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## **Usefulness to quantify serum KL-6 levels to follow up uveitic patients with sarcoidosis**

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## **Abstract**

Background: KL-6 is a human glycoprotein secreted by type II alveolar cells in lung, and its serum levels increase in pneumonia of various causes. We previously reported that serum KL-6 levels in the uveitis patients with sarcoidosis were significantly higher than that of other uveitis and healthy controls. And combined measurement of serum KL-6 and angiotensin converting enzyme (ACE) was useful to screen uveitic patients to diagnose sarcoidosis. The purpose of the present study is to investigate the clinical usefulness of quantifying serum KL-6 levels for following up the patients with sarcoidosis.

Patients & Methods: Sera were obtained from 36 uveitic patients diagnosed as sarcoidosis and same number of healthy volunteers. To examine the influence of systemic medication, we collected blood samples from four more sarcoidosis patients additionally, who were systemically treated with corticosteroid or angiotensin converting enzyme (ACE) inhibitor, an anti-hypertensive drug. Serum concentration of KL-6 was measured by a human KL-6 electrochemiluminescence immunoassay (ECLIA).

Results: The mean KL-6 concentrations of sarcoidosis patients and healthy controls were  $449.3 \pm 66.3$  (mean  $\pm$  SE) and  $192.1 \pm 11.3$ , respectively. The average levels of serum KL-6 were significantly elevated in sarcoidosis than healthy control subjects ( $p < 0.001$ ), and there were significant correlations between serum KL-6 and ACE levels in the patients with sarcoidosis ( $r = 0.70$  and  $p < 0.0001$ ). Moreover, serum KL-6 concentrations were less affected by systemic corticosteroid, and unaffected by ACE inhibitory drugs in contrast to ACE levels.

Conclusions: Measurement of serum KL-6 in the uveitic patients may be useful to follow up the diagnosed sarcoidosis as well as diagnosing sarcoidosis, because the serum KL-6 level was correlated to ACE well, and less affected by systemic medication than ACE levels.

## Introduction

KL-6 (Krebs von den Lungen-6) is a high molecular weight mucinous glycoprotein discovered as pulmonary adenocarcinoma-related antigen, and KL-6 monoclonal antibody reacts to sugar moiety of MUC-1 [22]. MUC-1 mucin exists in mucosal epithelium of the respiratory and digestive systems, as well as epithelial cells of cornea and conjunctiva [12]. It was reported that the biochemical properties of KL-6 are similar to those of other MUC-1 mucins [10]. In normal lung tissue, KL-6 appears on type II pneumonocytes, respiratory bronchiolar epithelial cells, and bronchial gland cells [22]. Today, it is classified as Cluster 9 antigen (MUC-1) under the pulmonary cell antigen cluster classification, proposed at the 3rd International Workshop on Lung Tumor and Differentiation Antigens [30].

Measurement of serum KL-6 levels is now widely accepted in Japan as a diagnostic examination to monitor the activity of lung diseases, such as idiopathic interstitial pneumonia [21], radiation pneumonia [5], pneumonia following bone marrow transplantation [1], *Pneumocystis carinii* pneumonia in the immunocompromised host [6], pediatric respiratory diseases [11, 26], breast cancer [29], Amiodarone-induced pulmonary toxicity [3], *Mycoplasma pneumoniae* [27], interstitial pneumonia associated with collagen diseases [25], and pulmonary sarcoidosis [21]. Also, we recently demonstrated that serum KL-6 levels are

elevated in uveitic patients with sarcoidosis [19]. Although there are only a few reports describing the influences of ocular disorders on serum KL-6 level, it was lately reported that impression cytology indicated higher KL-6 expression in ocular surface epithelium of the patients with dry eye than healthy subjects [7].

