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ORIGINAL ARTICLE

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Comparative evaluation of KL-6 and surfactant protein D as serum markers for interstitial pneumonia associated with collagen diseases

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Abstract We evaluated the usefulness of serum levels of KL-6 and surfactant protein D (SP-D) as markers of interstitial pneumonia (IP) associated with collagen diseases in 115 patients. KL-6 and SP-D levels in patients with IP (n = 38) were significantly higher than those in patients without IP (n = 77). Moreover, KL-6 and SP-D levels in patients with active IP (n = 8) were significantly higher than those in patients with inactive IP (n = 30). Both KL-6 and SP-D proved useful for the diagnosis of IP associated with collagen diseases and for the evaluation of disease activity. We classified IP into three groups according to the extent of the lesion on computed tomographs of the chest. There was a significant difference among three groups in serum levels of KL-6, but not in serum levels of SP-D. KL-6 proved to be useful as an indicator of the extent of the IP lesions. We monitored changes in KL-6 and SP-D levels in seven of eight patients with active IP. Chronological changes of serum KL-6 and SP-D in patients with active IP were different. In fatal cases in particular, serum levels of SP-D decreased at the terminal stage of IP while levels of KL-6 continued to increase.

Key words Collagen diseases · Interstitial pneumonia · KL-6 · Serum marker · SP-D

Introduction

Interstitial pneumonia (IP) is a severe life-threatening complication of collagen diseases. The activity of IP can be evaluated by lung biopsy, chest roentgenography, chest computed tomography (CT), pulmonary function tests, scintigraphy with ^{67}gallium citrate, bronchoalveolar lavage fluid (BALF), and determination of the serum level of lactate dehydrogenase.[1,2] However, the information provided is not specific to IP, and is often influenced by respiratory infection, other types of inflammatory processes, and liver dysfunction.

KL-6, a mucin-like high-molecular-weight glycoprotein, is expressed on proliferating regenerating type II pneumocytes in patients with IP.[3] Surfactant protein D (SP-D), a hydrophilic glycoprotein, is synthesized and secreted by type II pneumocytes.[4] Recently, these substances have attracted attention as new serum markers for the diagnosis and evaluation of the activity of IP.[5–10] However, there has been no report of comparative evaluations of KL-6 and SP-D in the same patients. In this study, we measured the serum levels of KL-6 and SP-D at the same time in patients with collagen diseases. We compared the usefulness of these two markers in the diagnosis of IP associated with collagen diseases, and in the evaluation of disease activity, the extent of IP lesions, and their clinical courses.

Methods

Study subjects

The study population consisted of 115 patients (85 women, 30 men) with collagen diseases who had visited the Center for Rheumatic Disease at Kagoshima Red Cross Hospital, from January to December 1999. The age range was 21 to 84 years (mean ± SD, 60.4 ± 13.0 years). There were 96 patients with rheumatoid arthritis, seven with systemic sclerosis, seven with dermatomyositis, two with polymyositis, and three with systemic lupus erythematosus. Chest CT scans

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were performed on all patients. There were 38 patients with IP (IP+) and 77 patients without IP (IP−). The diagnosis of IP was confirmed by chest CT and pulmonary function test in all patients. BALF and transbronchial lung biopsy were examined in five and three patients, respectively, to eliminate any other disease. IP was judged to be "active" when PaO_2 decreased 10 torr or more within 1 month, clinical and radiographic findings deteriorated progressively, and the possibility of an infection was unlikely. The disease was judged to be "inactive" when it did not meet these criteria. The clinical courses and changes in serum levels of KL-6 and SP-D after treatment were monitored in patients with active IP.

Measurement of serum levels of KL-6 and SP-D

Sera were stored at −80°C until assayed. Serum KL-6 was measured by enzyme immunoassay using commercially available kits (ED046, Eisai Corp., Tokyo, Japan),[11] and serum SP-D was measured by enzyme-linked immunosorbent assay also using commercially available kits (Yamasa, Yamasa Corp., Tokyo, Japan).[12] Normal values are less than 500 U/ml[13] for KL-6, and less than 110 ng/ml for SP-D.[14]

Chest CT findings

We evaluated chest CT findings in all patients. Some published reports describe a scoring system for IP findings on chest CT.[14–16] However, these methods are very difficult to use in a clinical setting. We classified IP into three groups according to the extent of the lesions on one slice of chest CT at the top level of the right diaphragm. In group 1 (Fig. 1A), lesions were observed only in the back subpleural area of the slice, in group 3 (Fig. 1C), the lesions comprised the whole area, and in group 2 (Fig. 1B), the lesions were in an area intermediate between those in groups 1 and 3.

