ANA (n)	3	4	0.32
SpO2/FIO2	426±6.3	431±4.1	0.88

n=number; M=Male; F=female; KL-6=Krebs von den Lungen-6; WBC=white blood cells; LDH= Lactate dehydrogenase; CRP=C-reactive protein; ANA=anti-nuclear antibody; SpO2=Oxygen saturation; FIO2= fraction of inspired oxygen concentration

CD4 CD29 lymphocytes in the BAL were within the normal range. The numbers of total cells in the BAL were not related to the serum KL-6 levels, CXR score or CT score.

Comparison of chest radiological images

As shown in Table 4, there were no significant differences in the CXR score or CT score between the groups. When the CXR score and serum KL-6

Table 3. Bronchoalveolar lavage fluid

	High KL-6 (n=11)	Normal KL-6 (n=11)	P value
Recovery rate %	38.5±1.4	42.5±1.6	0.58
Total cell counts (x104/mL)	70.3±7.5	45.9±3.9	0.40
Macrophages %	45.4±2.6	54.4±2.1	0.42
Lymphocytes %	49.6±2.2	38.8±2.0	0.29
Eosinophils %	2.0±0.31	2.2±0.21	0.88
Neutrophils %	2.4±0.51	4.3±0.65	0.49
Subtypes			
CD4+ lymphocytes %	39.4±2.0	46.3±1.9	0.46
CD8+ lymphocytes %	50.5±2.0	35.3±2.1	0.14
CD4/CD8	1.17±0.098	2.80±0.30	0.14
NKT cells %	2.51±0.16	2.77±0.25	0.79
NK cells %	2.83±0.20	2.48±0.24	0.74
CD4+CD29+ lymphocytes %	37.9±2.0	45.9±1.9	0.39
CD8+CD29+ lymphocytes %	46.0±2.4	34.3±2.1	0.28

n=number; M=Male; F=female; KL-6= Krebs von den Lungen-6; CD=cluster of differentiation; NKT; natural killer T lymphocytes; NK=natural killer cells

Table 4. Comparison of chest radiological images

	High KL-6 (n=11)	Normal KL-6 (n=10)	P value
CXR score	2.1±0.09	1.5±0.08	0.17
CT score	13.9±0.75	7.8±0.72	0.088
Dominant lesion			
Upper lobe	9	5	0.14
Middle lobe	10	6	0.13
Lower lobe	11	10	0.99
bilateral/unilateral	10/1	6/4	0.13
Infiltration pattern			
Parenchymal lesion	5	7	0.19
Ground glass opacity	10	9	0.52
Patchy infiltration	6	7	0.27
Micronodular lesion	0	0	0.99
Mass lesion	0	0	0.99
Reverse halo sign	1	1	0.52
Pleural effusion	2	3	0.32
Traction bronchiectasis	3	3	0.37
Interseptal thickening	1	2	0.37
Mediastinal lymphadenopathy	4	4	0.34