Uveitis in patients with systemic sarcoidosis is common in any race, gender, or age, and sarcoidosis is also an immunological and systemic disease involving almost all organs in the body [34]. It is one of the most frequently diagnosed diseases in patients with endogenous uveitis worldwide [8, 15, 23, 33]. Now it is known that angiotensin-converting enzyme (ACE) is a helpful serum marker to diagnose sarcoidosis. ACE is thought to be one of the most useful markers to diagnose sarcoidosis; however, while specific, it is not very sensitive. Another problem of ACE is that ACE inhibitory agents may be administrated into some patients with blood hypertension because it is one of the general antihypertensive drugs. When hypertension is treated with ACE inhibitors, their serum ACE levels should markedly decrease. In addition, ACE levels fluctuate with corticosteroid use [31]. Thus, clinically it is important to find another helpful laboratory parameter to diagnose sarcoidosis and/or to monitor the severity of the disease.

Sarcoidosis is a Th1-mediated disease, and recent reports identified some immunological

markers possibly indicating sarcoidosis; up-regulated intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecules-1 (VCAM-1) on macrophages, and/or the presence of inducible NO synthase (iNOS) in bronchoalveolar lavage fluid (BALF) [32]. We previously detected the high levels of serum macrophage migration inhibitory factor (MIF) in the uveitis patients with sarcoidosis [18, 24].

In the present study, we quantified serum KL-6 levels in the patients with sarcoidosis, and evaluated the usefulness in monitoring the disease severity.

## Materials & Methods

### Patients

After obtaining informed consent in accordance with the tenets of the Declaration of Helsinki, sera were obtained from 40 uveitis patients with sarcoidosis at the Uveitis Survey Clinic of the Hokkaido University Hospital, Sapporo, Japan. The mean age was 49.3 years old (25 to 68 years old), and 31 of 40 cases were women. Three of them were treated with systemic corticosteroid, and anti-hypertensive drug was given to another subject systemically. The criteria established by the Diffuse Pulmonary Disease Research Committee of Japan

were used to diagnose patients with sarcoidosis [9].

#### Measurement of KL-6

Human KL-6 levels were determined by PICOLUMI KL-6 kit from Eisai (Tokyo, Japan), an electrochemiluminescence immunoassay (ECLIA) specific for human KL-6, containing all necessary reagents for assay. Beads bound with anti-KL-6 mouse monoclonal antibodies (Eisai) were prepared as a solid phase and placed into a 96-well plate.

Venous blood samples were collected in sterile vacuum tubes and centrifuged for 30 minutes at the Hokkaido University Hospital as described previously [16, 17, 18]. The serum samples were diluted 1/50 and 20  $\mu$ l was mixed with 25  $\mu$ l of anti-KL-6 coated beads; the mixture was incubated for 9 minutes at 30°C. The beads were washed, mixed, and incubated for 9 minutes with ruthenium (Ru)-labeled anti-KL-6 monoclonal antibody, which should illuminate in response to electrochemical stimuli. An electric impulse was applied via an electrode using a PICOLUMI8220 automated immunoassay reader (Sanko Junyaku, Tokyo, Japan) followed by washing the beads. The level of luminescence for the Ru complexes bound to solid-phase antibodies reflects the amount of KL-6 in the sample. The concentration of KL-6 in the serum sample was calculated by comparing the sample's luminescence to that

of a calibrated solution of known KL-6 standards.

## Results

### Serum KL-6 and ACE levels in the patients with sarcoidosis

The mean KL-6 concentrations in sera of 36 sarcoidosis and sex/age-matched healthy volunteers were  $449.3 \pm 66.3$  (standard error) and  $192.1 \pm 11.3$  (U/ml), respectively.

The average level of KL-6 in sera of sarcoidosis patients was significantly higher ( $p < 0.001$ ) than that of healthy control subjects (Fig. 1).