Statistical analysis

Data are expressed as the mean ± SD, and/or median and range. Intergroup comparisons were performed using Student's t-test for continuous values, and the Mann–Whitney *U*-test or the Kruskal–Wallis test for ordinal values.

Results

The characteristics of the 115 patients with collagen diseases, with or without interstitial pneumonia, are shown in Table 1. There were no significant differences in sex, age, or disease duration between patients with IP and those without IP.

Serum KL-6 levels in patients with IP were 1018 ± 780 (781 (200–3980), median (range)) U/ml, and were significantly higher than those in patients without IP, which were 282 ± 105 (266 (100–687)) U/ml ($P < 0.0001$, Mann–Whitney *U*-test). The sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of KL-6 were 81.6%, 98.7%, 96.7%, 91.6%, and 93.0%, respectively. Serum SP-D levels in patients with IP were 191.0

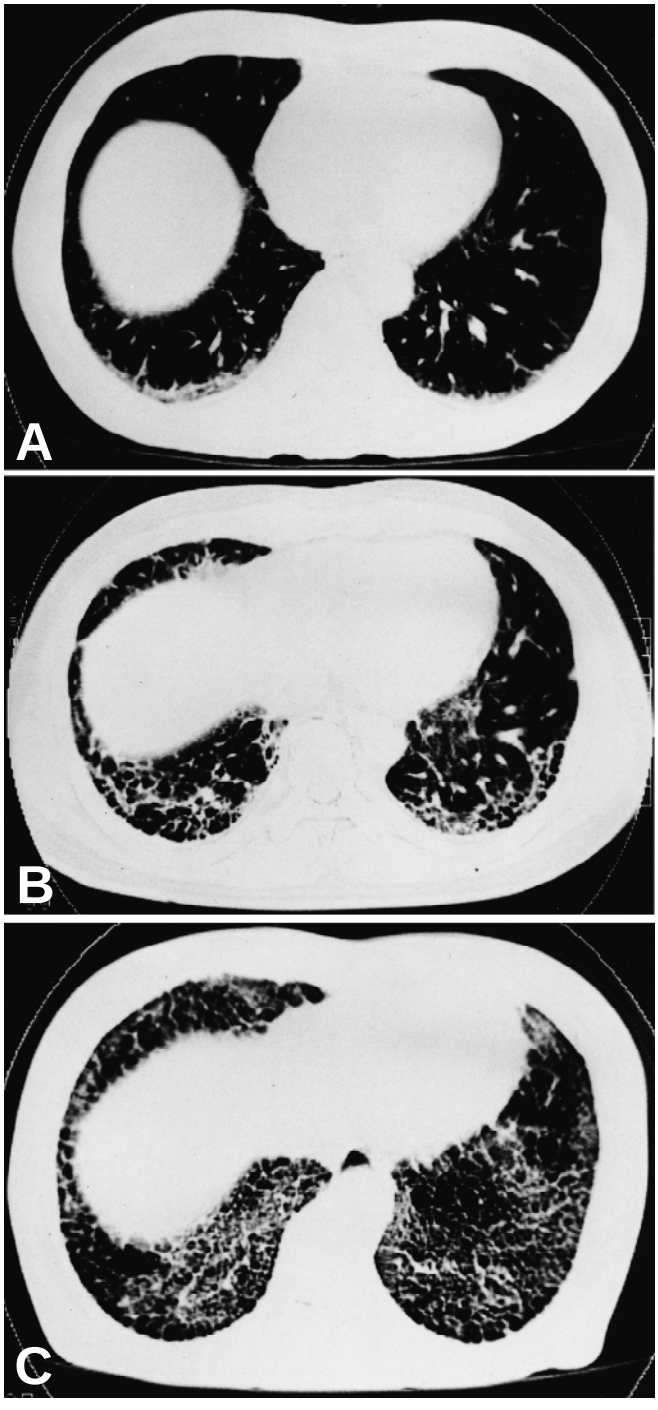

Fig. 1. Computed tomographs of the chest in patients with interstitial pneumonia were classified into three groups according to the extent of the lesions. **A** Group 1, the lesion was observed only in a back subpleural area of the slice. **C** Group 3, the lesion comprised the whole area. **B** Group 2, the lesion was in an area intermediate between groups 1 and 3

± 227.0 (147.5 (29.2–1450)) ng/ml, and were significantly higher than those in patients without IP, which were 47.0 ± 27.8 (38.3 (17.2–169)) ng/ml ($P < 0.0001$, Mann–Whitney *U*-test). The sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of SP-D were 63.2%, 97.4%, 92.3%, 84.3%, and 86.1%, respectively.

