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ORIGINAL ARTICLE

Clinical characteristics and risk factors for *Pneumocystis jirovecii* pneumonia in patients with rheumatoid arthritis receiving adalimumab: a retrospective review and case–control study of 17 patients

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Abstract

Objectives To investigate the clinical characteristics and risk factors of *Pneumocystis jirovecii* pneumonia (PCP) in rheumatoid arthritis (RA) patients treated with adalimumab.

Methods We conducted a multicenter, retrospective, case–control study to compare RA patients treated with adalimumab with and without PCP. Data from 17 RA patients who were diagnosed with PCP and from 89 RA

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patients who did not develop PCP during adalimumab treatment were collected.
Results For the PCP patients, the median age was 68 years old, with a median RA disease duration of eight years. The median length of time from the first adalimumab injection to the development of PCP was 12 weeks. At the onset of PCP, the median dosages of prednisolone and methotrexate were 5.0 mg/day and 8.0 mg/week, respectively. The patients with PCP were significantly older ($p < 0.05$) and had more structural changes ($p < 0.05$) than the patients without PCP. Computed tomography of the chest revealed ground-glass opacity without interlobular septal boundaries in the majority of the patients with PCP. Three PCP patients died.
Conclusions PCP may occur early in the course of adalimumab therapy in patients with RA. Careful monitoring, early diagnosis, and proper management are mandatory to secure a good prognosis for these patients.

Keywords Adalimumab · *Pneumocystis jirovecii* pneumonia · Rheumatoid arthritis · TNF antagonist

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by persistent synovitis and structural damage to multiple joints. Tumor necrosis factor (TNF) is abundantly produced in the inflamed synovium and contributes to the imuunopathogenesis of the disease. Adalimumab is the first fully human monoclonal antibody against TNF; treatment with this biologic agent has been well established in patients with RA in multiple clinical trials [1–3]. On the other hand, treatment with adalimumab, as well as infliximab and etanercept, has been associated with increased risk for opportunistic and serious infections in cohort studies using RA patient registries [4–7]. In Japan, strict post-marketing surveillance (PMS) programs have been conducted for patients with RA given TNF antagonists. The numbers of RA patients with *Pneumocystis jirovecii* (*P. jirovecii*) pneumonia (PCP) who were treated with infliximab, etanercept, or adalimumab were 22 (0.4 %) out of 5,000 patients, 25 (0.18 %) out of 13,894 patients, and 25 (0.33 %) out of 7,469 patients, respectively, in these PMS programs [6–8]. Note that these incidence rates of PCP in Japan are apparently higher than the corresponding figure (0.01 %) reported from the United States [9].

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We have previously described the clinical characteristics and risk factors for PCP in RA patients treated with infliximab [10, 11] and etanercept [12]. These risk factors included older age and presence of coexisting lung diseases for both TNF antagonists, a higher daily dose of prednisolone (PSL) for infliximab, and a higher weekly dose of methotrexate (MTX) for etanercept. Considering the similar incidence of PCP in the PMS programs among the three TNF antagonists, it is clinically important and intriguing to characterize PCP in RA patients given adalimumab and to compare the results with those obtained for RA patients treated with other TNF antagonists.

In this paper, we report detailed clinical, laboratory, and radiographic features of PCP that developed in RA patients during treatment with adalimumab. Furthermore, we compared 17 RA patients receiving adalimumab who developed PCP with 89 RA patients who did not develop PCP during treatment, and identified risk factors for PCP in patients with RA treated with adalimumab.

Materials and methods

Patients

Patients included in the present study fulfilled the 1987 American College of Rheumatology (formerly the American Rheumatism Association) criteria for RA [13] and received adalimumab (40 mg every two weeks) with or without concomitant MTX. Between April 2008 and April 2010, 17 patients with PCP (PCP group) were collected from 16 hospitals through either the PMS program for adalimumab ($n = 16$) or through a voluntary case report by attending physicians at a scientific meeting ($n = 1$). We convened a face-to-face meeting in March 2011 to discuss diagnosis and treatment for the collected cases among the investigators of this study. RA patients who did not develop PCP during adalimumab therapy for at least one year from the first dose of adalimumab (non-PCP group, $n = 89$) were randomly collected from the participating hospitals of this study. Other eligibility criteria for the non-PCP group were registration in the PMS program of adalimumab and the use of adalimumab five times or more. The median (range) observation period for the non-PCP group treated with adalimumab was 365 (63–365) days. To increase the statistical power of this case–control study, the number of patients in the non-PCP group was designed to be about five times as many as that in the PCP group [14].

Diagnostic criteria for PCP

Previously established diagnostic criteria for PCP [15, 16] were used in the present study [10]. A diagnosis of PCP was considered definitive if a patient fulfilled the following four conditions: clinical manifestations (fever, dry cough, or dyspnea), hypoxemia, interstitial infiltrates on chest radiographs, and microscopic detection of *P. jirovecii* in induced sputum or bronchoalveolar lavage fluid. The diagnosis of PCP was considered presumptive if a patient fulfilled all of these conditions except for the microscopic detection of *P. jirovecii* in the absence of other infectious diseases and the presence of either a positive polymerase chain reaction (PCR) test for *P. jirovecii* DNA or increased serum 1,3-β-D-glucan (BDG) levels (Fungitec G test MK; Seikagaku, Tokyo, Japan or Wako β-D-glucan test; Wako Pure Chemical Industries, Tokyo, Japan) [17, 18] along with a response to standard treatments for PCP. Both the PCR test for *P. jirovecii* DNA and that for serum BDG are commercially available, validated, and officially approved as clinical laboratory tests by the Ministry of Health, Labour, and Welfare in Japan.

Collection and analysis of clinical data

Clinical information was collected using a standardized format to evaluate demographic information, Steinbrocker's radiographic stage and functional class [19], comorbidities, concomitant drugs, laboratory data, radiographic data, treatment, and outcome. Chest radiographs and computed tomography (CT) scans were evaluated by a pulmonologist (H.S.) and a diagnostic radiologist (F.S.). CT findings were categorized into three patterns, as we did in previous studies [12, 20]: (a) diffuse ground-glass opacity (GGO) distributed in a panlobular manner; that is, GGO was sharply demarcated from the adjacent normal lung by interlobular septa (type A GGO); (b) diffuse GGO that is homogeneous or somewhat inhomogeneous in distribution but without the sharp demarcation caused by interlobular septa (type B GGO); (c) other patterns, such as mixed consolidation and GGO (type C).

Statistical analyses

Demographic data and baseline data were compared between the PCP and non-PCP groups using the χ^2 test for categorical variables and the Mann–Whitney test for continuous variables. To identify risk factors for PCP, the Cox proportional-hazards regression model was used. All analyses were performed using SPSS software, version 16.0 (SPSS Japan, Tokyo, Japan).

Ethics

The guidelines of the Declaration of Helsinki (revised in 2008) and the ethics guidelines for epidemiologic research in Japan were followed. The study protocol was approved

by the Institutional Ethics Committee of the Tokyo Medical and Dental University Hospital (#863 in 2010).