To determine whether serum KL-6 levels are associated with serum ACE levels, we plotted the two values in the patients with sarcoidosis. We previously reported that though both serum ACE and KL-6 levels were significantly higher than that of controls, these two values were not correlated among whole uveitic patients including sarcoidosis, Behçet's disease, Vogt-Koyanagi-Harada's disease, and HLA-B27 associated uveitis, unclassified uveitis, and healthy subjects [19]. In the present study, we picked up and plotted KL-6 and ACE values restricted in the patients with sarcoidosis (Fig. 2). Four of forty sarcoidosis patients were excluded because they were given corticosteroid and anti-hypertensive agents. Significant correlation was detected between the serum levels of KL-6 and those of ACE in sarcoidosis

(correlation coefficient was 0.70,  $t=5.715$ ,  $df=34$ , and  $p<0.0001$ ).

We then examined three patients with sarcoidosis before and after starting their systemic corticosteroid administrations. Their serum ACE and KL-6 levels were quantified before and 6 to 15 months after the initiation of corticosteroid administration (10 or 20 mg of prednisolone) (Fig. 3). Since serum ACE level is considered susceptible to corticosteroid administration, it is reasonable that their serum ACE levels decrease after treatment. In contrast, KL-6 levels were not very influenced by systemic corticosteroid treatment in two of three tested patients (Fig. 3).

Next, we found a case with sarcoidosis complicated with blood hypertension, and serially reviewed her serum KL-6 and ACE levels (Fig. 4). The patient was a 68-year-old woman, and she had already been treated with ACE inhibitory drug (5 mg/ day of imidaprilhydrochloride) when she visited our clinic at the first time. Her serum ACE levels were quite low (1.9 IU/l) at the first examination. Thereafter, her serum ACE levels once markedly increased (14.0 IU/l) due to change from the ACE inhibitor to valsartan (40 mg/ day), a specific angiotensin subtype 1 (AT1) receptor blocker. After a year, her ACE level decreased again (1.3 IU/l) because her primary care physician began a combined medication consisting of the ACE inhibitor and the AT1 blocker. However, her serum KL-6 level was not

influenced by imidaprilhydrochloride or valsartan (Fig. 4).

## Discussion

KL-6 is a member of the MUC-1 family of mucin and is recognized by a mouse monoclonal antibody against a sialylated carbohydrate chain [22]. The anti-KL-6 monoclonal antibody reacts with an antigen strongly expressed on type II alveolar pneumonocytes and bronchiolar epithelial cells, but not on granulomatous tissue [22]. Today, to quantify serum KL-6 level is considered useful to diagnose and examine the severity of interstitial pneumonia in Japan. However, its usefulness for ocular disorders has remained unclear. Though it is accepted that MUC-1 is expressed in normal human conjunctiva and cornea [2, 7, 12], the expression of MUC-1/KL-6 in other ocular tissues had been unknown. Recently, others and we reported that determination of the serum KL-6 levels is quite useful for both pulmonologists and ophthalmologists to care for patients with sarcoidosis [21, 23]. Since some sarcoidosis patients suffer from damage to alveolar cells in the lung, it is reasonable to suggest that the elevated serum levels of KL-6 in sarcoidosis are due to its production or release by alveolar pneumonocytes and bronchiolar epithelial cells. However, it had remained unclear whether pulmonary tissues are the only sources of elevated serum KL-6 in uveitic

patients with sarcoidosis. In the present study, we did not detect the KL-6 expression on uvea (iris, ciliary body, and choroid) but cornea/ conjunctiva (data not shown), and the volume of ocular tissue is much smaller than lung. These results suggest that the systemic elevation of serum KL-6 levels mainly depends upon the destruction of pulmonary tissues. Other possible sources of KL-6 in the inflamed eyes may be corneal and conjunctival cells. Though their contribution rates to raise systemic level of KL-6 might be small, sarcoid granulomas are seen in 7% to 17% of sarcoidosis patients with ocular involvement [13, 14, 28], suggesting that sarcoidosis frequently invades into ocular surface. Thus, it is possible that inflamed eye with sarcoidosis is one of the possible candidate organs to raise KL-6 level.