Eight patients with IP were found to have active interstitial pneumonia (active IP), 30 patients were found to have inactive interstitial pneumonia (inactive IP), and 77 patients were found to have no interstitial pneumonia (non-IP). Serum levels of KL-6 and SP-D in these patients are shown in Fig. 2. Serum levels of KL-6 in patients with active IP, inactive IP, and non-IP were 2032 ± 1090 (2105 (635–3980)) U/ml, 749 ± 362 (680 (200–1710)) U/ml, and 282 ± 105 (266 (100–687)) U/ml, respectively. There were significant differences among the three groups ($P = 0.0015$ for active vs. inactive IP, $P < 0.0001$ for inactive vs. non-IP, and $P < 0.0001$ for active vs. non-IP, using the Kruskal–Wallis test and the Mann–Whitney *U*-test). Serum levels of SP-D in patients with active IP, inactive IP, and non-IP were 387.3 ± 443.2 (229.5 (115–1450)) ng/ml, 138.7 ± 69.9 (132.5 (29.2–278)) ng/ml, and 47.0 ± 27.8 (38.3 (17.2–169)) ng/ml, respectively. There were significant differences among the three groups ($P = 0.0099$ for active vs. inactive IP, $P < 0.0001$ for inactive vs. non-IP, and $P < 0.0001$ for active vs. non-IP, using the Kruskal–Wallis test and the Mann–Whitney *U*-test).

Table 1. Characteristics of 115 patients with collagen diseases, with or without interstitial pneumonia

	IP+, $n = 38$	IP−, $n = 77$
Sex (female/male)	26/12	59/18
Age (years)	61.4 ± 12.6[a]	59.9 ± 13.2[a]
Disease duration (years)	8.4 ± 1.4[a]	9.4 ± 1.1[a]
Collagen diseases diagnosed		
Rheumatoid arthritis	21	75
Systemic sclerosis (D)	5	0
Systemic sclerosis (L)	1	1
Dermatomyositis	7	0
Polymyositis	2	0
Systemic lupus erythematosus	2	1

[a] Mean ± SD

IP, interstitial pneumonia; systemic sclerosis (D), diffuse cutaneous systemic sclerosis; systemic sclerosis (L), limited cutaneous systemic sclerosis

Nine patients with IP were classified into group 1, 17 patients into group 2, and 12 patients into group 3 according to the extent of the lesions shown on chest CT. Serum levels of KL-6 and SP-D in the three groups are shown in Fig. 3. Serum levels of KL-6 in groups 1, 2, and 3 were 410 ± 158 (456 (200–635)) U/ml, 885 ± 360 (804 (542–1710)) U/ml, and 1665 ± 1027 (1360 (611–3980)) U/ml, respectively. There were significant differences among these three groups ($P = 0.0002$ for group 1 vs. group 2, $P = 0.0090$ for group 2 vs. group 3, $P = 0.0002$ group 1 vs. group 3, using the Kruskal–Wallis test and the Mann–Whitney *U*-test). Serum levels of SP-D in groups 1, 2, and 3 were 114.9 ± 72.9 (102 (29.2–234)) ng/ml, 160.0 ± 74.3 (145 (39.6–278)) ng/ml, and 292.1 ± 379.5 (167.5 (83.4–1450)) ng/ml, respectively. There was no significant difference among these three groups ($P = 0.151$ using the Kruskal–Wallis test).