Results

Diagnosis and clinical characteristics of RA patients with PCP

We applied the above diagnostic criteria to the 17 RA patients in the PCP group. Of the 17 cases, three (patients 8, 14, and 17) met the criteria for definitive PCP, and 14 met the criteria for presumptive PCP. The clinical characteristics of each patient are summarized in Table 1. The median age of the 17 patients was 68 years (range 48–78 years), and 12 (71 %) were female. The median duration of RA was eight years. Fourteen patients were at Steinbrocker's stage III or IV. All patients received MTX and 13 (77 %) received corticosteroids from baseline to the onset of PCP. At the onset of PCP, the median dosages of prednisolone and MTX were 5.0 mg/day (range 2.5–9 mg/day) and 8.0 mg/week (range 4–15 mg/week), respectively. One patient was receiving another immunosuppressive drug, tacrolimus, at 3 mg/day. Eight patients had pulmonary comorbidities, including interstitial pneumonia ($n = 4$), chronic obstructive pulmonary disease ($n = 4$), and old pulmonary tuberculosis ($n = 2$). Four patients had diabetes mellitus. None of the patients received chemoprophylaxis for PCP at the time of PCP diagnosis. The median interval between the first injection of adalimumab and the onset of PCP was 12 weeks (range 4–38 weeks). Thirteen patients (76 %) developed PCP within 26 weeks after the first injection. Fever was the most common clinical symptom (it was observed in 15 patients; 88 %), followed by dyspnea on effort (82 %) and dry cough (41 %).

Laboratory and radiographic features of the PCP patients

Laboratory data at the onset of PCP are summarized in Table 2. Fourteen patients either had severe hypoxia (with $PaO_2 < 60$ mm Hg on room air) or required immediate oxygen therapy at the onset of PCP. Peripheral blood lymphocyte (PBL) counts at the onset of PCP were <500 cells/μl in three patients, 500–1,000 cells/μl in five patients, and $>1{,}000$ cells/μl in nine patients. *P. jirovecii* was microscopically identified in three patients. The polymerase chain reaction test for *P. jirovecii* DNA was positive in 13 patients, using either induced sputum (11 patients) or bronchoalveolar lavage fluid (four patients), but three patients were not examined. Serum levels of BDG, one of

Table 1 Characteristics of rheumatoid arthritis patients treated with adalimumab at the onset of PCP

Pt	Age/sex	Stage/class	Number of injections[a]	Treatment duration (days)[b]	MTX (mg/w)	PSL (mg/d)	Lung disease	DM	Clinical manifestations
1	48/F	III/I	7	105	8	2.5	–	–	Fever/DOE
2	69/M	IV/III	4	62	10	0	E	–	Cough/DOE
3	74/F	IV/II	9	131	8	5	IP E	–	DOE
4	52/M	III/II	5	59	4	8	IP	–	Fever/cough/DOE
5	61/F	IV/II	3	45	8	9	–	–	Fever
6	67/F	III/III	3	28	8	8	IP	–	Fever/cough/DOE
7	61/F	IV/II	4	59	6	0	Old TB	–	Fever/DOE
8	77/F	IV/II	6	129	6	5	–	+	Fever/DOE
9	52/F	III/I	3	55	8	5	–	–	Fever/DOE
10	78/M	III/III	6	86	8	0	IP	+	Fever/DOE
11	66/F	I/III	6	106	8	3	–	–	Fever/cough
12	70/F	II/II	2	23	8	5	Old TB	–	Fever/cough/DOE
13	68/M	I/II	3	28	8	0	E	+	Fever/DOE
14	71/F	III/II	15	214	8	7.5	–	–	Fever/DOE
15	73/M	III/II	18	268	15	3	–	+	Fever/cough/DOE
16	65/F	III/II	16	227	8	2	–	–	Fever/DOE
17	78/F	IV/II	16	252	4	4	–	–	Fever/cough

PCP Pneumocystis jirovecii pneumonia, *Pt* patient, *w* week, *d* day, *M* male, *F* female, *MTX* methotrexate, *PSL* prednisolone, *E* emphysema, *IP* interstitial pneumonia, *old TB* old tuberculosis, *DM* diabetes mellitus, *DOE* dyspnea on effort, *cough* dry cough

[a] Number of injections of ADA prior to the diagnosis of PCP

[b] Treatment duration with ADA before the onset of PCP

Table 2 Laboratory data of rheumatoid arthritis patients treated with adalimumab at the onset of PCP

Pt	WBC (/μl)	Lymphocytes (/μl)	SpO_2 or PaO_2 (Torr) [O_2, l/min][a]	Serum β-D-glucan (μg/ml) [normal range at the institute]	*Pneumocystis jirovecii* PCR
1	7,870	912	SpO_2 96 % [0]	289 [<11]	+
2	5,100	1,989	SpO_2 92 % [0]	30.5 [<11]	+
3	6,300	252	55.1 [0]	1041 [<11]	NA
4	6,200	874	68.0 [0]	25.76 [<11]	+
5	8,050	1,110	60.4 [0]	50.3 [<20]	NA
6	6,400	716	58.9 [0]	37.8 [<6]	+
7	5,660	1,041	71.8 [0]	22.1 [<11]	+
8	6,800	279	31.3 [0]	29 [<11]	+[b]
9	15,900	832	85.7 [3]	79.5 [<20]	+
10	7,500	1,350	65.4 [0]	22.3 [<20]	+
11	8,400	3,696	69.5 [0]	16.4 [<11]	+
12	11,700	1,029	26.1 [0]	21.06 [3.5]	+
13	7,950	1,761	SpO_2 85 % [2]	160 [<5]	+
14	9,580	34	56.7 [0]	13.0 [<11]	NA[b]
15	5,700	1,140	55.1 [0]	13.0 [<11]	–
16	7,000	1,330	56.1 [10]	21.38 [<11]	+
17	3,200	704	52.5 [0]	419 [<11]	+[b]
Median (IQR)	7,000 (5950–8225)	1,029 (710–1340)	Not applicable	Not applicable	Not applicable

PCP Pneumocystis jirovecii pneumonia, *Pt* patient, *WBC* white blood cell, *PCR* polymerase chain reaction, *NA* not assessed, *SpO2* oxygen saturation measured using a pulse oximeter, *IQR* interquartile range

[a] Oxygen therapy during the measurement of PaO_2

[b] *Pneumocystis jirovecii* microscopically detected in bronchoalveolar-lavage fluid

the major components of the cell walls of fungi and a serum maker for PCP [17, 18], were elevated in all patients. Results of sputum culture performed in 14 patients revealed no causative bacteria or fungi.

Chest radiographs and thoracic CT scans were analyzed for all 17 patients. The most common CT finding was ground-glass opacity (GGO) (in 17 patients), either with sharp demarcation by interlobular septa in one patient (type A GGO) (Fig. 1a) or without interlobular septal boundaries in 14 patients (type B GGO) (Fig. 1b). Two patients demonstrated mixed patterns (type C).

Treatment and clinical course of PCP in patients with RA receiving adalimumab

All patients were hospitalized on the same day that PCP was suspected. Fourteen patients (all except for patients 2, 5, and 11) received oxygen therapy on admission. MTX and adalimumab were immediately discontinued in all patients. All patients received therapeutic doses of trimethoprim/sulfamethoxazole (TMP/SMX). Because of adverse drug reactions that included skin eruptions, liver dysfunction, thrombocytopenia, and hyperpotassemia, TMP/SMX was reduced or stopped in eight patients. One patient was changed to pentamidine isethionate. Sixteen patients were concomitantly treated with high-dose corticosteroids within a few days after admission. Eleven patients were empirically treated with antibiotics and four with antifungal agents. Three patients (patients 1, 3, and 8) were intubated on the day of admission because of progressive respiratory failure; two of these patients responded to treatment and were successfully weaned from artificial ventilation. One patient (patient 17) died because of PCP with progressive respiratory failure. Two patients died because of multiple organ failure (patient 12) and gastrointestinal bleeding, cytomegalovirus infection, and multiple organ failure (patient 3) after improvement of PCP.

