Serum KL-6 Elevation and Possible Pulmonary Involvement in Patients with Rheumatoid Arthritis Treated with Biological Agents

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Abstract

Backgrounds: Interstitial lung disease (ILD) is associated with rheumatoid arthritis (RA) itself and is also induced by biological and non-biological disease-modifying antirheumatic drugs. The glycoprotein Krebs von den Lungen-6 (KL-6) is reported to be a marker for the activity of ILD.

Objectives: To elucidate the relationship between serum KL-6 levels in patients with RA treated with biological agents and pulmonary involvement on computed tomography of the chest.

Methods: The subjects were 307 patients with RA treated with infliximab, etanercept, adalimumab, or tocilizumab. Medical records were reviewed to investigate serum KL-6 levels, disease activity, and pulmonary imaging findings.

Results: Levels of KL-6 were abnormally elevated in 25 patients (8.1%): 15 patients (11.2%) treated with infliximab, 6 patients (4.4%) treated with etanercept, and 4 patients (22.2%) treated with adalimumab, but in no patients treated with tocilizumab. However, no clinical pulmonary events developed. Computed tomography of the chest showed the start or progression of interstitial fibrotic change in 5 of 25 (20%) patients with abnormal KL-6 values. The changes in disease activity did not differ significantly between patients who showed elevated KL-6 values and those who did not.

Conclusions: Serum KL-6 levels were elevated in 8.1% of patients with RA treated with biological agents. Careful observation is necessary for these patients regarding lung fibrosis. (J Nippon Med Sch 2014; 81: 364–371)

Key words: biological agent, Krebs von den Lungen-6, interstitial pneumonia, rheumatoid arthritis

Introductionarthritis (RA) in recent years has resulted in
significant improvement of clinical outcomes, even in
refractory patients with high disease activity, who
could hardly have been managed previously.

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However, attention should be paid to infectious complications due to immunosuppression induced by such agents¹. In particular, pulmonary infections including septic pneumonia, tuberculosis and pneumocystis pneumonia (PCP) often cause severe and life-threatening conditions. In addition to infectious lung diseases, interstitial lung disease (ILD) is associated with RA itself (RA-related ILD). In patients with RA, the rate of ILD is as high as 80% at biopsy, 50% on computed tomography, and 5% on chest radiography²³. The 10-year survival rate of RA-related ILD is reported to be 76.6% in patients who have undergone surgical lung biopsy⁴.

ILD is also induced by biological and nonbiological disease-modifying antirheumatic drugs, and the mortality rates are 35% and 13%, respectively⁵. The reported prevalence of symptomatic ILD during treatment with anti-tumor necrosis factor (TNF) agents is 0.5% to 0.6% in Japan⁶⁻³. The diagnosis of ILD is usually made on the basis of symptoms and radiographic findings, and prompt cessation of the relevant medication is required.

Krebs von den Lungen-6 (KL-6) is a mucinlike high molecular weight glycoprotein expressed on regenerating type II pneumocytes. After type I pneumocytes have been damaged or reduced in number owing to alveolar epithelial injury, KL-6 production increases during the proliferation of type II pneumocytes. Therefore, KL-6 is considered to reflect the degree and severity of alveolar epithelial injury⁹. Consequently, KL-6 is believed to be a marker for the activity of interstitial pneumonia (IP) and hypersensitivity pneumonia, in which alveolitis is the main pathological process¹⁰. Regardless of the pathologic marker, elevation of serum KL-6 levels were noted in some reports with or without pulmonary manifestations¹¹⁻¹³.

The purpose of this study is to examine serum KL-6 levels in RA patients who were treated with biological agents retrospectively and reveal the significance of KL-6 elevation during biological treatment.

Materials and Methods

Patients

The subjects were 323 patients with RA treated with infliximab (INF) (n=142), etanercept (ETN) (n= 143), adalimumab (ADA) (n=19), or tocilizumab (TCZ) (n=19) in the Department of Rheumatology, Nippon Medical School, from January 2003 through October 2011. Medical records were reviewed to investigate serum KL-6 levels, disease activity, and pulmonary imaging findings.

Methods

Serum levels of KL-6 were measured with an enzyme-linked immunosorbent assay kit (Eisai, Tokyo, Japan; normal range, <500 ng/mL). The serum KL-6 values were measured at baseline and monitored at intervals of several months during treatment with biological agents. If levels of KL-6 were abnormal, measurements could be repeated at shorter intervals at the physician's discretion. Serum KL-6 values were considered to be abnormally elevated when 500 U/mL or greater.