KL-6=Krebs von den Lungen-6; CXR=chest X-ray

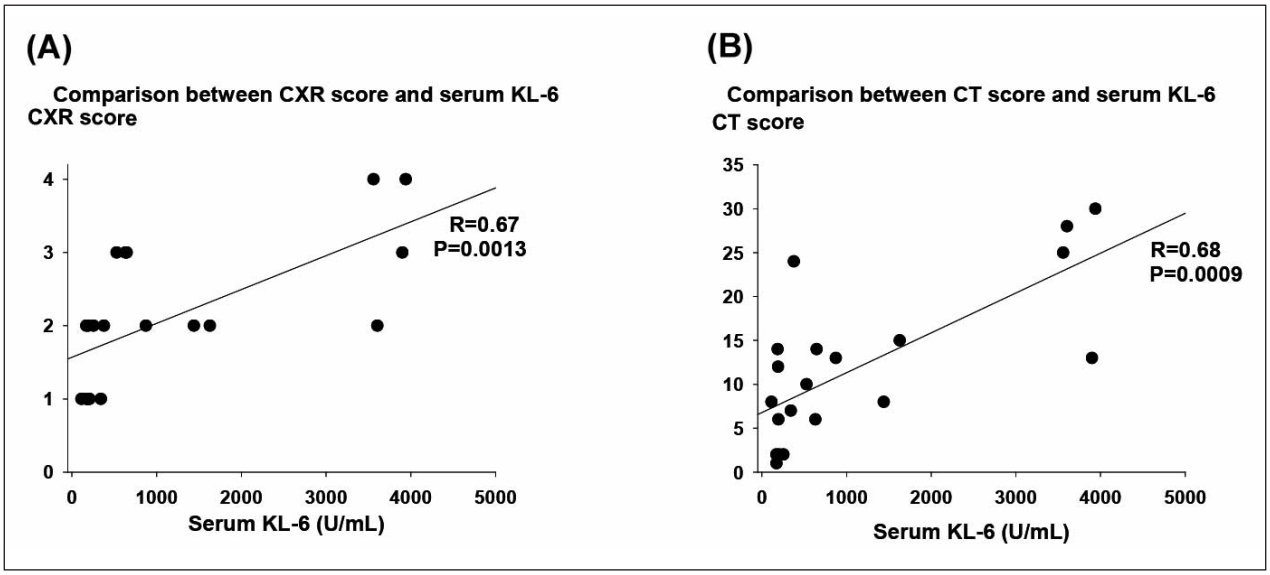

Fig. 1. (A) The relationship between the CXR scores and serum KL-6 levels. The serum KL-6 levels were correlated with the CXR scores (P=0.0013). (B) The relationship between the CT scores and serum KL-6 levels. The serum KL-6 levels were correlated with the CT scores (P=0.0009).

levels were compared (Figure 1a), it was found that the CXR scores were correlated with the serum KL-6 levels (R=0.67, p=0.0013). The CT scores were also correlated with the serum KL-6 levels(R=0.68, p=0.0009) (Figure 1b). There were no significant differences in the dominant abnormal pulmonary lesions or infiltration patterns on chest CT scans between the high KL-6 and normal KL-6 groups. Micronodular lesions and mass lesions were not detected on the chest CT findings in any of the present patients. The high KL-6 group showed a tendency to have worse interstitial changes on chest CT findings and higher CT scores on admission than the normal KL-6 group.

Comparison of clinical parameters after treatment

prednisolone was administered for treatment to 18 patients. Four patients did not need treatment because they had no symptoms or evidence of respiratory failure, and had improved laboratory data and radiological findings without treatment (Table 5). Eleven patients who needed to be treated with prednisolone were included in the high KL-6 group (p=0.045). With regard to the therapeutic effect after a month of treatment, there were no significant differences in the serum KL-6 and SpO_2/F_1O_2 before or after administration. The CXR score after administration showed a significant improvement in both the high KL-6 and normal KL-6 groups ($p<0.0001$ and p=0.010, respectively). The CXR score after treatment did not correlate with the serum KL-6 level (p 0.75). The infiltration shadows on chest X-ray findings completely disappeared in 4 of the 11 patients in the high KL-6 group and 5 of the 11 patients with a normal KL-6 level after a month.

Discussion

As described in a previous report, there were no significant differences in the clinical data between patients with COP and SOP (1). With regard to the relationship between the KL-6 levels and therapeutic effects on OP, the patients with high KL-6 levels more frequently needed treatment with prednisolone, although there were no differences in the clinical data regarding the need for treatment or KL-6 levels based on whether patients had SOP or COP. In our enrolled patients with OP, the high KL-6 patients with higher CXR scores and CT scores showed more severe respiratory conditions and expansion of interstitial changes although CXR score and CT score did not show significant difference between normal KL-6 and high KL-6

Table 5. Comparison of clinical parameters after treatment

	(A) High KL-6 (n=11)	(B) Normal KL-6 (n=11)	P value (A) vs. (B)
Treatment			0.045
Prednisolone	11	7	
No treatment	0	4	
KL-6			
Before	1838±142	215±6.6	0.0020
After	1080±67.2	264±11.8	0.015
P value, before vs. after	0.15	0.31	
SpO2/FIO2			
Before	426±6.3	431±4.1	0.88
After	461±0.34	457±1.9	0.50
P value, before vs. after	0.10	0.10	
CXR score	(n=11)	(n=11)	
Before	2.1±0.09	1.5±0.08	0.17
After	0.73±0.06	0.64±0.06	0.75
P value, before vs. after	<0.0001	0.010	
Complete improvement (n)	4	5	0.32

KL-6=Krebs von den Lungen-6; CXR=chest X-ray

groups. Therefore, prednisolone therapy was required for OP patients with a high KL-6 level with high CXR and CT scores, and in those demonstrating abnormal KL-6 levels with high CXR or CT scores.The serum KL-6 and SP-D levels are useful biomarkers for the diagnosis of various types of interstitial lung disease (ILD) (14). LDH has also been studied as a marker for pulmonary inflammation (15) and could reflect the cellular destruction of pulmonary cells. We reviewed the clinical characteristics of the 22 patients with OP in the present study and analyzed them to determine whether the KL-6 and LDH levels were useful biomarkers for OP. As a result, they were both found to be effective for evaluating the respiratory condition of OP patients and for predicting the need to start treatment for OP patients with respiratory failure. Therefore, patients with high KL-6 and LDH levels can be considered to be indicated for treatment with prednisolone.

In the previous reports, the relationship between smoking and OP was controversial. Although OP has usually been considered to be unrelated to smoking (16-18), there was a review that showed that an inverse relationship might exist (19). In our present study, there were 11 smokers in the 22 patients. This was a relatively high percentage of patients, suggesting that smoking might be related to the occurrence of OP. There were, however, no relationships between smoking and the serum KL-6 levels, although previous studies suggested that KL-6 also increases with aging and a chronic smoking history (20).