Case–control study

In order to characterize the PCP group more precisely, we compared demographic information, comorbidities, treatments, and laboratory data at baseline (i.e., at the initiation of treatment with adalimumab) between the PCP and non-PCP groups using a univariate analysis (Table 3). The PCP group was significantly older ($p = 0.003$) and had a more advanced radiographic stage (Steinbrocker's stage III or IV) ($p = 0.010$) than the non-PCP group. Although the rates of patients with preexisting pulmonary diseases and diabetes mellitus in the PCP group were numerically higher, these differences were not statistically significant. There were no differences in disease duration and the dosages of prednisolone and methotrexate between the two groups. None of the patients in the PCP group and fourteen patients in the non-PCP group received prophylaxis for

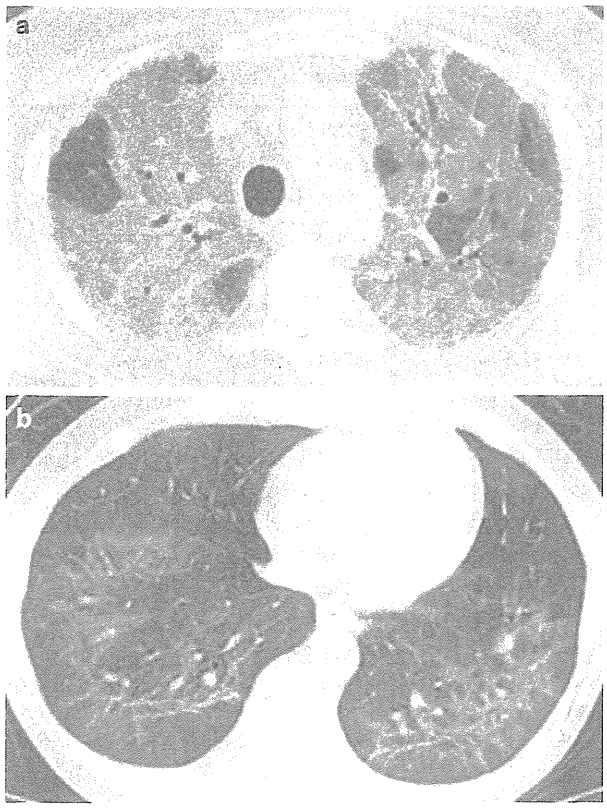

Fig. 1 Representative thoracic computed tomography findings of rheumatoid arthritis patients who developed *Pneumocystis jirovecii* pneumonia while receiving adalimumab. a Ground-glass opacity (GGO) with sharp demarcation by interlobular septa (type A) (patient 12). b Inhomogeneous GGO without obvious demarcation by interlobular septa (type B) (patient 1)

PCP for at least three months during the observation period. Twelve patients used TMP/SMX and two used aerosolized pentamidine.

Based on the results of the univariate analysis, age, sex, pulmonary comorbidities and Steinbrocker's stage of RA were analyzed as candidate predictors for the development of PCP. The Cox proportional-hazards regression analysis revealed a significant association between advanced radiographic stage (stage III or IV) and development of PCP (hazard ratio (HR) 3.76, 95 % confidence interval (CI) 1.03–7.30, $p = 0.045$). While the hazard ratios of older age and preexisting pulmonary diseases tended to be higher, they did not reach statistical significance (Table 4).

Because 14 patients in the non-PCP group received prophylaxis for PCP, we performed the multivariate analysis after excluding these 14 patients, and found a significant association between older age and development of PCP (HR 3.31, 95 % CI 1.09–10.0, $p = 0.034$). The HR of the radiographic stage did not reach statistical significance (HR 2.82, 95 % CI 0.74–10.7) in this model.

Discussion

We accumulated the largest possible number of patients with RA who developed PCP during treatment with adalimumab, and described the clinical and radiologic characteristics of the 17 patients that we found.

Adalimumab is the third TNF antagonist to be approved in Japan. We have already reported the clinical characteristics and risk factors for PCP in RA patients treated with infliximab or etanercept [10–12]. The median interval (range) between the first dose of TNF antagonists and the onset of PCP was 12 weeks (range 4–38) for adalimumab, nine weeks (range 2–90) for infliximab [11], and 14 weeks (range 3–43) for etanercept [12]. PCP developed within six months in the majority of RA patients after the initiation of each TNF antagonist: 90 % for infliximab, 80 % for etanercept, and 76 % for adalimumab.

Previous studies have revealed that patients without HIV infection develop PCP abruptly and progress to fulminating pneumonia with acute respiratory failure [21, 22]. We also reported that RA patients treated with infliximab or etanercept developed PCP rapidly and progressed to severe respiratory failure [10–12]: 18 out of 21 PCP patients using infliximab, all 15 PCP patients using etanercept, and 14 of 17 PCP patients in this study showed severe hypoxemia and required oxygen therapy. The mortalities of the patients with PCP given infliximab (0 %) or etanercept (6.7 %) are numerically lower than the mortality of this study, in which three patients (17.6 %) died. Walzer etal. [23] identified older age, second or third episode of PCP, low hemoglobin level, low PaO_2 breathing room air at admission, pulmonary Kaposi sarcoma, and presence of medical comorbidity as early predictors of mortality of PCP in HIV-infected patients. Although such prognostic factors in non-HIV PCP patients are unknown, all three patients in our study who died were females over 70 years old, and their PaO_2 on admission was less than 60 Torr. Two of these patients had pulmonary comorbidities. One patient had a quite high serum level of BDG, and one was positive for both microscopic detection and the PCR test for the organism. These data would suggest severe pulmonary injury at presentation and a high burden from *P. jirovecii*.

In our study, all patients received therapeutic doses of TMP/SMX. However, eight patients (47.1 %) were obliged to reduce the dosage or stop using the drug due to adverse drug reactions, such as gastrointestinal symptoms and hematological abnormalities. Kameda et al. [24] also reported that more than one-third of the patients could not complete the standard protocol of the TMP/SMX treatment. These data indicate that the optimal dosage and treatment period of TMP/SMX for PCP should be investigated. The clinical benefit of adjunctive corticosteroid

Table 3 Baseline characteristics of patients with rheumatoid arthritis treated with adalimumab

Characteristic	PCP group ($n = 17$)	Non-PCP group ($n = 89$)	p value
Age (years)[a]	68 (48–78)	60 (24–79)	0.003
Female (%)	70.6	80.9	0.255
Disease duration (years)[a]	8.0 (0.7–36)	9.5 (3–40)	0.491
Chronic pulmonary disease (%)	47.1	22.5	0.107
Diabetes mellitus (%)	23.5	7.9	0.074
Steinbrocker's radiographic stage (III or IV) (%)	82.4	48.3	0.010
Steinbrocker's functional class (III or IV) (%)	17.6	19.1	0.596
MTX (%)	100	86.5	0.108
MTX (mg/week)[a]	8.0 (4–10)	8.0 (4–15)	0.119
MTX ≥ 8 mg/week (%)	11.8	28.1	0.228
PSL (%)	76.5	56.2	0.118
PSL (mg/day)[a]	5.0 (3–12)	5.0 (1–17)	0.529
PSL ≥ 5 mg/day (%)	52.9	33.7	0.131
WBC <4,000/μl (%)	0	2.2	0.731
Serum IgG (mg/dl)[a]	1421 (846–1954)	1316 (827–3165)	0.817

PCP Pneumocystis jirovecii pneumonia, *MTX* methotrexate, *PSL* prednisolone, *Chronic pulmonary disease* = interstitial pneumonia, bronchiectasis, chronic obstructive pulmonary diseases, bronchial asthma, middle lobe syndrome, old pulmonary tuberculosis

p values were calculated using the Mann–Whitney test for continuous variables or χ^2 test for categorical variables

[a] Median (range)

Table 4 Cox regression analysis of risk factors for the development of PCP in rheumatoid arthritis patients treated with adalimumab

	Hazard ratio (95 % CI)	p value
Age (≥ vs. <65 years old)	2.38 (0.80–7.05)	0.119
Gender (female vs. male)	0.53 (0.18–1.58)	0.258
Chronic pulmonary disease (yes vs. no)	2.14 (0.79–5.76)	0.133
Steinbrocker's radiographic stage (III/IV vs. I/II)	3.76 (1.03–7.30)	0.045