Routine hematological studies were performed, and disease activity was measured as Disease Activity Score in 28 joints with ESR (DAS28) every visit or once a month.

Before treatment with biological agents, computed tomography (CT) of the chest was performed to exclude active pulmonary diseases and was performed again when lung diseases, such as IP, were suspected or when KL-6 levels were abnormally elevated. When one biological agent was replaced with another, the patient was re-classified as being treated with the new agent.

Statistical Analysis

Data are presented as the mean±SD. Differences between treatment groups were analyzed with analysis of variance, the Mann-Whitney U-test, the chi-square test, or Fisher's exact test. When multiple tests of association were performed, a post-hoc Bonferroni correction was made.

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	Infliximab (IFN)	Etanercept (ETN)	Adalimumab (ADA)	Tocilizumab (TCZ)
Patients, n	135	135	18	19
Age, years	59.0 ± 12.2	58.1 ± 13.6	61.1 ± 11.9	57.3 ± 14.1
Disease duration, years	15.2 ± 10.4	17.4 ± 12.1	13.4 ± 10.0	11.9 ± 8.9
DAS28	5.3 ± 1.5	5.10 ± 1.26	4.72 ± 1.12	4.95 ± 0.98
KL-6, U/mL	321 ± 89.7	325 ± 91.0	354 ± 19.6	308 ± 22.4
Methotrexate, mg/week (%)	6.49 ± 1.46 (100%)	6.18±1.88 (46%)	5.87 ± 1.86 (95%)	5.60 ± 1.58 (53%)
Prednisolone, mg/day (%)	6.59 ± 3.30 (76%)	5.82 ± 2.82 (51%)	4.17 ± 2.66 (78%)	6.29±2.75 (74%)

Table 1 Demographic and clinical features at baseline of patients with RA treated with biological agents

Table 2 Prevalence of abnormal KL-6 elevation in 4 biological agents

	Infliximab (IFN)	Etanercept (ETN)	Adalimumab (ADA)	Tocilizumab (TCZ)
KL-6 elevation	15 of 135 patients (11.2%)	6 of 135 patients (4.4%)	4 of 18 patients (22.2%)	0 of 19 patients (0%)
The prevalence was significantly different (P<0.05) according to Fisher's exact test.				

Ethics

The present study was performed in accordance with the guidelines of the Helsinki Declaration and the ethics guidelines for epidemiological research in Japan. The ethics guidelines for epidemiological research in Japan require the notification of eligible patients with RA of this study and allows implementation of this study without obtaining individual written informed consent. This study was announced by posters in the outpatient clinic of the Department of Rheumatology, Nippon Medical School Hospital. Patients who expressed unwillingness to participate in the present study were excluded.

Results

Demographic Features

Of 323 patients with RA treated with biological agents, 16 who had elevated KL-6 levels (>500 U/mL) at baseline were excluded from this study. Of these 16 patients, 9 had already received a diagnosis of a lung disease, such as IP, sarcoidosis, or asthma. Demographic and clinical features of the remaining 307 patients are shown in **Table 1**. There were no significant differences between the treatment groups in mean age, mean disease duration, DAS28 or serum KL-6 levels at baseline (**Table 1**). The percentages of patients receiving methotrexate differed between the groups, but the average doses

of methotrexate did not. The mean dose of prednisolone in the ADA group was significantly lower than doses in the other groups.

Prevalence of KL-6 Elevation

The average follow-up period was 55 months (range, 8–95 months). Serum KL-6 levels increased to greater than 500 U/mL in 25 patients, and the maximum values ranged from 538 to 2,007 U/mL. In total, 25 patients (8.1%) showed elevated levels of KL-6: 15 patients (11.2%) treated with INF, 6 patients (4.4%) treated with ETN, and 4 patients (22.2%) treated with ADA, but no patients treated with TCZ. The percentage of patients with KL-6 elevation differed significantly between the groups (P<0.05) (**Table 2**). The highest prevalence was 22.2% in the ADA group, and the lowest prevalence was 0% in the TCZ group.

