□ORIGINAL ARTICLE□

Serum Levels of KL-6 Reflect Disease Activity of Interstitial Pneumonia Associated with ANCA-related Vasculitis

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Abstract

Objective **KL-6 is reported to be excreted from the lung alveolar and bronchial epithelial cells and may be a good marker for monitoring disease activity of interstitial pneumonia. This study was designed to ascertain the clinical significance of serum KL-6 levels in interstitial pneumonia associated with anti-neutrophil cytoplasmic antibody (ANCA)-related vasculitis.**

Methods **Serum KL-6 levels were determined by an enzyme-linked immunosorbent assay.**

Patients **We examined 20 healthy subjects, 13 patients with perinuclear (myeloperoxidase, MPO) ANCA-related vasculitis and 12 dermatomyositis (DM)/polymyositis (PM) patients as disease controls in this study. Six out of 13 patients with ANCA-related vasculitis had interstitial pneumonia.**

Results **Serum levels of KL-6 in ANCA-positive patients with interstitial pneumonia were significantly elevated, while they remained as low as those of healthy subjects in ANCA- positive patients without interstitial pneumonia. Similarly, KL-6 levels in sera were higher in 12 dermatomyositis/polymyositis patients with interstitial pneumonia, while they remained low in DM/PM patients without interstitial pneumonia. Moreover, the elevated serum KL-6 level was reduced during the convalescence induced by glucocorticoid therapy and reflected the disease activity of interstitial pneumonia associated with ANCA-related vasculitis.**

Conclusion **These data suggest that the measurement of serum KL-6 levels may be a good monitoring system for the diagnosis and follow-up of interstitial pneumonia of patients with ANCA-related vasculitis.**

(Internal Medicine 40: 1093–1097, 2001)

Key words: marker, interstitial lung disease, vascular disease

Introduction

Interstitial pneumonia is a life threatening disease, and often immunosuppressive therapy including methylprednisolone pulse therapy is necessary (1). Clinical monitoring systems for disease activity, therefore, are needed for patients with interstitial pneumonia. KL-6, MUC1 mucin, is expressed on type II alveolar pneumocytes and bronchial epithelial cells (2). Recent studies have revealed that serum levels of KL-6 reflect the disease activity of interstitial pneumonia and serve as a useful clinical marker (3–7).

Perinuclear anti-neutrophil cytoplasmic antibody (ANCA)-related vasculitis, whose antigen is myeloperoxidase (MPO), often affects the lung as well as the kidney. Recent studies have revealed the molecular mechanisms and pathogenesis in interstitial pneumonia followed by the presence of ANCA. Circulating ANCA attaches to the primed neutrophil surface and destroys the neutrophilic cell membrane, leading to the subsequent release of intracytoplasmic toxic products with resultant matrix and endothelial injury. Then, neutrophils accumulate in an intact interstitial space and undergo oxidative burst, resulting in the destruction of the alveolar wall (8). Based on these types of injury, pulmonary lesions associated with ANCA include pulmonary capillaritis, alveolar hemorrhage and lung fibrosis, all of which show a poor prognosis despite immunosuppressive therapy (9–12). Therefore, the development of a clinical monitoring system for diagnosis and as a means to monitor the disease activity of interstitial pneumonia in patients with ANCA-related vasculitis seems necessary.

Here, we evaluated the serum levels of KL-6 in patients with ANCA-related vasculitis to determine whether or not the KL-6 levels in sera reflect disease activity of interstitial pneumonia associated with ANCA.

Materials and Methods

Patients

We evaluated 13 patients with perinuclear ANCA-related

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Received for publication February 15, 2001; Accepted for publication July 17, 2001

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vasculitis, proven by the presence of ANCA in serum and microscopic polyangiitis in 12 patients by kidney biopsy and in 1 patient by skin biopsy (mean age 70.1 years old, range 51 to 83 years old). Six out of 13 patients with ANCA were affected with interstitial pneumonia (Table 1). Twelve patients (1 male and 11 females) with DM/PM with/without interstitial pneumonia (mean age 66.3 years old, range 48 to 86 years old) were included as disease controls. In addition, 20 healthy volunteers were also included as controls (mean age 72.5 years old, range 66 to 84 years old). The diagnosis of interstitial pneumonia was based on the typical clinical findings of chest radiographs and chest computed tomography (CT), and findings in spirometory such as the diffusion capacity (11). Patients in a clinically active state were treated with glucocorticoid including methylprednisolone pulse therapy 500–1,000 mg/day for 3 days. Measurement of serum KL-6 levels was performed with the consent of all the patients.