PCP Pneumocystis jirovecii pneumonia, *CI* confidence interval

Chronic pulmonary disease = interstitial pneumonia, bronchiectasis, chronic obstructive pulmonary diseases, bronchial asthma, middle lobe syndrome, old pulmonary tuberculosis

therapy for PCP patients without HIV infection has not been established [25]. All patients except for one in this study received adjunctive corticosteroid therapy with various treatment durations and dosages, including intravenous methylprednisolone pulse therapy. Nineteen out of 21 PCP patients who used infliximab and nine out of 15 PCP patients who used etanercept used adjunctive corticosteroid therapy as well [11, 12]. Pareja etal. [26] retrospectively analyzed the clinical courses of 30 cases of severe PCP without HIV infection, among which 16 cases who received high doses of adjunctive corticosteroid therapy presented a good clinical outcome. Considering the intense inflammatory response to the organism in non-HIV PCP patients [25] and the favorable effectiveness of adjunctive corticosteroid therapy in previous studies, it is necessary to consider treatment with corticosteroids for PCP patients with RA who show hypoxemia at presentation or during their clinical courses.

In the present study, using the Cox proportional-hazards analysis, Steinbrocker's radiographic stage III or IV was identified as a statistically significant risk factor for the development of PCP in patients receiving adalimumab. Although there was no significant difference in Steinbrocker's functional class, it is plausible that advanced radiographic stages associated with decreased physical function contributed to the development of PCP. Steinbrocker's functional class may be less sensitive to the detection of such differences in physical function. On the other hand, older age was a significant risk factor in another Cox proportional-hazards regression analysis after excluding those who received TMP/SMX or aerosolized pentamidine for prophylaxis at least three months from the non-PCP group. The different results from the Cox proportional-hazards regression analyses can be explained by the fact that nine out of 14 patients given prophylaxis were aged 65 or older. Pulmonary diseases were not significant risk factors for PCP in either Cox proportional-hazards analysis, perhaps because of the small number of PCP cases enrolled.

None of the 17 patients had received prophylaxis for PCP. Vananuvat etal. [27] conducted a retrospective cohort study for patients with connective tissue diseases (CTD) who were at risk for PCP in order to examine the effectiveness of primary prophylaxis with TMP/SMX and the incidence of adverse drug reactions (ADR) of TMP/SMX. Six patients without and none with prophylaxis developed PCP; the overall incidence rate was 4.3 % and the relative risk reduction was 100 %. Five patients (8.5 %) developed ADR: four had drug eruptions and one had mild hepatitis. These data indicate that TMP/SMX can be used effectively for primary prophylaxis against PCP.

There are definite limitations to our study. First, we included definite and presumptive cases of PCP in our analysis. It has been well documented that the microscopic detection of *P. jirovecii* is difficult in non-HIV PCP [28, 29], as confirmed in this and our previous studies. To increase the specificity of the diagnosis of PCP without detecting the organism microscopically, we utilized composite diagnostic criteria, including clinical symptoms, laboratory tests, radiological findings, and the clinical

course. Kameda etal. found no difference in clinical characteristics of PCP in RA patients between definite PCP (i.e., acute-onset diffuse interstitial lung disease and microscopic positivity for *P. jirovecii* or positivity in both PCR test and BDG) and probable PCP (acute-onset diffuse interstitial lung disease and positivity in either PCR test or BDG) [24]. Their data support the use of composite diagnostic criteria for PCP in patients with RA. Second, we had only 17 RA patients with PCP, which decreased the sensitivity of the Cox proportional-hazards analysis for detecting statistically significant risk factors. Third, a higher incidence of PCP in Japanese RA patients receiving TNF antagonists and their risk factors have gained widespead recognition in the past few years by Japanese rheumatologists who use TNF antagonists; this may have affected the characteristics of the patients who were treated with adalimumab. For example, we found a significant difference in the daily dose of PSL between the PCP and non-PCP groups in our previous two studies, but not in this study.

In summary, the results of this study show that PCP is a serious complication in patients with RA who receive treatment with adalimumab. The majority of the patients developed PCP early in the course of adalimumab treatment and progressed to respiratory failure. Treating physicians should therefore take prophylaxis with TMP/SMX or other agents into consideration in RA patients with a high risk for PCP. Careful monitoring of clinical manifestations and laboratory tests for early diagnosis and treatment of PCP are strongly recommended.

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ORIGINAL ARTICLE

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

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Abstract
Objective The associations between elevated levels of serum Krebs von den Lungen-6 (KL-6) and treatment of rheumatoid arthritis (RA) with tumor necrosis factor (TNF) inhibitors were investigated in five Japanese clinical trials.

Methods Percentages and incidence rates were calculated for elevated serum KL-6 levels. Adverse events associated with elevated levels of serum KL-6 were investigated.

Results In RISING, a clinical trial for infliximab, 15.6 % of the enrolled patients met criterion B (KL-6 ≥500 U/ml and >1.5-fold increase over the baseline value) by week 54. In HIKARI, 7.8 % of the certolizumab pegol (CZP) group and 0 % of the placebo group met criterion B during the double-blind (DB) period ($p = 0.003$). In J-RAPID, 8.4 % of the methotrexate (MTX) + CZP and

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3.9 % of the MTX + placebo groups met criterion B during the DB period. In GO-MONO, 1.8 % of the golimumab (GLM) and 1.3 % of the placebo groups met criterion B during the DB period. In GO-FORTH, 7.1 % of the MTX + GLM and 0 % of the MTX + placebo groups met criteron B during the DB period ($p = 0.017$). No adverse events accompanied the elevation of serum KL-6 levels in 95.7 % of these patients.

Conclusion Serum KL-6 levels may increase during anti-TNF therapy without significant clinical events. In these patients, continuing treatment with TNF inhibitors under careful observation is a reasonable option.

Keywords Biological disease modifying antirheumatic drug · KL-6 · Rheumatoid arthritis · Interstitial pneumonia · *Pneumocystis jirovecii* pneumonia

Introduction

During the last decade, the introduction of tumor necrosis factor (TNF) inhibitors for the treatment of rheumatoid arthritis (RA) has completely changed the treatment strategy and management of this intractable disease. In Japan, four TNF inhibitors have been approved for the treatment of RA and are widely used in clinical practice: infliximab (IFX) in 2003, etanercept (ETN) in 2005, adalimumab (ADA) in 2008, and golimumab (GLM) in 2011. Certolizumab pegol (CZP) is now under clinical development, and phase 3 and phase 2/3 trials have already been completed. For IFX, ETN, and ADA, post-marketing surveillance (PMS) programs have revealed short-term safety profiles of these biological disease-modifying antirheumatic drugs (DMARDs) in Japanese RA patients [1, 2]. Infection was the most frequently reported adverse drug reaction for IFX and ETN, and the second most for ADA. About half of these infectious events developed in the respiratory system. The results of the PMS and other clinical studies indicated that clinically important pulmonary infections in Japanese RA patients given TNF inhibitors encompassed bacterial pneumonia, tuberculosis, and *Pneumocystis jirovecii* pneumonia (PCP) [1–4].

