Serum KL-6 Concentrations in Dairy Farmers*

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Study objectives: Serum KL-6 (Krebs von den Lungen-6) has been recognized to be a marker for the activity of diffuse interstitial lung diseases. The purpose of the study is to evaluate serum KL-6 measurement as a marker for farmer’s lung disease (FLD).

Design: A cross-sectional survey of a cohort of dairy farmers. Retrospective measurement of KL-6 stored serum samples from those dairy farmers previously screened for FLD.

Setting: University hospital screening project for FLD within a dairy-farming community in Japan.

Participants: Four hundred seventy-two dairy farmers were invited to attend a local clinic.

Measurements and results: We examined serum KL-6 concentrations in 272 farmers. Subjects were classified into three groups: (1) 5 farmers with FLD, (2) 30 farmers with positive serum precipitating antibodies to *Saccharopolyspora rectivirgula* and/or *Thermoactinomyces vulgaris* without FLD (Ab⁺), and (3) 237 farmers without these antibodies (Ab⁻). Serum KL-6 concentrations in the FLD group were significantly higher than those in the Ab⁺ and the Ab⁻ groups (1,263 ± 288 [SEM], 328 ± 57, and 207 ± 6 U/mL, respectively, p < 0.001). Serum KL-6 concentrations in those with FLD were significantly higher than KL-6 concentrations from stored screening samples from the same individual when FLD was not diagnosed (1,263 ± 288 and 419 ± 209 U/mL, respectively, p < 0.05). Serum KL-6 concentrations of the Ab⁻ group were significantly higher than those of the Ab⁺ group (p < 0.001). In the Ab⁺ group, farmers with high serum KL-6 concentrations had lower permeability coefficients than farmers with normal serum KL-6 concentrations (p < 0.05). These results may suggest that subclinical FLD can be detected in farmers with high KL-6 concentrations and precipitating antibodies.

Conclusion: Serum KL-6 concentration can be a useful marker for assessing the activity of FLD and may be able to be used to detect subclinical disease. (CHEST 2000; 118:445–450)

Key words: farmer’s lung disease; glycoprotein; hypersensitivity pneumonitis; interstitial pneumonia; mucin

Abbreviations: Ab = antibody; BALF = BAL fluid; DLCO = carbon monoxide diffusing capacity; Dl/Va = permeability index; FLD = farmer’s lung disease; IPF = idiopathic pulmonary fibrosis; ROC = receiver operating characteristic

Farmer’s lung disease (FLD) is a hypersensitivity pneumonitis caused by the inhalation of thermoophilic actinomycetes that grow in moldy hay or straw. In a cohort study, the conventional diagnosis of FLD is usually made on the basis of clinical symptoms, chest roentgenographic abnormalities, and positive precipitins to moldy antigens.¹ However, in dairy-farming communities, some farmers have continuously positive precipitin to moldy antigens without clinical symptoms or lung opacities,² and some of these farmers have lymphocytic alveolitis revealed by BAL.³–⁷ These findings suggest that the combination of clinical symptoms, chest roentgenogram, and serum-precipitating antibody (Ab) may not be sensitive enough to detect FLD. At present, there are few diagnostic procedures available to confirm the diagnosis and to evaluate the disease activity of FLD. Pulmonary function tests, high-resolution CT scan, BAL, transbronchial biopsy, and open lung biopsy are too invasive and costly to use for widespread screening of FLD.

KL-6 (Krebs von den Lungen-6), a mucinlike high-molecular-weight glycoprotein, was discovered as a tumor marker, and its concentration is known to...
be elevated in patients with lung cancer, especially adenocarcinoma. Recently, it has been found to serve as a marker for the activity of interstitial lung diseases. Patients with idiopathic pulmonary fibrosis (IPF) have elevated KL-6 concentrations in serum and BAL fluid (BALF), which reflect disease activity. KL-6 concentrations in sera and BALF are elevated in active summer-type hypersensitivity pneumonitis. It is not known whether serum KL-6 concentrations can be a useful indicator of disease activity in FLD. To evaluate serum KL-6 as a marker for FLD, we examined serum KL-6 concentrations in 272 farmers in a dairy-farming community.