KL-6 measurements

The concentration of KL-6 in sera (normal value <500 U/ml) was detected by a sandwich type enzyme-linked immunosorbent assay (ELISA) kit (ED046; Eisai Co., Ltd., Tokyo) (3, 13). In brief, polystyrene cups coated with KL-6 antibody were incubated with 0.1 ml of ten-fold diluted serum at 25°C for 1 hour. Then the cups were washed with 0.85% NaCl and incubated at 25°C for 1 hour with 0.1 ml of 1,000-fold diluted horseradish peroxidase-conjugated KL-6 antibody. Next, the cups were washed again, 0.1 ml of ABTS solution (1.5 mg/ml 2,2'-azino-bis 3-ethylbenz-thiazoline-6-sulfonic acid), 0.02% H_2O_2, and 0.1 M citrate buffer at a pH of 4.2 was added, and incubation was performed at 25°C for 1 hour. Finally, 0.013 M NaN_3 was added to inhibit the peroxidase reaction and the absorbance at 405 nm was measured.

Table 1. Patients' Profile of ANCA Related Vasculitis

Subject	Age (year)	Sex	IP	KL-6 (U/ml)	Therapy	Outcome
1	78	F	+	553	P, M	deceased
2	51	F	+	3,643	P, M	surviving
3	83	M	+	1,773	P, M, C	deceased
4	69	M	+	891	P, M, C	surviving
5	64	M	+	628	P, M, C	surviving
6	74	F	+	1,714	P, M, C	surviving
7	68	F	–	79	P, M	surviving
8	69	M	–	386	P, M	surviving
9	74	M	–	255	P, M	deceased
10	76	M	–	153	P, M	surviving
11	75	M	–	178	P, M	surviving
12	69	F	–	292	P, M	surviving
13	61	M	–	415	P, M, C	surviving

IP: interstitial pneumonia, P: prednisolone, M: methylprednisolone pulse treatment, C: cyclophosphamide.

MPO-ANCA measurements

The presence of perinuclear ANCA in circulation was determined by ELISA using microtiter plates coated with myeloperoxidase extracts (SRL Co., Tokyo), where normal values are less than 10 EIA units.

Statistical analysis

Data are expressed as mean±standard error. Statistical comparison of the data was carried out using the ANOVA test (StatView, Abacus Concepts, Inc., Berkeley, CA, USA). Statistical significance was defined as $p< 0.05$.

Results

KL-6 levels in sera in patients with ANCA-related vasculitis

Among 13 patients with ANCA-related vasculitis, 6 patients suffered from interstitial pneumonia (Table 1). Serum levels of KL-6 in all the patients with ANCA was 843.1±278.9 U/ml (range, 79.0–3,643.0). KL-6 levels in sera in ANCA patients with interstitial pneumonia were significantly higher than those of the patients without interstitial pneumonia and healthy subjects (1,533.7±473.8 U/ml vs. 251.1±46.6 U/ml and 212.2±24.9 U/ml, $p<0.05$, respectively) (Fig. 1). Serum levels of KL-6 in all the patients with DM/PM was 1,421.9±540.9 U/ml (range, 167.0–6,638.0). Similarly, KL-6 levels in sera in 7 DM/PM patients with interstitial pneumonia were significantly elevated compared to those of the patients without interstitial pneumonia (1,984.6±873.8 U/ml vs. 648.6±258.9 U/ml, $p<0.05$, respectively).