Though clinicians already use serum ACE level as a laboratory marker for sarcoidosis, ACE measurement identifies only 50 - 67 % of uveitis patients with sarcoidosis at our clinic [4, 19].

As it is thought that the granulomatous tissue can release ACE, serum ACE levels may be elevated in other granulomatous conditions such as tuberculosis, lymphoma, and asbestosis.

Furthermore, some patients, who are suffering from hypertension and/or heart failure, may be treated with ACE inhibitors, a kind of anti-hypertensive drugs. Since we have demonstrated in the present study that serum KL-6 levels are unaffected by ACE-inhibitory drugs different from serum ACE levels, and less affected by corticosteroid than by ACE, it may be

worthwhile to add measurement of serum KL-6 levels to the procedures to manage sarcoidosis. It is also possible that ACE measurement may be substituted by serum KL-6 levels for sarcoidosis patients when serum ACE values are unreliable in some other reasons.

Today, histological examinations of lung, conjunctiva, or lacrimal gland biopsy specimens are also accepted for diagnosing sarcoidosis. However, in the respect that biopsy sometimes causes much discomfort and pain to the patients, to add another laboratory parameter, KL-6, may provide a less invasive and a less expense laboratory test to screen and monitor sarcoidosis. Despite the fact that it remains unclear how much KL-6 should be released from ocular tissue in the patients with sarcoidosis, we would like to emphasize that KL-6 is considered a new and useful laboratory parameter to diagnose and manage uveitis of sarcoidosis, even when the patients are treated with systemic corticosteroid and/or anti-hypertensive drugs.

## Figure legends

### Figure 1

Mean concentrations of KL-6 in sera of uveitic patients with sarcoidosis and healthy controls.

The average level of KL-6 in sera of patients with sarcoidosis ( $449.3 \pm 66.3$  U/ml) is significantly higher than that of healthy control subjects ( $192.1 \pm 11.3$ ) ( $p < 0.001$ ).

### Figure 2

Plots of serum KL-6 and ACE levels in uveitis patients with sarcoidosis. Each circle shows individual subjects. Significant correlation was found between the serum levels of KL-6 and those of ACE in 36 patients with sarcoidosis (correlation coefficient was 0.70).

### Figure 3

Serial observation of serum KL-6 and ACE levels. Three sarcoidosis patients were treated with systemic corticosteroid. Each line shows individual subjects. Serum KL-6 levels (top) were less affected by systemic corticosteroid administration than ACE (bottom).

#### Figure 4

Serial observation of serum KL-6 and ACE levels. A case of sarcoidosis was given ACE inhibitory drug, an anti-hypertensive agent, at her first visit to our clinic. When her medication was changed to AT1 blocker, another type of anti-hypertensive drug, her ACE level once increased. Then, since her hypertension was treated with combination of ACE inhibitor and AT1 blocker, her ACE level decreased again. However, neither ACE inhibitor nor AT1 blocker affected serum KL-6 levels.

1. Ashida, T., Higashishiba, M., Sumimoto, Y., Sano, T., Miyazato, H., Shimada, T., Miyatake, J., Kawanishi, K., Tatsumi, Y., & Kanamaru, A. (2001). Serum KL-6 levels in patients with pulmonary complications after allogeneic bone marrow transplantation. *Int J Hematol* 74: 464-468.
2. Berry, M., Ellingham, R.B., & Corfield, A.P. (2000). Membrane-associated mucins in normal human conjunctiva. *Invest Ophthalmol Vis Sci* 41: 398-403.
3. Endoh, Y., Hanai, R., Uto, K., Uno, M., Nagashima, H., Narimatsu, A., Takizawa, T., Onishi, S., & Kasanuki, H. (2000). KL-6 as a potential new marker for amiodarone-induced pulmonary toxicity. *Am J Cardiol* 86: 229-231.
4. Goda, C., Kotake, S., Sasamoto, Y., Yoshikawa, K., Okamoto, T., & Matsuda, H. (1998). [Clinical manifestations and diagnosis of patients with sarcoidosis]. *Nippon Ganka Gakkai Zasshi* 102: 106-110.
5. Hamada, H., Kohno, N., Akiyama, M., & Hiwada, K. (1992). Monitoring of serum KL-6 antigen in a patient with radiation pneumonia. *Chest* 101: 858-860.
6. Hamada, H., Kohno, N., Yokoyama, A., Hirasawa, Y., Hiwada, K., Sakatani, M., & Ueda, E. (1998). KL-6 as a serologic indicator of *Pneumocystis carinii* pneumonia in immunocompromised hosts. *Intern Med* 37: 307-310.