**Materials and Methods**

**Subjects**

We have been performing screening for FLD in dairy farmers from 1978 to 1998 in the northernmost district of Hokkaido, Japan. We invited all 472 farmers in the community to a clinic during the course of 4 days in February 1995 and 1996. A total of 272 farmers attended. They were currently working as dairy farmers and filled out a detailed questionnaire. A physical examination, respiratory function tests, and a chest roentgenograph were performed, and a blood sample was taken.

The diagnosis of FLD was based on the following conventional diagnostic criteria: (1) exposure to moldy hay or straw; (2) characteristic symptoms (fever, cough, dyspnea) at the time of hay handling and after a period of exposure to moldy hay, or various combinations of these symptoms; (3) positive serum precipitins to *Saccharopolyspora rectivirgula* and/or *Thermoactinomyces vulgaris*; and (4) diffuse small nodular or ground-glass opacities on chest roentgenogram. When the subjects fulfilled all the criteria, active FLD was diagnosed. According to the conventional diagnostic criteria, subjects were classified into three groups: farmers with FLD (FLD group), farmers with serum precipitin Abs to *S rectivirgula* and/or *T vulgaris* without FLD (Ab1 group), and farmers without precipitating Abs (Ab- group). Serum KL-6 concentrations were measured in all subjects. Furthermore, in the FLD group, serum KL-6 was measured in stored serum from a previous screening consultation when subjects had no symptoms, no fine crackles on auscultation, and no abnormalities on the chest roentgenogram (inactive period). Patients with other diseases in which serum KL-6 concentrations are known to be elevated, such as IPF and malignant tumors, were excluded from the study.

**Questionnaire**

Information about systemic and respiratory symptoms, including fever, malaise, chills, dyspnea, and a dry cough, were obtained using a questionnaire based on the standard questionnaire of the American Thoracic Society epidemiology standardization project (ATS-DLD 78). The timing of such symptoms after handling moldy hay was also asked about. Smoking habits were recorded. In addition, information on farming conditions, including years on the farm, pasture area, number of dairy cows, working hours per day inside barns, and hay-handling time per day was also obtained.

**Clinical Evaluation**

For the clinical background, age, sex, dairy-farming history, clinical symptoms (such as fever, cough, dyspnea), and pulmonary crackles were analyzed. For respiratory function tests, FVC, FEV1, carbon monoxide diffusion capacity (DLCO), and DLco adjusted for alveolar volume (permeability index [Dlco/VA]) were analyzed. Spirometric measurements were performed using an autopsirometer (AS300; Minato; Osaka, Japan) for all farmers. Measurements of DLCO were performed by a single-breath technique, using transfer factor equipment (CHESTAC-55V; Chest; Tokyo, Japan) for the farmers of both the FLD group and the Ab- group. Standard values of vital capacity were estimated by the equations of Baldwin and associates. Three pulmonary specialists who were blinded to the clinical and functional findings examined the chest roentgenograms independently. Small nodules with a lower-lung predominance or ground-glass opacities in the peripheral lung zones were judged as positive roentgenographic abnormalities only when all three pulmonary physicians concurred.

**Measurement of Serum Precipitating Ab**

Stored serum samples frozen at −80°C were tested for serum precipitins to *S rectivirgula* and *T vulgaris* by the double-diffusion gel method described by Ouchterlony. The antigens were obtained from Hollister-Stier Laboratory (Spokane, WA). They were examined by counter-immunoelectrophoresis with a 1.5-mm-thick film of 1% agar gel in veronal phosphate buffer (pH 8.6; viscosity index = 0.05) on a 10 × 13-cm glass plate, using a constant 35-mA current for 90 min. The diameter of the wells for each patient's serum and the *S rectivirgula* and *T vulgaris* antigen solutions was 4 mm. These wells were filled with 10 µL of tested sera or antigen solutions. The plates were immersed in a 5% sodium citrate solution for 30 min and then in a 0.3 M NaCl solution overnight after electrophoresis to remove arcs resulting from nonspecific reactions.