Effects of glucocorticoid therapy on serum levels of KL-6

Serum KL-6 levels in ANCA patients with interstitial pneu-

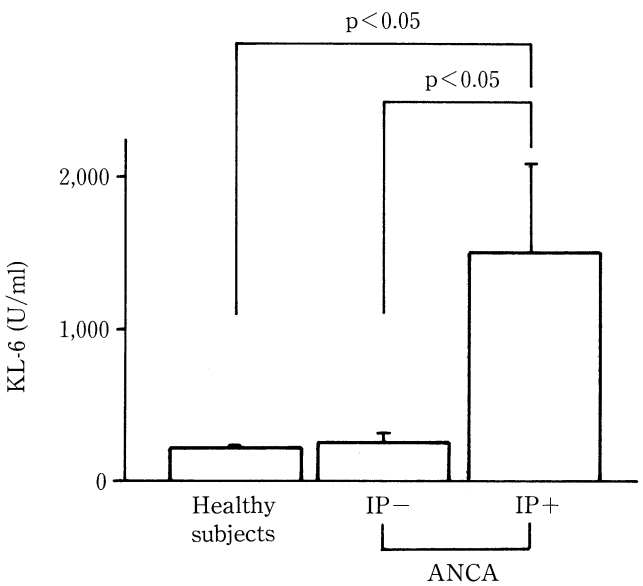


Figure 1. Serum levels of KL-6. ANCA: anti-neutrophil cytoplasmic antibody, IP: interstitial pneumonia.

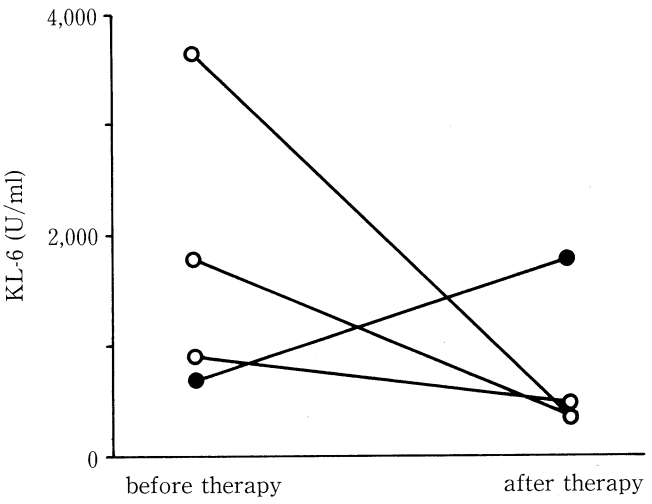


Figure 2. Alteration of serum levels of KL-6 after glucocorticoid therapy. Open circles indicate surviving patients, while closed circles indicate the deceased patient.

monia were examined in four patients after glucocorticoid therapy. Serum levels of KL-6 in 3 out of 4 ANCA patients decreased significantly during the convalescent period. However, one patient who died due to severe interstitial pneumonia showed an increase in KL-6 levels despite therapy (Figs. 2, 3A).