The Krebs von den Lungen-6 (KL-6) antigen is a mucinous high-molecular-weight glycoprotein primarily derived from a lung adenocarcinoma cell line and classified as a cluster 9 mucin-1 of lung tumors and differentiation antigens [5]. KL-6 is produced by type II alveolar epithelial cells and is reported to be elevated in patients with idiopathic interstitial pneumonia (IIP), interstitial pneumonia (IP) associated with collagen diseases, other interstitial lung diseases, PCP, and malignancies [6–14]. Among the clinical trials for biological DMARDs, the "impact on Radiographic and clinical response of Infliximab therapy concomitant with methotrexate in patients with rheumatoid arthritis by the trough Serum level in the dose-escalatING study" (the RISING study) [15] systematically measured serum KL-6 levels for the first time. In a report to the Pharmaceuticals and Medical Devices Agency of Japan, the RISING study describes its findings of an abnormal elevation of this serum marker in RA patients receiving IFX without any development or exacerbation of pulmonary disease or malignancies. However, no peer review journal report of the details of the elevation of serum KL-6 has been published, and it has not been determined whether this adverse event is truly related to treatment with IFX, is common among treatment with TNF inhibitors or other biological DMARDs, or is related to treatment with MTX. A report of elevated serum KL-6 levels in three RA patients treated with ADA has been recently published [16].

In Japan, the measurement of serum KL-6 levels is an officially approved and widely used clinical laboratory test in the field of rheumatology. The Japan College of Rheumatology convened an ad hoc committee for the safety of biological DMARDs to investigate the abnormal elevation of serum KL-6 levels in RA patients given biological DMARDs. The committee implemented two studies to investigate this issue, one for clinical trial data and the other for clinical practice data. The results from the analyses of the clinical trial data are reported here; those from the study of clinical practice data will be reported separately.

Patients and methods

Clinical trials

Serum KL-6 levels were measured in clinical trials in Japan for three TNF inhibitors, IFX, CZP, and GLM. For our analyses, we utilized the RISING study for IFX [15], a pHase 3 study to assess the effIcacy, safety and phamacoKinetics of CDP870 (CZP) in rheumatoid ArthRItis patients (HIKARI; ClininalTrials.gov, NCT00791921) and the Japanese RA PreventIon of structural Damage (J-RAPID; ClininalTrials.gov, NCT00791999) for CZP, and the GO-MONO [17] and GO-FORTH [18] for GLM. Although the study period of these clinical trials lasted more than 1 year, including extension studies for CZP and GLM, our study evaluated data only for 54 weeks of the RISING study and 52 weeks for the other four clinical trials. The measurement of serum KL-6 levels was originally scheduled in RISING, HIKARI, and J-RAPID, and the protocols and informed consent forms of GO-MONO and GO-FORTH were amended during these clinical trials to measure serum KL-6.

RISING study

The first clinical trial of biological DMARDs that included serum KL-6 as a laboratory test was RISING [15]. Electronic Supplementary Material (ESM) Fig. S1 shows the design and ESM Table S1 shows the baseline characteristics of the patients enrolled in RISING. In this trial, established RA patients with mean disease duration of 8.2 years received IFX for 54 weeks with concomitant stable doses of MTX. After a screening period, 327 patients entered the open-label period (3 mg/kg at weeks 0, 2, and 6) and 307 patients proceeded to the double-blind (DB) trial period. These patients were randomly allocated to 3, 6, or 10 mg/kg IFX groups and received an infusion of IFX every 8 weeks through to week 54. The percentage of patients with elevated serum KL-6 levels higher than 500 U/ml at baseline was 3.1 %.

HIKARI and J-RAPID

Two clinical trials for CZP have been implemented in Japan—HIKARI (phase III) and J-RAPID (phase II/III). In HIKARI, 230 RA patients who had an inadequate response to or who were intolerant of MTX were DB randomly assigned either to placebo or CZP without MTX for 24 weeks, followed by an open extension period until approval of the drug (ESM Fig. S2-A). In J-RAPID, 316 RA patients who had inadequate response to treatment with MTX were DB randomly allocated either to the placebo or to one of three dosage groups of CZP with concomitant MTX at stable dosages for 24 weeks, followed by an open extension period until approval of the drug (ESM Fig. S2-B). Both trials allowed for early escape (EE) at week 16 if a patient did not meet ACR20 response criteria at both weeks 12 and 14. Demographic characteristics of the enrolled patients to these trials were similar, with a mean disease duration of about 6 years (ESM Table S2). The percentage of patients with IP in HIKARI was 12.2 % and in J-RAPID 2.2 %. The percentage of patients with elevated serum KL-6 levels of $\geq$500 U/ml at baseline was 8.8–11.2 % in HIKARI and 2.4–6.1 % in J-RAPID (ESM Table S3).

GO-MONO and GO-FORTH

Serum KL-6 levels were evaluated in two randomized controlled trials of GLM implemented in Japan—GO-MONO [17] and GO-FORTH [18]. Patients who participated in either of these studies and who gave consent for measurements of serum KL-6 level were enrolled in our study. In GO-MONO, 308 RA patients who had an inadequate response to DMARDs were DB randomly assigned to placebo, GLM 50 mg, or GLM 100 mg monotherapy for 16 weeks, followed by an open extension period until week 116 (ESM Fig. S3-A). In GO-FORTH, 261 RA patients who had an inadequate response to MTX were DB randomly assigned to placebo, GLM 50 mg, or GLM 100 mg with concomitant MTX at stable dosages for 24 weeks, followed by an open extension period until week 152 (ESM Fig. S3-B). Baseline characteristics of the enrolled patients are summarized in Table ESM S4. The mean disease duration of the enrolled patients was about 9 years and patients with IP were not eligible for either study. At baseline for GO-MONO, 3.8 % of the patients in the GLM 50 mg group, 0 % in the GLM 100 mg group, and 1.3 % in the placebo group had KL-6 levels of >500 U/ml. In GO-FORTH, 2.9 % of the patients in the MTX + GLM 50 mg group, 0 % in the MTX + GLM 100 mg group, and 4.2 % in the MTX + placebo group had KL-6 levels of >500 U/ml (ESM Table S5).

Data collection

The chairperson (M.H.) and the committee members (A.T., T.A., M.D., S.H., H.N., and Y.S.) reviewed the data on elevations of serum KL-6 levels in the five Japanese clinical trials. M.H. and A.T. requested that the pharmaceutical companies Mitsubishi Tanabe Pharma Corporation, Otsuka Pharmaceutical, UCB Japan, and Janssen Pharmaceutical provide data in a systematic and predetermined format. The data were analyzed by the committee only; the pharmaceutical companies were not involved in data analysis. The committee did not have direct access to the database of the clinical trials. The final version of this report was reviewed by the pharmaceutical companies to enable data validation.

Measurement of serum KL-6 levels

Serum KL-6 levels were centrally measured in each clinical trial. In the RISING study, serum KL-6 levels were measured at weeks 0, 2, and then every 4 weeks until week 54. In HIKARI and J-RAPID, serum KL-6 levels were measured at weeks 0 and 1, every other week (EOW) from weeks 2 to 16, and at weeks 20 and 24 during the double-blind trial period, and every 4 weeks from weeks 28 to 52. Serum KL-6 levels were retrospectively measured at weeks 0, 12, 24, 36, and 52 for GO-MONO and GO-FORTH using stored serum samples. Serum KL-6 levels were available for 250 and 212 patients in GO-MONO and GO-FORTH, respectively. Serum KL-6 levels were also measured after week 52 in the clinical trials for CZP and GLM, but these data were not analyzed for our study. Serum KL-6 levels were measured using the Picolumi KL-6 kit (Eidia Co., Tokyo, Japan) in all five clinical trials.

Definition of elevated/reduced serum KL-6 levels

Criteria A, B, and C for the elevation of serum KL-6 levels were developed by the committee and are shown in Table 1. We defined three criteria based on the serum KL-6 value at the initiation of TNF inhibitor therapy and the maximum value thereafter because some patients with RA have elevated serum KL-6 levels due to concurrent pulmonary diseases at baseline. In this study, our primary focus was criterion B. We also defined criterion R for the significant reduction of serum KL-6 levels in RA patients after achieving criterion B (Table 1).