**Measurement of Serum KL-6 Concentrations**

The serum KL-6 concentration was measured by a sandwich-type enzyme-linked immunosorbent assay using a KL-6 Ab kit (ED046; Eisai; Tokyo, Japan). Polystyrene cups coated with KL-6 Abs were incubated at room temperature for 2 h with 100 µL of serum diluted 201-fold by dilution solution (1% bovine serum albumin, 0.1% NaN3, and 0.05 M Tris-HCl buffer, pH 7.5). They were then washed with 0.85% NaCl and incubated at room temperature for 1 h with 100 µL of 1,000-fold diluted horseradish peroxidase-conjugated KL-6 Ab. The cups were washed again, 100 µL of ABTS solution (1.5 mg/mL 2,2'-azino-bis 3-ethyl-benz-thiazoline-6-sulfonic acid), 0.02% H2O2, and 0.15 M citrate-phosphate buffer, pH 4.2, was added, followed by incubation at room temperature for 30 min. Finally, 100 µL of 1 N sulfuric acid was added to inhibit the peroxidase reaction, and the absorbance at 405 nm was measured.

**Statistical Analysis**

The comparisons of continuous variables among the three groups were performed by the Kruskal-Wallis test. The comparisons of discontinuous variables among three groups were performed by χ2 analysis with Yates correction as needed. The comparison of serum KL-6 during active and inactive periods was performed by Student’s paired t test. The sensitivity and specificity were calculated for arbitrary KL-6 values to analyze the optimal criteria for discrimination between farmers with high
KL-6 concentrations and those with normal KL-6 concentrations. Furthermore, the receiver operating characteristic (ROC) curve analysis was performed using calculated values for sensitivity and specificity. The comparison of clinical characteristics in the \( \text{Ab}^+ \) group was performed using Mann-Whitney U test. Differences with a p value < 0.05 were considered significant.

**Results**

**Subjects Characteristics**

The mean (± SEM) age of the 272 farmers was 50.2 ± 0.8 years, and the sex distribution was 142 men and 130 women. A total of 78 farmers were smokers, 36 were ex-smokers, and 158 were non-smokers. The smokers’ average life-long cigarette consumption was 29.8 ± 2.6 pack-years. They had been engaged in farming for 27.4 ± 0.8 years, had handled hay for 46.4 ± 2.8 min/d, and had spent 6.1 ± 0.1 h/d in the barn. They raised 103.4 ± 5.5 cows, and their pasture area was 66.9 ± 7.7 hectares (1 hectare = 0.1 km by 0.1 km). A total of 157 farmers (57.7%) complained of dry cough, sputum, or shortness of breath; 8 farmers (2.9%) had small nodular opacities on chest roentgenogram; and fine crackles were audible in 6 farmers (2.2%). There were 35 farmers (12.9%) who had precipitating Abs to \( S \) rectivirgula and/or \( T \) vulgaris.

According to the conventional diagnostic criteria, the subjects were classified into three groups. The FLD group was composed of five farmers who fulfilled all the diagnostic criteria. The \( \text{Ab}^+ \) group was composed of 30 farmers who were positive for anti-\( S \) rectivirgula and/or anti-\( T \) vulgaris Abs but did not fulfill the diagnostic criteria. The \( \text{Ab}^- \) group was composed of 237 farmers who were negative for these Abs.