Chest CT Image

Twenty-five patients underwent CT of the chest soon after KL-6 elevation was recognized. Five (20%) of these patients (3 patients treated with INF, 1 treated with ETN, and 1 treated with ADA) showed progression of fibrosis, whereas the others showed no definite changes (**Fig. 1**). There was no significant difference between the groups in the prevalence of CT evidence of the progression of fibrosis (**Table 3**).

To examine whether KL-6 elevation is a specific marker for progression of fibrosis, the findings of CT

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Fig. 1 The representative chest CT images of the progression of fibrosis. A: at baseline, B: at the time point after KL-6 elevation. The fibrotic change was increased (arrow).

Table 3 Prevalence of fibrotic progression in patients with KL-6 elevation

	Infliximab (IFN)	Etanercept (ETN)	Adalimumab (ADA)	Tocilizumab (TCZ)
Prevalence of fibrotic progression	3 of 15 patients	1 of 6 patients	1 of 4 patients	0 of 0 patients

There was no significant difference in the prevalence between the groups calculated with Fisher's exact test

performed because of respiratory symptoms, such as severe cough, cough producing sputum, or shortness of breath, from 17 patients treated with INF but without KL-6 elevation were also assessed. The radiological diagnosis was bacterial pneumonia in 3 patients, bronchiectasis in 1 patient, and obstructive pneumonitis in 1 patient. Progression of fibrosis was not observed.

KL-6 Elevation and Concomitant Use of Methotrexate

The mean methotrexate dosage did not differ significantly between patients with KL-6 elevation $(5.1\pm2.9 \text{ mg/week}, n=25)$ and those without $(4.2\pm3.3 \text{ mg/week}, n=282)$.

Timing of KL-6 Elevation and Serum KL-6 Values

In most cases, KL-6 elevation was observed within 20 months after the start of treatment (Fig. 2). The KL-6 elevation appeared within 9 months after the start of treatment in 4 of 5 patients with evidence of the progression of fibrosis on CT (Fig. 2). Similarly, progression of fibrosis was seen in 4 of 13 patients (30.8%) who showed KL-6 elevation within 9 months. Neither maximum serum KL-6 values nor the

increase in KL-6 over the baseline value was correlated with the progression of fibrosis; no patient with CT evidence of the progression of fibrosis had maximum KL-6 values greater than 1,000 U/mL (**Fig. 3**).

KL-6 Elevation and Disease Activity

At the time of KL-6 elevation, 12 patients had a good response, 8 had a moderate response, and 5 had no response, according to the European League Against Rheumatism response criteria¹⁵. Ultimately, 20 of 25 patients with KL-6 elevation responded to biological agents, and the prevalence of response did not differ from that in patients without KL-6 elevation (data not shown).

Clinical Course of Patients with KL-6 Elevation

In 2 of the 5 patients with KL-6 elevation and the progression of fibrosis on chest CT, the biological agent was changed to another one while methotrexate was continued (**Table 4**). In the remaining 3 patients both the biological agent and methotrexate were discontinued. The KL-6 values in these 3 patients decreased soon after the discontinuation. In 19 of the 20 patients in whom chest CT images showed no evidence of progression





Fig. 2 Time point of KL-6 elevation and serum KL-6 values The horizontal axis indicates time point when abnormal KL-6 values were first observed after induction of biological agents. A: serum KL-6 values, B: increase of KL-6 values from baseline. The solid black circle indicates patients with fibrotic progression, and open circle indicates those without fibrotic progression.



Fig. 3 Serum KL-6 levels and the progression of fibrosis Serum KL-6 levels showed no significant difference between patients with and without fibrotic progression on chest CT. A: serum KL-6 levels, B: increase of KL-6 values from baseline. FP+: with fibrotic progression (n=5), FP-: without fibrotic progression (n=20).

of fibrosis, the originally prescribed biological agent and concomitant methotrexate were continued and KL-6 levels remained greater 500 U/mL without

pulmonary manifestations. For the 20^{th} patient, ADA was discontinued soon after the KL-6 value increased to 2,007 U/mL. The KL-6 value did not

Chest CT		Biological agents	Methotrexate	Prognosis
Progression of fibrosis	2 patients	switch	continue	decline promptly*1
	3 patients	discontinue	discontinue	decline promptly *1
No remarkable change	1 patient	discontinue	continue	1 year until normal range*2
	19 patients	continue	continue	persistent KL-6 elevation* ³

Table 4 Clinical course of patients with abnormal KL-6 elevation

*1: KL-6 values declined promptly.