Association of elevated serum KL-6 level with pulmonary events

We analyzed the association of elevated serum KL-6 levels with pulmonary events through week 54 for IFX and week 52 for CZP and GLM. Pulmonary events of this study were defined using preferred terms of the MedDRA ver 12.0 including PCP (10064108), interstitial lung disease (10022611), pulmonary fibrosis (10037383), and pulmonary interstitial emphysema syndrome (10037415). In this study, we used the diagnosis of pulmonary events that were made during the clinical trials by the original investigators. Newly diagnosed or exacerbated pulmonary events between 4 weeks before and 4 weeks after the first elevation of serum KL-6 levels meeting criterion B were counted.

Statistical analysis

Percentages and incidence rates were calculated per 100 patient-years (PY) for patients who met the criteria for elevated serum KL-6 levels. Denominators were: 327 patients who received open-label treatment in RISING; full-analysis-set patients for whom data on KL-6 were available in HIKARI (230 patients) and J-RAPID (316 patients); patients who gave informed consent to serum KL-6 level measurements and for whom available data were available in GO-MONO (250 patients) and GO-FORTH (212 patients).

Because RISING did not have a placebo group and three different doses of IFX were compared for 54 weeks, the primary and secondary endpoints of our study for RISING were percentage and incidence rates per 100 PY of patients who met criterion B by week 54, respectively. Taking the time points when treatments were changed for open extension periods in HIKARI, J-RAPID, GO-MONO, and GO-FORTH into account (ESM Figs. S2, S3), we defined the primary endpoints as percentages of patients who met criterion B by week 28 for HIKARI and J-RAPID, by week 16 for GO-MONO, and by week 24 for GO-FORTH. Secondary endpoints for these four trials were percentages of patients who met criteria A and C and incidence rates per 100 PY of patients who met criteria A, B, and C by the same time points given above, and percentages and incidence rates per 100 PY of patients who met criteria A, B, and C by week 52 in each trial. Percentages among treatment groups were compared using the Fisher's exact probability test for the primary endpoints, but statistical comparisons were not calculated for secondary endpoints. In clinical trials comparing different dosage groups, the TNF inhibitor groups combined were first compared with the placebo group. If a significant difference was observed, each dosage group was then compared with the placebo group. We took these measures to avoid type I errors derived from multiple comparisons.

Ethics

The study protocols of the five clinical trials were approved by the local institutional review board of each study institution and were carried out in accordance with the Helsinki Declaration and Good Clinical Practice. In GO-MONO and GO-FORTH, patients provided additional informed consent after amendment of the study protocols to measure serum KL-6 levels using stored serum samples.

Table 1 Criteria for the elevation or reduction of serum KL-6 levels

Criteria	Definition
A	≥500 U/ml and ≥1.25-fold higher than baseline value
B	≥500 U/ml and ≥1.5-fold higher than baseline value
C	≥1,000 U/ml and ≥3-fold higher than baseline value
R	Decrease in serum KL-6 levels to <500 U/ml or less than [baseline + 0.5 × (maximum baseline)] after achieving criterion B and reaching the maximum level of a patient

KL-6 Krebs von den Lungen-6 antigen

Criteria A, B, and C are for the elevation of serum KL-6 levels, and criterion R is for the reduction of serum KL-6 levels after achieving criterion B and reaching the maximum level of a patient

Results

Elevation of serum KL-6 levels in RISING

Among the 327 patients who received open-label treatment with IFX, the percentage (incidence rate/100 PY) of patients by week 54 who met criterion A was 18.7 % (20.0/100 PY), criterion B 15.6 % (16.7/100 PY), and criterion C 1.5 % (1.6/100 PY) (Table 2). The percentages of patients meeting all three criteria in the 3 mg/kg group were not significantly different from those in the 6 or 10 mg/kg groups.

Table 2 Percentage and incidence rate/100 PY of patients meeting the criteria for elevated serum KL-6 levels at least one time by week 54 in RISING

Treatment group	Number of patients	Percentage and incidence rate		
		Criterion A	Criterion B	Criterion C
IFX (3 mg/kg)	99	16.2 % (16.6/100 PY)	14.1 % (14.5/100 PY)	1.0 % (1.0/100 PY)
IFX (6 mg/kg)	104	21.2 % (21.6/100 PY)	15.4 % (15.7/100 PY)	1.0 % (0.98/100 PY)
IFX (10 mg/kg)	104	18.3 % (18.4/100 PY)	16.3 % (16.5/100 PY)	2.9 % (2.9/100 PY)
All patients	327	18.7 % (20.0/100 PY)	15.6 % (16.7/100 PY)	1.5 % (1.6/100 PY)

Criteria A, B, and C for elevation of serum KL-6 levels are defined in Table 1. Among 327 patients who received open-label treatment with IFX (3 mg/kg), 20 patients did not enter the double-blind (DB) period. No significant difference exists in percentages of the patients meeting the three criteria in the 3 mg/kg group compared to the 6 or 10 mg/kg groups by the Fisher's exact probability test. Lengths of exposure were 96.4 PY for the 3 mg/kg IFX group, 101.7 PY for 6 mg/kg IFX group, 103.2 PY for 10 mg/kg IFX group, and 304.6 PY for all patients

IFX infliximab, *PY* patient-year

We analyzed the association between elevated serum KL-6 levels and the predefined pulmonary events described in "Patients and methods". Of the 51 cases meeting criterion B by week 54, three pulmonary events in three patients were reported (ESM Table S6). The serum KL-6 level of a suspected case of PCP at week 12 (withdrawn from the trial before entering the DB period) increased from 269 (week 0) to 996 U/ml (week 14), that of a patient who developed IP at week 6 (withdrawn from the trial before entering the DB period) increased from 468 (week 0) to 935 U/ml (week 6), and that of a patient developing IP at week 50 increased from 205 (week 0) to 1,470 U/ml (week 50). The remaining 48 patients did not develop any of the predefined pulmonary events, and we could not identify other specific reasons, including malignancy, for the elevated KL-6 levels in these patients.

Changes in serum KL-6 levels over time in RA patients meeting criterion B ($n = 51$) are shown in Fig. 1. In 29 (60.4 %) of the 48 RA patients who met criterion B without developing a predefined pulmonary event, serum KL-6 levels spontaneously decreased to meet criterion R by week 54. Of these 48 RA patients, 33 had serum KL-6 data available after reaching their maximum level of whom 29 (87.9 %) met criterion R by week 54.

Elevation of serum KL-6 levels in HIKARI

In HIKARI, patients who entered EE received 200 mg of CZP EOW on and after week 16, while treatments of patients who did not enter EE were changed at week 28 (ESM Fig. S2-A). We therefore performed on-drug analysis for weeks 0–28: the exposure period of patients who entered EE at week 16 included only the first 16 weeks in their originally allocated treatment group. The exposure period of patients who did not enter EE was 28 weeks or until withdrawal from the trial, whichever came first. Between weeks 0 and 28, 16 (13.8 %) of the patients who received CZP 200 mg without MTX satisfied criterion A and 9 (7.8 %) satisfied criterion B, while 4 (3.5 %) and 0 % of patients who received placebo without MTX met criteria A and B, respectively ($p = 0.009$ for criterion A; $p = 0.003$ for criterion B vs. placebo group by the Fisher's exact probability test) (Table 3). By week 52, of the 219 patients, 12.8 % (19.3/100 PY) met criterion A, 9.2 % (13.8/100 PY) met criterion B, and 1.4 % (2.1/100 PY) met criterion C. For this 52-week analysis, the exposure period of patients who were initially assigned to the placebo group included only the period of time they received CZP, that of patients who were assigned to the CZP 200 mg group was counted from weeks 0 to 52, and that of patients who were withdrawn from the clinical trial before week 52 included only the period before withdrawal.