**Clinical Background**

No significant differences were observed among these three groups in age, sex, or agricultural factors, including years on the farm, hours in the barn, pasture area, persons engaged in work, number of cows, and hay-handling time. The proportion of smokers was significantly higher in the \( \text{Ab}^- \) group than in the FLD and the \( \text{Ab}^+ \) groups (31.2, 20.0, and 10.0%, respectively; p < 0.05). The incidence of symptoms in the FLD group was significantly higher than in the \( \text{Ab}^- \) group (p < 0.05). The incidence of lung opacities in the FLD group was significantly higher than in the \( \text{Ab}^+ \) and \( \text{Ab}^- \) groups (p < 0.001). The incidence of fine crackles in the \( \text{Ab}^+ \) group was significantly higher than in the \( \text{Ab}^- \) group (p < 0.01). In respiratory function tests, there were no significant differences in percent FVC and FEV\(_1\)/FVC among the three groups. The FLD group had significantly reduced both DL\(_{\text{CO}}\) and DL\(_{\text{VA}}\) than the \( \text{Ab}^+ \) group (14.2 ± 0.5 mL/min/mmHg vs 20.0 ± 1.3 mL/min/mmHg, p < 0.01; 3.98 ± 0.38 mL/min/mmHg/L vs 5.02 ± 0.21 mL/min/mmHg/L, p < 0.01, respectively; Fig 1). Of the 30 farmers in the \( \text{Ab}^+ \) group, 13 had symptoms such as cough and dyspnea, and another 1 farmer had lung opacities.

**Serum KL-6 Concentrations**

The serum concentrations of KL-6 in the three groups are shown in Figure 2. The mean (± SEM) serum KL-6 concentrations were 1,263 ± 288, 328 ± 57, and 207 ± 6 U/mL for the FLD, \( \text{Ab}^+ \), and \( \text{Ab}^- \) groups, respectively. The FLD group had a significantly higher serum KL-6 concentration than the \( \text{Ab}^+ \) and \( \text{Ab}^- \) groups (p < 0.001). Furthermore, the \( \text{Ab}^+ \) group had a significantly higher serum KL-6 concentration than did the \( \text{Ab}^- \) group (p < 0.001).

The serum KL-6 concentrations in the five patients of the FLD group were measured in the active and inactive periods of the disease (Fig 3). All of their serum KL-6 concentrations were elevated when FLD was diagnosed, and the concentration was significantly higher during the active period than the inactive period (1,263 ± 288 U/mL and 419 ± 200 U/mL, respectively; p < 0.05). During
the inactive period, four of the five farmers with FLD had serum KL-6 concentrations < 500 U/mL.

Further Analysis of the Ab\(^+\) Farmers Without FLD

Because the Ab\(^+\) farmers without FLD had significantly higher serum KL-6 concentrations than the Ab\(^-\) farmers, the Ab\(^+\) group was divided into two categories, farmers with high-KL-6 concentrations and those with normal-KL-6 concentrations. Normal range of serum KL-6 concentrations was determined on the basis of the results obtained from the Ab\(^-\) group, who were considered to be normal farmers without lung disease. The mean (± SD) concentrations of serum KL-6 in the Ab\(^-\) group were 223 ± 100 U/mL in 127 male farmers and 188 ± 83 U/mL in 110 female farmers. Serum KL-6 concentrations were affected significantly by sex (p < 0.05) but not by smoking habits. The sensitivity and specificity values were used for ROC analysis. The ROC curves for male and female farmers were drawn. The optimal criterion was defined as the closest point to that indicating 100% sensitivity and 100% specificity. According to the ROC curve, the KL-6 value corresponding nearest to the optimal criterion was from 440 to 450 U/mL in male farmers and from 410 to 420 U/mL in female farmers.