Serum levels of KL-6 and SP-D in seven of eight patients with active IP were examined three times during the first 4 weeks after treatment (Fig. 4). One fatal case was examined twice because he died on the 10th day, after acute deterioration. The seven cases were divided into three groups (improved cases, unchanged cases, and fatal cases) according to their clinical courses, which were evaluated on the basis of subjective symptoms and laboratory tests including chest radiography, pulmonary function tests, and arterial blood gas analyses after treatment. In all three improved cases (two with rheumatoid arthritis and one with dermatomyosi-

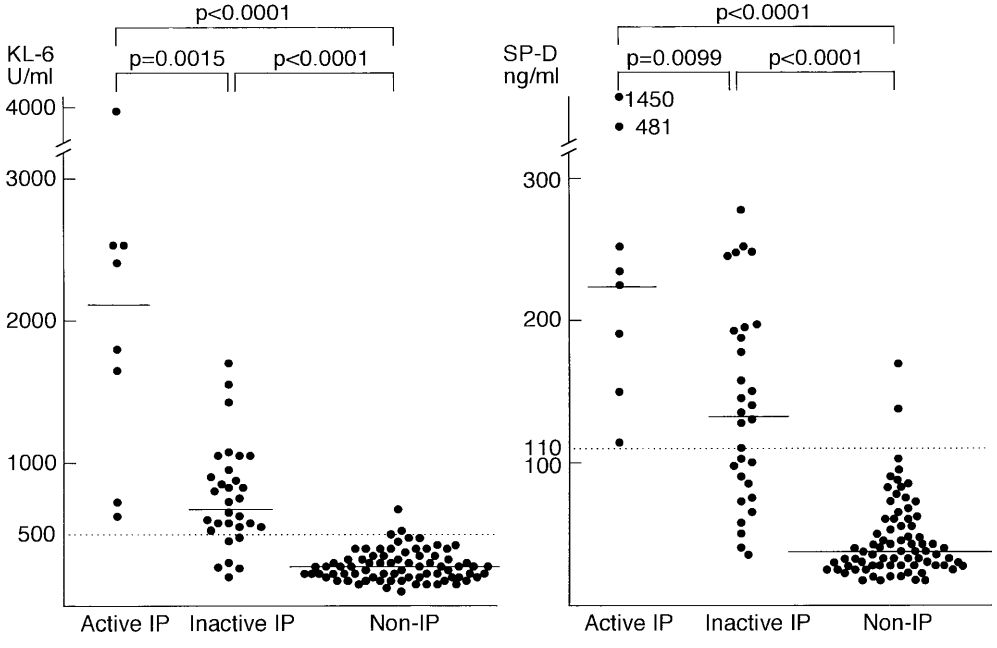

Fig. 2. Serum levels of KL-6 and surfactant protein D (SP-D) in eight patients with active interstitial pneumonia (active IP), 30 patients with inactive interstitial pneumonia (inactive IP), and 77 patients without interstitial pneumonia (non-IP). There were significant differences among these groups in serum levels of both KL-6 and SP-D (Kruskal–Wallis test and Mann–Whitney *U*-test). *Horizontal bars* indicate the median values. *Dotted lines* indicate cut-off values