There were no significant differences in the KL-6 levels based on the symptoms. Fever was more frequently reported in OP patients with normal KL-6 levels. Laboratory abnormalities, such as mild anemia and an elevated CRP level, were more common in SOP patients with a normal KL-6 level. We supposed that the SOP patients in the normal KL-6 group likely included cases with malignancies and, connective tissue disorders, and that the symptoms were probably associated with the underlying disease. Although there were no significant differences between the hemoglobin and ESR when comparing patients with COP and SOP in a previous study (1), the results obtained in this study might have been influenced by the severity of MDS and CRF in the SOP patients. ANA positivity was recognized regardless of the serum KL-6 levels, and ANA positivity was previously reported in OP patients (21).

There were no significant differences between the SpO_2/F_IO_2 and serum KL-6 levels in patients with OP, although there was a previously report of a significant correlation between the serum KL-6 levels and indices of lung injury severity (oxygenation index and PaO_2/ F_IO_2) in patients with acute respiratory distress syndrome (22). Rapidly progressive OP with severe respiratory failure is rare (23). In our study, although the serum KL-6 level was related to the CT score and CXR score, the values of CT score and CXR score themselves were not useful markers of the need for predonisolone treatment and oxygen supplementation, because even if our OP patients showed high CT and CXR scores, they did not need oxygen supplementation.

The BAL indicates characteristic clinical features of OP. The proportion of macrophages is lower in OP than in normal subjects, although the total cell counts are higher (24). Although the relationship between BAL in OP and the serum KL-6 level is unclear, there have been several reports about the BAL in ILD patients. For example, the KL-6 levels in the BAL correlated with the serum KL-6 levels and the number of total cells, lymphocytes and neutrophils in the BAL of patients with interstitial pneumonia (25) .In our cases with OP, there were no differences between the serum KL-6 levels and the total cell counts or the number of lymphocytes in the BAL. NKT cells comprise a unique subgroup of lymphocytes that express T cell receptors (TCRs), as well as markers associated with NK cells, such as CD56 and/or CD161 (26). Although the role of NKT cells in humans is still not completely understood, they have mainly been connected with priming and regulating the immune responses. There have been reports that higher frequencies of NKT cells are present in the BAL of patients with hypersensitivity pneumonitis (27) and eosinophilic pneumonia (28). In a comparison of the numbers of NKT cells in the BAL of ILD patients, the COP patients had the lowest numbers of NKT cells. No significant differences were found in the percentages of NK cells in ILD patients. Similar to previous reports, the NKT and NK cells in the BAL of the patients with OP in our study was not associated with the pathogenesis of the disease.

The CXR score is one of the lung injury scores (LIS). The LIS score is commonly used to determine the severity of ARDS. The LIS value was previously reported to be positively related to the KL-6 levels (29). In our present study, the CXR score was significantly correlated with the KL-6 levels in patients with OP. High KL-6 levels might therefore indicate the severity of OP. The findings on CT scans of traction bronchoectasis and architectural distortion in COP patients has been reported to correlate with the KL-6 levels (30). The CT score indicated the prognosis in ARDS patients complicated with fibrous changes (31). In our study, the CT score in patients with OP was also significant related to the serum KL-6 levels, although there were no significant differences in the CT scores between the normal KL-6 and high KL-6 groups.

The standard treatment for patients with OP is the administration of predonisolone. The dose of predonisolone has not been standardized, but generally, the recommended initial dose is 0.5 to 1.0 mg/kg per day of prednisolone for four to six weeks. The predonisolone is then tapered every 2 to 4 weeks to a dose of 10 mg/day, and takes from six to 12 months before patients are no longer receiving the steroid (2)(19)(32)(33). Relapses are common upon stopping or reducing the predonisolone, often leading to prolonged treatment (34). In a previous report of patients with OP, 65% of patients needed corticosteroid therapy and 13% of patients were followed up without treatment (24). When patients are diagnosed with OP, they are generally administered prednisolone according to the grade of severity of their clinical and radiological data. In this study, the patients with normal KL-6 levels did not need predonisolone treatment, and they showed low CXR and CT scores. We suggest that measuring the KL-6 level on admission is the most useful marker to determine the appropriate treatment for patients with OP.

This study had some limitations, because the number of reviewed patients in the study is small, surgical lung biopsies were not performed in most patients, because there have been several reports that clinical and radiological data, combined with TBLB and BAL, can accurately diagnose OP. However, surgical lung biopsy remains the gold standard for the diagnosis of OP (33). Moreover, the laboratory data may have been influenced because the OP patients included both COP and SOP patients.

In conclusion, the serum KL-6 levels were correlated with the CXR score and CT score, and they also correlated with the severity of LIS. We found that patients who needed to be treated for OP showed

higher CXR and CT scores accompanied with high serum KL-6 levels on thier initial admission. The initial level of serum KL-6 is therefore a useful marker for determine whether prednisolone treatment should be started.

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