We analyzed the association between elevated serum KL-6 levels and the occurrence of the defined pulmonary events described in "Patients and methods". One case of IP and two cases of PCP were reported among the 21 cases meeting criterion B by week 52. The serum KL-6 levels of the patient who developed IP at week 50 (CZP 200 mg group) increased from 428 (week 0) to 663 U/ml (week 52), those of the patient who developed PCP at week 6 (CZP 200 mg group) increased from 945 (week 0) to 3,610 U/ml (week 6), and those of the patient who developed PCP at week 24 (placebo group, but receiving CZP 200 mg at the development of PCP) increased from 383 (week 0) to 1,600 U/ml (week 30). The remaining 18 patients did not develop the predefined pulmonary events nor could we identify other specific reasons, including malignancy, for the observed elevation in KL-6 levels in these patients.

Changes in serum KL-6 levels in 6 patients in the placebo group and 15 in the CZP 200 mg group meeting criterion B are shown in Fig. 2. All patients from the placebo group met criterion B after their treatments were changed to 200 mg of CZP. In 7 (38.9 %) of the 18 RA patients who met criterion B without developing any of the

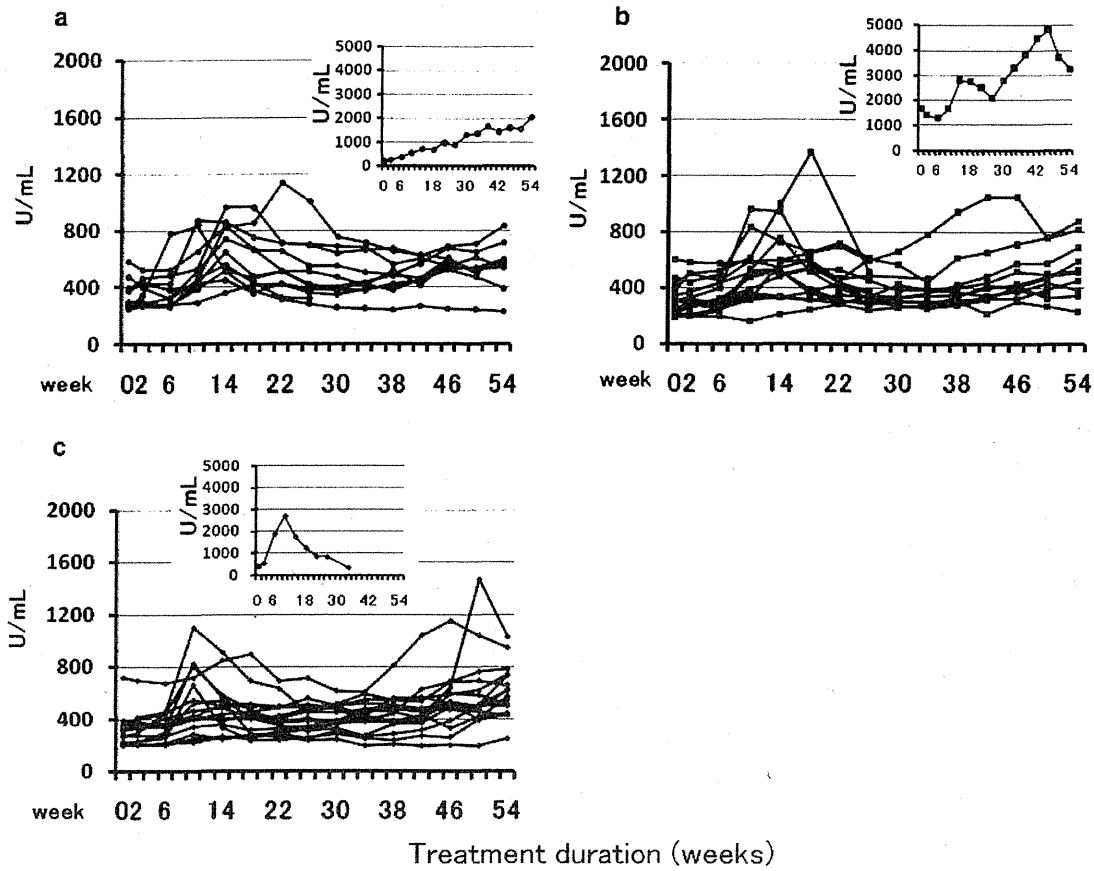

Fig. 1 Changes in serum Krebs von den Lungen-6 (KL-6) antigen levels over time in 51 rheumatoid arthritis (RA) patients who met criterion B at least one time by week 54 in the RISING study. Data from the infliximab (IFX) 3 mg/kg group ($n = 14$) and from patients who did not enter the double-blind (DB) period ($n = 4$) (**a**), data from the IFX 6 mg/kg group ($n = 16$) (**b**), and data from the IFX 10 mg/kg group ($n = 17$) (**c**) are shown separately. Data from patients whose maximum serum KL-6 level reached >2,000 U/ml are shown in the *insets* of the figures. For definition of criterion B, see Table 1

Table 3 Percentage and incidence rate/100 PY of patients who met the criteria for elevated serum KL-6 levels at least one time by week 28 in HIKARI

Treatment group	Number of patients[a]	Percentage and incidence rate		
		Criterion A	Criterion B	Criterion C
CZP (200 mg)	116	13.8 % (29.9/100 PY)*	7.8 % (16.8/100 PY)**	1.7 % (3.7/100 PY)
Placebo	114	3.5 % (10.6/100 PY)	0 % (0.0/100 PY)	0 % (0.0/100 PY)

Criteria A, B, and C for elevation of serum KL-6 levels are defined in Table 1. The exposure period of patients who entered early escape (EE) at week 16 was considered to be 16 weeks. The exposure period of patients who did not enter EE was considered to be 28 weeks or until withdrawal from the trial. Lengths of exposure were 53.5 PY for the CZP 200 mg group and 37.8 PY for the placebo group

CZP certolizumab pegol

Significance: * $p = 0.009$, ** $p = 0.003$ (CZP vs. placebo groups, by the Fisher's exact probability test)

[a] All patients assigned to each group with available data for serum KL-6 levels were evaluated

predefined pulmonary events, serum KL-6 levels spontaneously decreased to meet criterion R by week 52. Of these 18 RA patients, 14 had serum KL-6 data available after reaching their maximum levels of whom 7 (50.0 %) met criterion R by week 52.

Elevation of serum KL-6 levels in J-RAPID

Patients in J-RAPID who entered EE received 200 mg of CZP EOW with MTX on and after week 16, while treatments of patients who did not enter EE were changed at

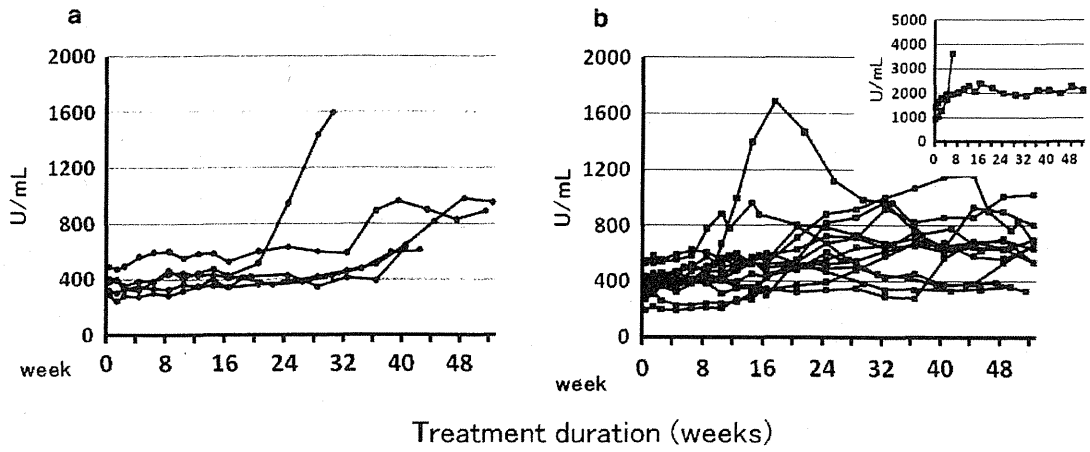

Fig. 2 Changes in serum KL-6 levels over time in 21 RA patients given certolizumab pegol (CZP) who met criterion B at least one time by week 52 in the HIKARI study. Data from the placebo group ($n = 6$) (**a**) and the CZP 200 mg group ($n = 15$) (**b**) are shown separately. The treatment for each patient was changed as described in ESM Fig. S2-A. Data from patients whose maximum serum KL-6 level surpassed 2,000 U/ml are shown in the *insets* of the figures