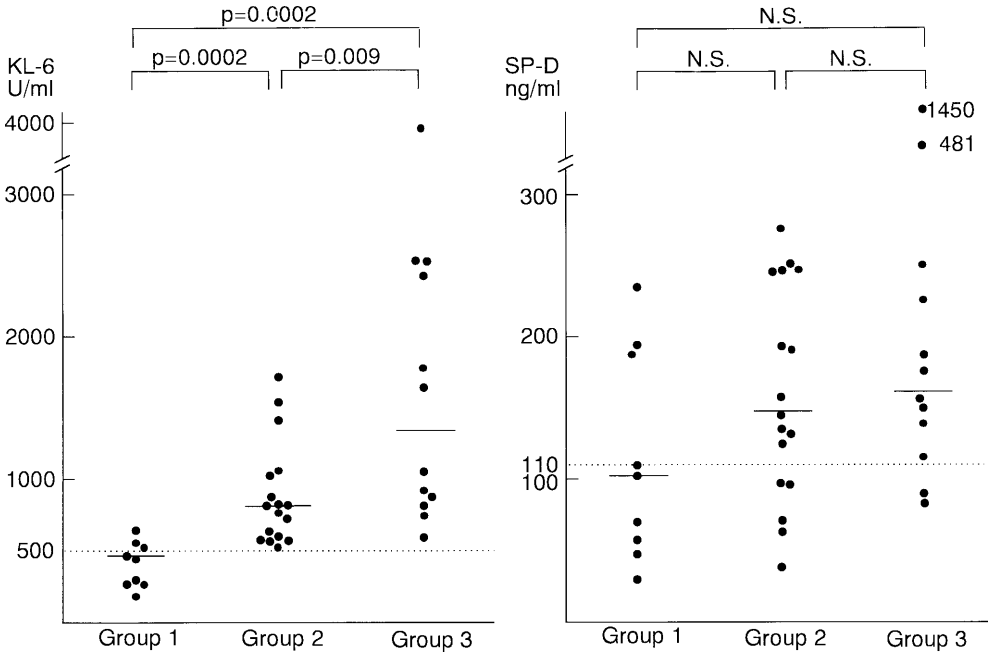

Fig. 3. Serum levels of KL-6 and SP-D in patients with interstitial pneumonia classified into three groups according to the extent of the lesion as shown on computed tomographs of the chest. There were significant differences among these groups in serum levels of KL-6 (Kruskal–Wallis test and Mann–Whitney *U*-test), but there were no significant differences in serum levels of SP-D (Kruskal–Wallis test). *Horizontal bars* indicate the median values. *Dotted lines* indicate cut-off values. N.S., not significant

tis), serum levels of SP-D decreased soon after treatment, while serum levels of KL-6 increased once and then decreased. In the two unchanged cases (one with dermatomyositis and one with systemic lupus erythematosus), serum levels of KL-6 and SP-D changed only slightly. In the two fatal cases (one with rheumatoid arthritis and one with dermatomyositis), serum levels of KL-6 continued to increase until death, whereas serum levels of SP-D decreased just before death. In addition, KL-6 increased markedly to levels above 5000 U/ml.

Discussion

KL-6, a mucin-like high-molecular-weight glycoprotein, is expressed on proliferating, regenerating type II pneumocytes in IP.[3–7] However, SP-D, a hydrophilic glycoprotein, is synthesized and secreted by type II pneumocytes.[4] Both KL-6 and SP-D are reported to be found at high circulating levels, and to reflect the degree of disease activity in patients with IP associated with collagen diseases as well as in those with idiopathic IP.[5–10]

Our data showed that serum levels of both KL-6 and SP-D in patients with IP associated with collagen diseases were significantly higher than those in patients without IP. We calculated the sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of KL-6 and SP-D levels in patients with IP. Both KL-6 and SP-D were found to have high specificity and to have a positive predictive value of >90%. These results suggest that KL-6 and SP-D are useful for the diagnosis of IP associated with collagen diseases.

Our data also showed that serum levels of both KL-6 and SP-D in patients with active IP were significantly higher than those in patients with inactive IP. These results suggest that KL-6 and SP-D would be useful for evaluating the activity of IP associated with collagen diseases.

We classified IP into three groups according to the extent of the lesion on chest CT, and compared serum levels of KL-6 and SP-D among the three groups. There was a significant difference among the three groups in serum levels of KL-6, but not in serum levels of SP-D. Nakajima et al.[9] reported that the greater the extent of the IP lesions according to chest CT findings, the higher the serum KL-6 level. These results may indicate that KL-6 levels would allow us to evaluate the extent of the lesion related to fibrotic changes. It has been reported that KL-6 is strongly expressed on regenerating type II pneumocytes when the basement membrane is injured.[5] Therefore, KL-6 could be a marker of IP, which would reflect the degree of alveolar injury and the remodeling of the alveolar structure.[8] These reports might explain why serum levels of KL-6 were high in patients with advanced honey-combing lung even if they were in the inactive clinical stage of the disease. On the other hand, serum levels of SP-D did not vary significantly among the three groups. SP-D might not be useful in evaluating fibrotic changes in IP.

In seven of eight patients with active IP, the clinical courses and changes in serum levels of KL-6 and SP-D were monitored. Serum levels of SP-D decreased soon after treatment, but serum levels of KL-6 increased once and then decreased in the improved cases. These observations raise the possibility that the half-life period of SP-D might be shorter than that of KL-6, or that the production rate of SP-D might decrease faster than that of KL-6.