A representative patient with interstitial pneumonia

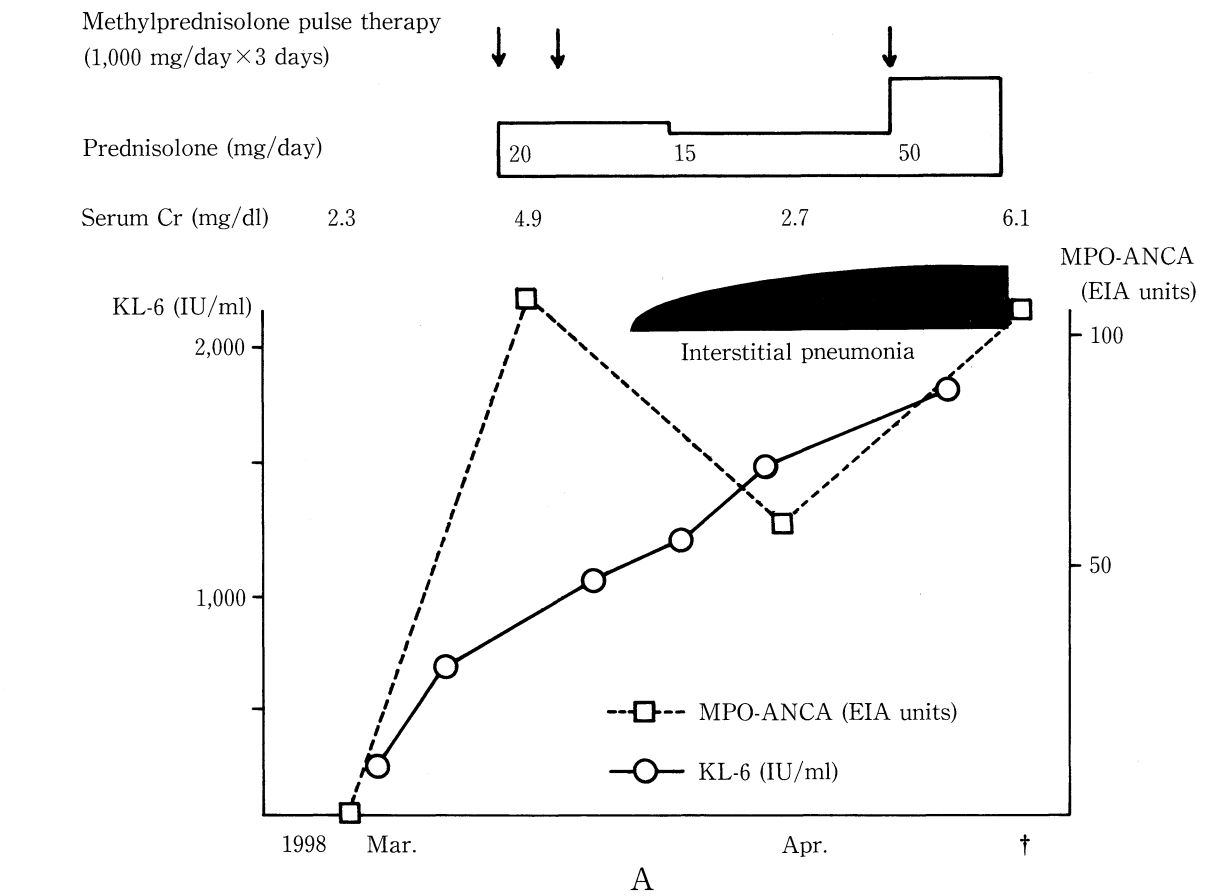
An 83-year-old man was admitted to Kanazawa University Hospital because of renal dysfunction in February 1998 (Fig. 3A). Serum creatinine (Cr) levels were increased from 2.3 mg/dl to 4.9 mg/dl in accordance with the elevation of MPO-ANCA (117 EIA units, normal; <10 EIA units). There was no evidence of interstitial pneumonia and the serum level of KL-6 was 290 IU/ml on admission (Fig. 3B). Glucocorticoid treatment led to the improvement of renal function and decrease of MPO-ANCA titers. However, 1 month after methylprednisolone pulse therapy, Cr was increased from 2.7 mg/dl to 6.1 mg/dl. Chest X-ray and CT showed active interstitial pneumonia and pulmonary hemorrhage in accordance with the increase of KL-6 and MPO-ANCA titers (Fig. 3C). In parallel with deteriorated renal function and lung lesions, KL-6 levels and MPO-ANCA increased and reached a peak at death. These data suggest that the measurement of serum levels of KL-6 reflect disease activity in interstitial pneumonia.

Discussion

KL-6 was reported in 1989 as a serum indicator of interstitial pneumonia (2). KL-6 is expressed on type II pneumocytes and bronchial epithelial cells. KL-6 is detected in sera at the time of injury such as interstitial pneumonia, berylliosis and radiation pneumonitis (3–7). In contrast, in patients with bacterial pneumonia, bronchial asthma and chronic bronchitis, the serum levels of KL-6 remain low. Therefore, the elevation of KL-6 in sera may specifically reflect lung injury. In this study, the serum levels of KL-6 were significantly higher in ANCA patients with interstitial pneumonia. In comparison, the serum levels of KL-6 in ANCA patients without interstitial pneumonia remained similar to levels in healthy subjects. These data suggest that serum levels of KL-6 were not simply reflected by the presence of ANCA. Similarly, KL-6 levels in serum were elevated in 12 DM/PM patients with interstitial pneumonia, as disease controls, while they remained low in DM/PM patients without interstitial pneumonia. Taken together, the elevation of serum KL-6 levels was commonly observed in accordance with the disease activity of interstitial pneumonia in patients with ANCA. Therefore, it may be a good diagnostic marker of interstitial pneumonia of ANCA-related vasculitis.

We measured the serum levels of KL-6 serially in 4 ANCA patients with interstitial pneumonia. KL-6 levels in sera decreased during the convalescence with glucocorticoid therapy in 3 patients. In contrast, 1 patient showed an increase in KL-6 levels despite the therapy and eventually died due to interstitial pneumonia and pulmonary hemorrhage. In that case, the initial KL-6 level was normal, but it became elevated before the onset of clinical symptoms and increased in accordance with the severity of lung involvement. Methylprednisolone pulse therapy and oral prednisolone treatment improved the renal function and titer of ANCA. On the other hand, the levels of KL-6 increased progressively in spite of the immunosuppressive therapy. These data suggest that KL-6 levels may reflect the disease activity of interstitial pneumonia associated with ANCA. ANCA-related vasculitis often affects renal function. However, as shown in Fig. 3, serum KL-6 levels remained low when the renal function was deteriorated, suggesting that serum KL-6 levels may be less affected by renal function. Collectively, the measurement of KL-6 may be a useful marker to assess the effect of therapy on interstitial pneumonia in ANCA patients even in those with renal dysfunction.