Eight farmers in the Ab\(^+\) group had serum KL-6 concentrations above the normal range (high KL-6 farmers), and the rest of 22 farmers had normal KL-6 concentrations (normal KL-6 farmers). No significant difference was recognized in the agricultural factors, the incidence of clinical symptoms, lung opacities, and fine crackles. In respiratory function tests, DLCO and DLVA were significantly lower in high KL-6 farmers than in normal KL-6 farmers (16.2 ± 2.1 vs 21.9 ± 1.3, p < 0.01; 4.66 ± 0.33 vs 5.22 ± 0.25, p < 0.05, respectively; Fig 4).

DISCUSSION

Major findings in this study were that the FLD group, with conditions diagnosed according to the conventional diagnostic criteria, had significantly higher serum KL-6 concentrations than the Ab\(^+\) and Ab\(^-\) groups. Serum KL-6 concentrations were significantly higher in those individuals with active disease than when previously screened for FLD. The Ab\(^+\) group without FLD had a significantly higher serum KL-6 concentration than the Ab\(^-\) group. Finally, the farmers with Ab\(^+\) but without FLD had a significantly lower DLCO if the KL-6 was above the concentration found in normal farmers.

Recently, it has been recognized that serum KL-6 concentrations could be good markers for disease activity in interstitial lung diseases, such as IPF, interstitial pneumonia associated with collagen vaso-

Figure 2. Serum KL-6 concentrations of the three groups of farmers. The FLD group had a significantly higher serum KL-6 concentration than the Ab\(^+\) and Ab\(^-\) groups, and the Ab\(^+\) group had a significantly higher serum KL-6 concentration than the Ab\(^-\) group. Vertical line with small horizontal line indicates mean ± SEM. *p < 0.001.

Figure 3. Serum KL-6 concentrations during active and inactive periods of the FLD group. In patients with FLD, serum KL-6 concentrations in the active period are significantly higher than those in the inactive period. Four of five patients with FLD had serum KL-6 concentrations < 500 U/mL during the inactive period. *p < 0.05.
cular disease, and radiation pneumonitis. In IPF, serum KL-6 concentrations correlate well with uptake of $^{67}$Ga-citrate in the lung field and the clinical activity of the disease. KL-6 concentrations in BALF are also elevated in patients with IPF and correlate well with the number of lymphocytes and neutrophils in BALF. Immunohistochemical studies demonstrated that KL-6 is expressed on type II pneumocytes and respiratory bronchiolar epithelial cells in normal lungs, and that in IPF lungs, it is strongly expressed on regenerating type II pneumocytes and alveolar macrophages. From these findings, increased concentrations of serum KL-6 in patients with pneumonitis are considered to reflect the production levels of KL-6 derived from damaged or regenerating type II pneumocytes in the lower respiratory tract.

However, whether KL-6 concentrations are elevated in patients with FLD and correlate with disease activity was previously unknown. In this study, we observed that farmers with FLD had significantly higher serum KL-6 concentrations than the Ab$^+$ group. Furthermore, in the Ab$^+$ group, 8 of the 30 subjects had higher serum KL-6 concentrations than normal upper limits. In addition, their DL$\text{CO}$ and DL$\text{VA}$ values were 53% and 24% lower, respectively, than those values in farmers with normal KL-6 concentrations. These findings, together with the observation of KL-6 concentrations in FLD patients, may suggest the possible existence of subclinical FLD in the farmers with high KL-6 concentrations and precipitating Ab.

Because FLD is a major occupational hazard in dairy farmers, health screening of dairy farmers seems to be important. A significant question in screening is how to select the subjects who might have FLD and need further invasive examinations such as BAL, high-resolution CT, and lung biopsy. Previous studies revealed that the conventional diagnostic criteria of FLD may not be satisfactory for this purpose. The results of the present study suggest that serum KL-6 concentration can be a noninvasive and low-cost marker for disease activity, and that the inclusion of KL-6 measurements in a...
cross-sectional survey of FLD may improve the ability to detect early FLD. To confirm the sensitivity and specificity of serum KL-6 concentration in the diagnosis of FLD, further prospective study may be required.

REFERENCES