In the fatal cases, the serum levels of KL-6 continued to increase and showed markedly high levels (>5000 U/ml) just before death. These findings suggest that the continuous and remarkable increase in KL-6 after acute deteriora-

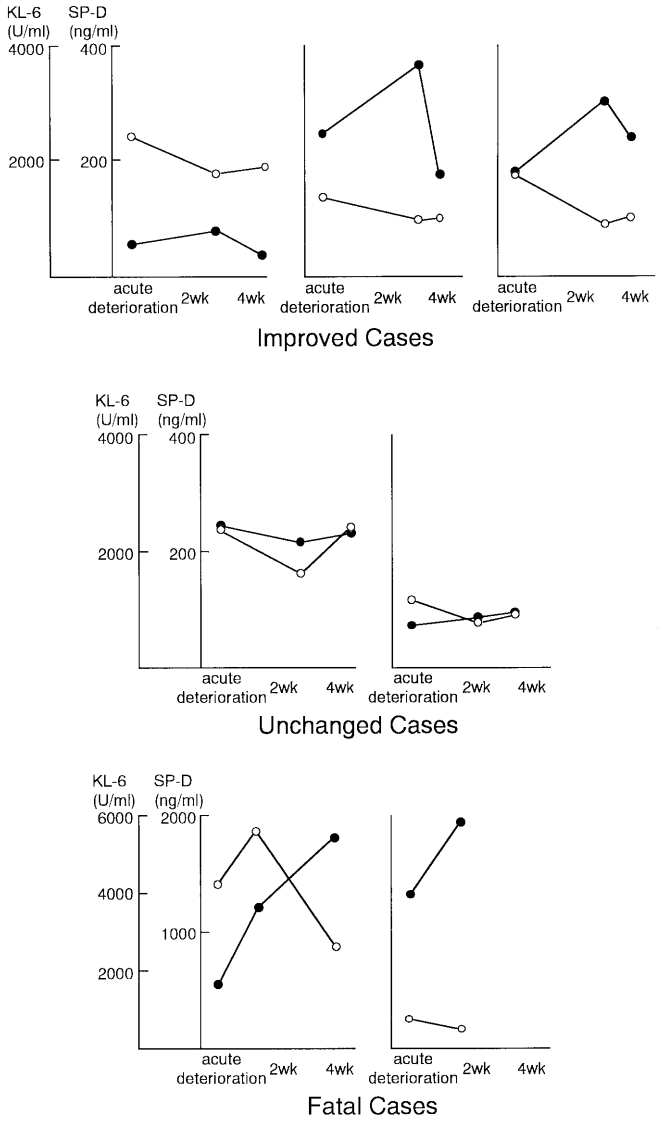

Fig. 4. Changes in KL-6 and SP-D levels in patients with active interstitial pneumonia. The seven cases were classified into three groups (improved cases, unchanged cases, and fatal cases) according to their clinical course. In all three improved cases, serum levels of SP-D decreased soon after treatment, while serum levels of KL-6 increased once and then decreased. In the two unchanged cases, serum levels of both KL-6 and SP-D changed only slightly. In the two fatal cases, serum levels of KL-6 continued to increase until their death, whereas serum levels of SP-D decreased just before their death. In addition, KL-6 increased markedly to levels of over 5000 U/ml. *Closed circles* indicate serum levels of KL-6. *Open circles* indicate serum levels of SP-D

tion may be associated with an early poor outcome. It has been reported that a remarkable increase in KL-6 during acute deterioration led to an early poor outcome, and that a decrease in KL-6 was indicative of a favorable outcome.[9,17] KL-6 was reported to be a chemotactic factor for most fibroblasts in previous studies.[18] As a result, the markedly high level of KL-6 may lead to severe pulmonary fibrosis and a poor outcome. On the other hand, SP-D decreased in the terminal stage of IP in fatal cases despite its high levels after acute deterioration. These findings suggest that serum levels of SP-D might not reflect the clinical activity of the disease in patients with the terminal stage of IP. The mechanism by which serum levels of SP-D increase in IP has not yet been clarified. It has been reported that there is a negative significant correlation between the concentrations of SP-D in serum and those in BALF.[4,10] This suggests that SP-D, which is primarily secreted by type II pneumocytes into the alveolar lumen, can enter the bloodstream easily due to injury at the alveolocapillary membrane.[10] In fatal cases, almost all SP-D stored in the alveolar lumen might enter the bloodstream soon after acute deterioration, or the production rate of SP-D might decrease faster than that of KL-6. As a result, little SP-D might remain in the alveolar lumen, and serum levels of SP-D would decrease in the terminal stages of IP.

In conclusion, both KL-6 and SP-D proved useful for the diagnosis of IP associated with collagen diseases, and also to evaluate disease activity. Furthermore, KL-6 proved useful as an indicator of the extent of IP. Chronological changes in serum KL-6 and SP-D in patients with active IP were different. In fatal cases in particular, SP-D levels decreased in the terminal stages of IP despite KL-6 levels continuing to increase.

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