7. Hayashi, Y., Kao, W.W., Kohno, N., Nishihara-Hayashi, M., Shiraishi, A., Uno, T., Yamaguchi, M., Okamoto, S., Maeda, M., Ikeda, T., Hamada, H., Kondo, K., & Ohashi, Y. (2004). Expression patterns of sialylated epitope recognized by KL-6 monoclonal antibody in ocular surface epithelium of normals and dry eye patients. *Invest Ophthalmol Vis Sci* 45: 2212-2217.
8. Henderly, D.E., Genstler, A.J., Smith, R.E., & Rao, N.A. (1987). Changing patterns of uveitis. *Am J Ophthalmol* 103: 131-136.
9. Hiraga, Y. (1989). Annual report of diffuse lung disease research committee. (Tokyo: The Ministry of Health and Welfare, Japan).
10. Hirasawa, Y., Kohno, N., Yokoyama, A., Inoue, Y., Abe, M., & Hiwada, K. (1997). KL-6, a human MUC1 mucin, is chemotactic for human fibroblasts. *Am J Respir Cell Mol Biol* 17: 501-507.
11. Imai, T., Takase, M., Takeda, S., & Kougo, T. (2002). Serum KL-6 levels in pediatric patients: reference values for children and levels in pneumonia, asthma, and measles patients. *Pediatr Pulmonol* 33: 135-141.
12. Inatomi, T., Spurr-Michaud, S., Tisdale, A.S., & Gipson, I.K. (1995). Human corneal and conjunctival epithelia express MUC1 mucin. *Invest Ophthalmol Vis Sci* 36: 1818-1827.

13. Jabs, D.A., & Johns, C.J. (1986). Ocular involvement in chronic sarcoidosis. *Am J Ophthalmol* 102: 297-301.
14. James, D.G., Neville, E., & Langley, D.A. (1976). Ocular sarcoidosis. *Trans Ophthalmol Soc U K* 96: 133-139.
15. Kijlstra, A., Rothova, A., Baarsma, G.S., Zaal, M.J., Fortuin, M.E., Schweitzer, C., Glasius, E., & de Jong, P.T. (1987). Computer registration of uveitis patients. *Doc Ophthalmol* 67: 139-143.
16. Kitaichi, N., Kotake, S., Mizue, Y., Matsuda, H., Onoé, K., & Nishihira, J. (2000). Increase of macrophage migration inhibitory factor in sera of patients with iridocyclitis. *Br J Ophthalmol* 84: 1423-1425.
17. Kitaichi, N., Kotake, S., Mizue, Y., Sasamoto, Y., Goda, C., Iwabuchi, K., Onoé, K., Matsuda, H., & Nishihira, J. (2000). High-dose corticosteroid administration induces increase of serum macrophage migration inhibitory factor in patients with Vogt-Koyanagi-Harada's disease. *Microbiol Immunol* 44: 1075-1077.
18. Kitaichi, N., Kotake, S., Sasamoto, Y., Namba, K., Matsuda, A., Ogasawara, K., Onoé, K., Matsuda, H., & Nishihira, J. (1999). Prominent increase of macrophage migration inhibitory factor in the sera of patients with uveitis. *Invest Ophthalmol Vis Sci* 40: 247-250.