*2: KL-6 value increased to 2,007 U/mL but decreased to less than 500 U/mL 1 year after discontinuation of biological agents.

*3: KL-6 values remained >500 U/mL during follow-up

decrease to less than 500 U/mL until 1 year later.

Discussion

Pulmonary disorders accompanying RA include IP, pleural lesions, bronchial lesions, pulmonary infection, and drug-induced pulmonary disorders. In particular, IP is observed as a complication in approximately 5% of patients with RA upon chest Xray and in 30% to 50% upon chest CT^{2,3}. On the other hand, methotrexate, leflunomide, and even biological agents induce ILD or worsen preexisting ILD. Moreover, drug-induced ILD can have a fatal outcome ⁵. Patients with RA treated with methotrexate or biological agents are immunocompromised hosts and are prone to infectious lung diseases, such as bacterial pneumonia, tuberculosis, and pneumocystis pneumonia. Pneumocystis pneumonia is associated with IP, and KL-6 is a useful marker for its development¹⁶.

KL-6 is a chemotactic factor for lung fibroblasts and is considered to be an index for the degree and activity of pulmonary fibrosis or inflammation or both. In the present study, serum KL-6 values were abnormally elevated in 8.1% of patients with RA who were treated with biological agents, and 20% of patients with elevated KL-6 showed progression of fibrosis on chest CT. However, neither the maximum KL-6 level nor the increase in KL-6 over baseline levels was correlated with the progression of fibrosis. Doishita et al. analyzed 240 patients with connective tissue diseases and reported that a serum KL-6 level of 500 U/mL or more was a marker for IP and that a level of 1,000 U/mL or more was a marker for active IP¹⁷. Yanabe et al. reported in a longitudinal study that a sudden increase in serum KL-6 level is related to an increase in the activity of pulmonary fibrosis¹⁸. Ichiyasu et al. have also reported that CT and the serum KL-6 concentration indicate similar levels of IP severity¹⁹. Despite these previous reports, in the present study only 20% of patients with KL-6 elevation showed the progression of fibrosis on chest CT. Although its cause is unclear, KL-6 elevation during treatment with biological agents may be induced by complicated mechanisms in addition to alveolar epithelial injuries.

Interestingly, KL-6 levels increased within 9 months after the administration of biological agents. This early elevation of serum KL-6 levels might be due in part to a pharmacological effect or an allergic reaction to the biological agents. This possibility is supported by the fact that the prevalence of KL-6 elevation differed significantly with the type of biological agent. We have previously reported 3 cases in which the serum KL-6 levels increased owing to ADA but then decreased with ADA discontinuation of and subsequent administration of TCZ11. Elevation of KL-6 has been reported in clinical trials of TNF inhibitors; patients with RA met a criteria of the elevation in 15.6% of INF trial, in 7.8% of certolizumab trial and in 1.8% and 7.1% of regarding golimumab trials¹². Takamura et al. have also reported a lower incidence of KL-6 elevation with TCZ than with TNF inhibitors¹³. The possible mechanisms include the following: 1) damage to type I pneumocytes due to biological agents, 2) stimulation of the production or release of KL-6 from type II pneumocytes by biological agents,

3) an effect of biological agents on the KL-6 assay system, 4) development of subclinical pulmonary infections under the influence of biological agents, and 5) IP caused by concomitant methotrexate. Regarding the last possible mechanism, the present study did not find a significant association between treatment with methotrexate and abnormal KL-6 elevation. In a retrospective study of patients with RA, the prevalence of KL-6 elevation was higher in patients treated with biological agents than in treated with methotrexate¹³. Taken patients together, KL-6 elevation is specific to biological agents.

Harigai et al. have shown that serum KL-6 levels are increased during anti-TNF therapy but are not associated with significant clinical events and spontaneously decrease in the majority of patients¹². However, they recommend continuing treatment with biological agents under careful observation. However, they did not examine chest CT scans from patients with KL-6 elevation. In the present study, the progression of fibrosis was observed upon chest CT in 20% of patients with elevated KL-6 levels. Although KL-6 elevation was not associated with clinical events in the present study, discontinuation of biological therapy should be considered after careful observation with chest CT.

In conclusion, KL-6 elevation was found in patients with RA treated with biological agents. Although the development of clinical pulmonary manifestations was rare, careful observation with chest CT is necessary.