It is hypothesized that continuous interstitial endothelial cell damage causes interstitial inflammation, resulting in interstitial pneumonia (14). In PM/DM associated interstitial pneumonia, it is reported that immune complex in alveoli may be associated with the development of interstitial pneumonia and type III allergic reaction may play an important role in the pathogenesis (15, 16). Although the detailed mechanisms of interstitial pneumonia in DM/PM and ANCA-related vasculitis may differ, the destruction of alveolar pneumocytes is followed by an increase of KL-6 in sera. In ANCA-related vasculitis, the interaction of ANCA and neutrophils may result in endothelial damage, leading to increased permeability and inflammation. Supporting this notion, Inoue et al reported that measurements of KL-6 in the peripheral blood may reflect not only epithelial cell injury in the lung but also the degree of leakage from the alveolus to the capillary in berylliosis (7). Therefore, the elevation of serum KL-6 levels in interstitial pneumonia in patients with ANCA-related vasculitis may also reflect both the leakage resulting from endothelial injury and the presence of epithelial injury. In addition, serum KL-6 levels may contrib-



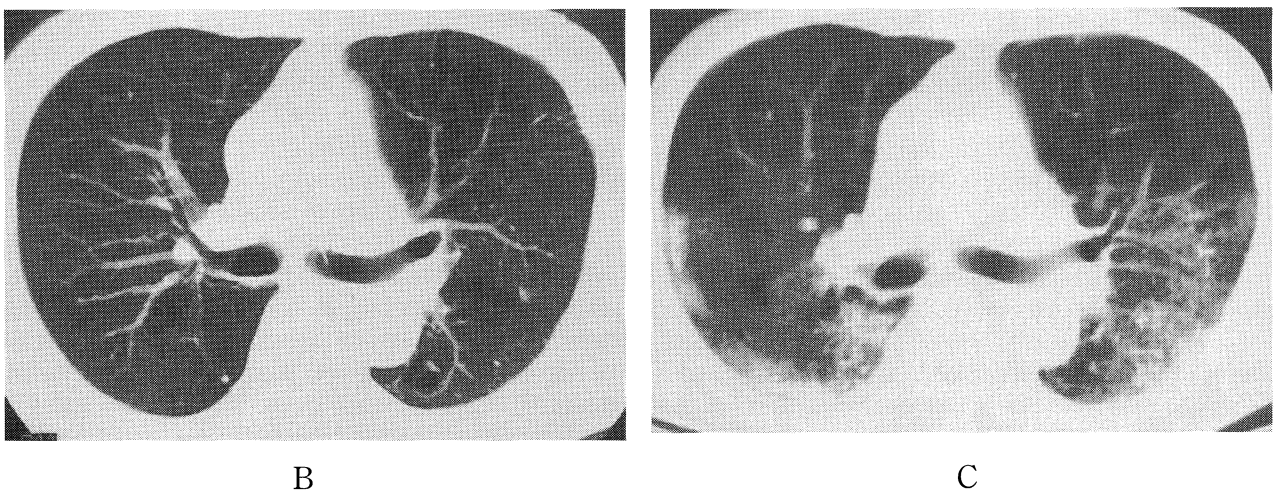


Figure 3. A representative MPO-ANCA patient with interstitial pneumonia. Clinical course (A). Findings of CT scans on admission (B) and at the time of active interstitial pneumonia (C).

ute to the early identification of patients with a high probability of resistance to corticosteroid therapy and may predict the outcome of rapidly progressive idiopathic pulmonary fibrosis (4). Although it remains unclear whether or not KL-6 levels in serum reflect the outcome of interstitial pneumonia associated with ANCA-related vasculitis, it is reasonable to speculate that the measurement of KL-6 levels in sera may be an indicator of disease activity of interstitial pneumonia associated with ANCA-related vasculitis.

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