19. Kitaichi, N., Kotake, S., Shibuya, H., Yamada, Y., Chiba, H., Namba, K., & Ohno, S. (2003). Increase of KL-6 in sera of uveitis patients with sarcoidosis. *Graefes Arch Clin Exp Ophthalmol* 241: 879-883.
20. Kobayashi, J., & Kitamura, S. (1995). KL-6: a serum marker for interstitial pneumonia. *Chest* 108: 311-315.
21. Kobayashi, J., & Kitamura, S. (1996). Serum KL-6 for the evaluation of active pneumonitis in pulmonary sarcoidosis. *Chest* 109: 1276-1282.
22. Kohno, N., Akiyama, M., Kyoizumi, S., Hakoda, M., Kobuke, K., & Yamakido, M. (1988). Detection of soluble tumor-associated antigens in sera and effusions using novel monoclonal antibodies, KL-3 and KL-6, against lung adenocarcinoma. *Jpn J Clin Oncol* 18: 203-216.
23. Kotake, S., Furudate, N., Sasamoto, Y., Yoshikawa, K., Goda, C., & Matsuda, H. (1996). Characteristics of endogenous uveitis in Hokkaido, Japan. *Graefes Arch Clin Exp Ophthalmol* 234: 599-603.
24. Kotake, S., Kitaichi, N., & Ohno, S. (2002). Macrophage migration inhibitory factor in uveitis. *Int Ophthalmol Clin* 42: 99-103.
25. Nakajima, H., Harigai, M., Hara, M., Hakoda, M., Tokuda, H., Sakai, F., Kamatani, N.,

- & Kashiwazaki, S. (2000). KL-6 as a novel serum marker for interstitial pneumonia associated with collagen diseases. *J Rheumatol* 27: 1164-1170.
26. Narita, M., Nakayama, M., Yamada, S., & Togashi, T. (2001). Elevated KL-6 levels in fatal measles pneumonia. *Eur J Pediatr* 160: 454-455.
27. Narita, M., Tanaka, H., Abe, S., Yamada, S., Kubota, M., & Togashi, T. (2000). Close association between pulmonary disease manifestation in *Mycoplasma pneumoniae* infection and enhanced local production of interleukin-18 in the lung, independent of gamma interferon. *Clin Diagn Lab Immunol* 7: 909-914.
28. Obenauf, C.D., Shaw, H.E., Sydnor, C.F., & Klintworth, G.K. (1978). Sarcoidosis and its ophthalmic manifestations. *Am J Ophthalmol* 86: 648-655.
29. Ogawa, Y., Ishikawa, T., Ikeda, K., Nakata, B., Sawada, T., Ogisawa, K., Kato, Y., & Hirakawa, K. (2000). Evaluation of serum KL-6, a mucin-like glycoprotein, as a tumor marker for breast cancer. *Clin Cancer Res* 6: 4069-4072.
30. Rolf, A., Walter, R., Hans, P., & Thomas, S. (1994). Third International Workshop on Lung Tumor and Differential Antigens: overview of the results of the central data analysis. *Int J Cancer (Suppl)* 8: 8-26.
31. Rothova, A. (2000). Ocular involvement in sarcoidosis. *Br J Ophthalmol* 84: 110-116.

32. Semenzato, G., Bortoli, M., & Agostini, C. (2002). Applied clinical immunology in sarcoidosis. *Curr Opin Pulm Med* 8: 441-444.
33. Tran, V.T., Auer, C., Guex-Crosier, Y., Pittet, N., & Herbort, C.P. (1994). Epidemiological characteristics of uveitis in Switzerland. *Int Ophthalmol* 18: 293-298.
34. Uyama, M. (2002). Uveitis in sarcoidosis. *Int Ophthalmol Clin* 42: 143-150.