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References

- 1. Miyasaka N, Takeuchi T, Eguchi K: Guidelines for the proper use of etanercept in Japan. Mod Rheumatol 2006; 16: 63–67.
- 2. Gabbay E, Tarala R, Will R, et al.: Interstitial lung

disease in recent onset rheumatoid arthritis. Am J Respir Crit Care Med 1997; 156: 528–535.

- Kim EA, Lee KS, Johkoh T, et al.: Interstitial lung diseases associated with collagen vascular diseases: radiologic and histopathologic findings. Radiographics 2002; 22: 151–165.
- 4. Nakamura Y, Suda T, Kaida Y, et al.: Rheumatoid lung disease: prognostic analysis of 54 biopsy-proven cases. Respir Med 2012; 106: 1164–1169.
- Roubille C, Haraoui B: Interstitial lung diseases induced or exacerbated by DMARDS and biologic agents in rheumatoid arthritis: A systematic literature review. Semin Arthritis Rheum 2013; 43: 613–626.
- Takeuchi T, Tatsuki Y, Nogami Y, et al.: Postmarketing surveillance of the safety profile of infliximab in 5,000 Japanese patients with rheumatoid arthritis. Ann Rheum Dis 2008; 67: 189– 194.
- Koike T, Harigai M, Inokuma S, et al.: Postmarketing surveillance of safety and effectiveness of etanercept in Japanese patients with rheumatoid arthritis. Mod Rheumatol 2011; 21: 343–351.
- 8. Koike T, Harigai M, Ishiguro N, et al.: Safety and effectiveness of adalimumab in Japanese rheumatoid arthritis patients: postmarketing surveillance report of the first 3,000 patients. Mod Rheumatol 2012; 22: 498–508.
- Kohno N, Kyoizumi S, Awaya Y, et al.: New serum indicator of interstitial pneumonitis activity. Sialylated carbohydrate antigen KL-6. Chest 1989; 96: 68–73.
- Kobayashi J, Kitamura S: KL-6: a serum marker for interstitial pneumonia. Chest 1995; 108: 311–315.
- Koiwa M, Goto S, Takenouchi K, et al.: Elevation of serum KL-6 levels in 3 patients with rheumatoid arthritis treated with adalimumab. Mod Rheumatol 2012; 22: 147–151.
- 12. Harigai M, Takamura A, Atsumi T, et al.: Elevation of KL-6 serum levels in clinical trials of tumor necrosis factor inhibitors in patients with rheumatoid arthritis: a report from the Japan College of Rheumatology Ad Hoc Committee for Safety of Biological DMARDs. Mod Rheumatol 2013; 23: 284– 296.
- 13. Takamura A, Hirata S, Nagasawa H, et al.: A retrospective study of serum KL-6 levels during treatment with biological disease-modifying antirheumatic drugs in rheumatoid arthritis patients: a report from the Ad Hoc Committee for Safety of Biological DMARDs of the Japan College of Rheumatology. Mod Rheumatol 2013; 23: 297–303.
- Imokawa S, Colby TV, Leslie KO, et al.: Methotrexate pneumonitis: review of the literature and histopathological findings in nine patients. Eur Respir J 2000; 15: 373–381.
- Van Gestel AM, Prevoo ML, Van't Hof MA, et al.: Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Arthritis Rheum 1996; 39: 34– 40.
- Hamada H, Kohno N, Yokoyama A, et al.: KL-6 as a serologic indicator of Pneumocystis carinii pneumonia in immunocompromised hosts. Intern Med 1998; 37: 307–310.

- 17. Doishita S, Inokuma S, Asashima H, et al.: Serum KL-6 level as an indicator of active or inactive interstitial pneumonitis associated with connective tissue diseases. Intern Med 2011; 50: 2889–2892.
- Yanaba K, Hasegawa M, Hamaguchi Y, et al.: Longitudinal analysis of serum KL-6 levels in patients with systemic sclerosis: association with the activity of pulmonary fibrosis. Clin Exp Rheumatol 2003; 21: 429–436.
- 19. Ichiyasu H, Ichikado K, Yamashita A, et al.:

Pneumocyte Biomarkers KL-6 and Surfactant Protein D Reflect the Distinct Findings of High-Resolution Computed Tomography in Nonspecific Interstitial Pneumonia. Respiration 2012; 83: 190–197.

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