Table 4 Percentage and incidence rate/100 PY of patients who met the criteria for elevated serum KL-6 levels at least one time by week 28 in J-RAPID

Treatment group[a]	Number of patients[b]	Percentage and incidence rate		
		Criterion A	Criterion B	Criterion C
CZP (100 mg)	72	11.1 % (24.1/100 PY)	5.6 % (12.0/100 PY)	2.8 % (6.0/100 PY)
CZP (200 mg)	82	12.2 % (25.5/100 PY)	9.8 % (20.4/100 PY)	2.4 % (5.1/100 PY)
CZP (400 mg)	85	9.4 % (20.0/100 PY)	9.4 % (20.0/100 PY)	2.4 % (5.0/100 PY)
CZP (combined)	239	10.9 % (23.1/100 PY)	8.4 % (17.8/100 PY)	2.5 % (5.3/100 PY)
Placebo	77	6.5 % (18.0/100 PY)	3.9 % (10.8/100 PY)	0 % (0.0/100 PY)

Criteria A, B, and C for elevation of serum KL-6 levels are described in Table 1. The exposure period of patients who entered EE at week 16 was considered to be 16 weeks. The exposure period of patients who did not enter EE was taken to be 28 weeks or until withdrawal from the trial. Lengths of exposure were 33.2 PY for the MTX + CZP 100 mg group, 39.3 PY for the MTX + CZP 200 mg group, 39.9 PY for the MTX + CZP 400 mg group, and 27.7 PY for the MTX + placebo group. Percentages of the patients meeting the three criteria in the CZP groups combined did not differ significantly from the placebo group by the Fisher's exact probability test

MTX methotrexate

[a] All patients received placebo, CZP 100, 200, or 400 mg with concomitant MTX. CZP (combined) refers to the total of all CZP treatment group patients

[b] All patients who were assigned to each group with available data for serum KL-6 level were evaluated

week 28, the same as in HIKARI (ESM Fig. S2-B). We therefore performed on-drug analysis for weeks 0–28 as described for HIKARI. Between weeks 0 and 28, 4 (5.6 %) patients from the MTX + CZP 100 mg group, 8 (9.8 %) from the MTX + CZP 200 mg group, 8 (9.4 %) from the MTX + CZP 400 mg group, and 20 (8.4 %) from the MTX + CZP groups combined met criterion B, while 3 (3.9 %) patients from MTX + placebo group met criterion B (Table 4). No significant difference was found between the CZP groups combined and the placebo group. By week 52, of the 309 patients, 12.0 % (15.5/100 PY) met criterion A, 9.7 % (12.6/100 PY) met criterion B, and 2.6 % (3.4/100 PY) met criterion C. For this 52-week analysis, the exposure periods were the same as those described for HIKARI.

We analyzed the association between elevated serum KL-6 levels and the pulmonary events defined in "Patients and methods". Among the 32 cases meeting criterion B by week 52, no patients developed any of the predefined pulmonary events. We could not identify any other specific reasons, including malignancy, for the elevation of KL-6 serum levels in these 32 patients.

Changes in serum KL-6 levels in these 32 patients in the MTX + placebo (5 patients), the MTX + CZP 100 mg group (8), the MTX + CZP 200 mg group (9), and in the MTX + CZP 400 mg group (10) meeting criterion B are

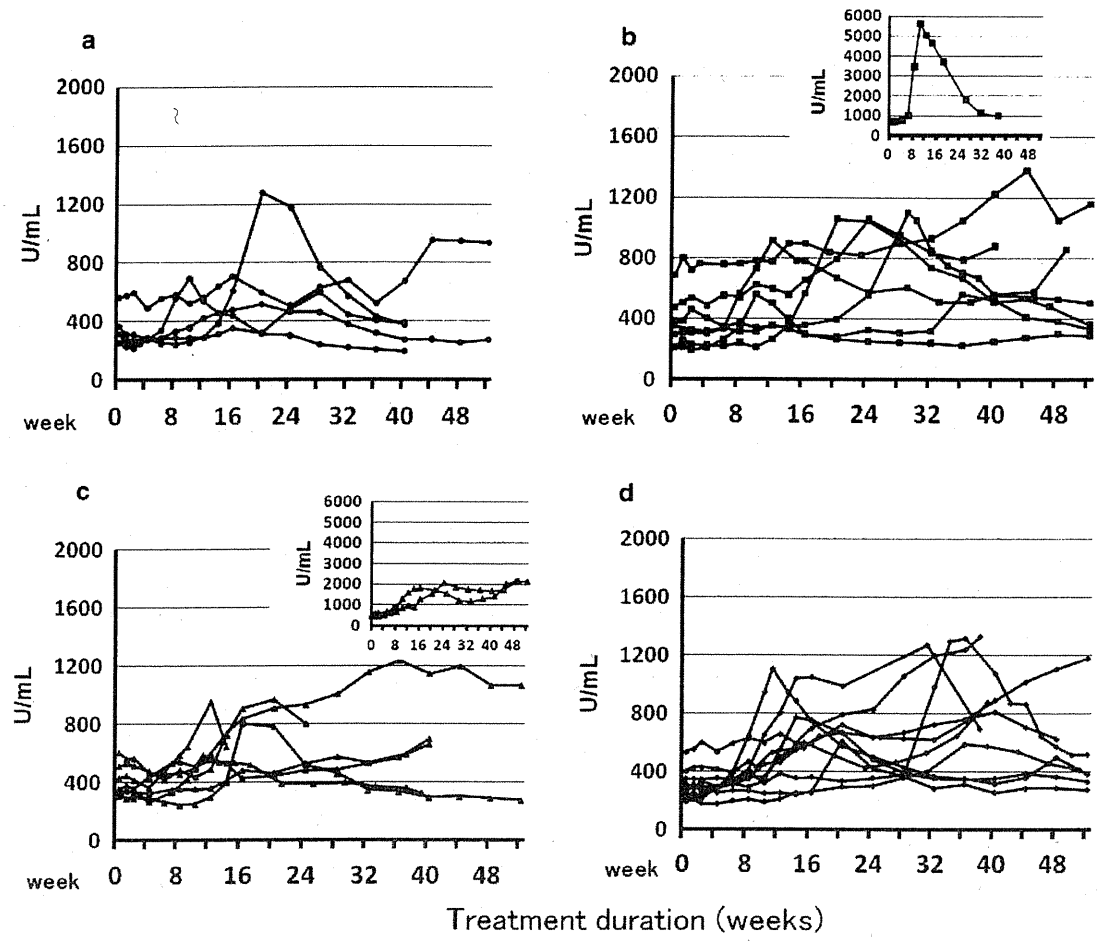

Fig. 3 Changes in serum KL-6 levels over time in 32 RA patients given CZP who met criterion B at least one time by week 52 in the J-RAPID study. Data from the methotrexate (MTX) + placebo group ($n = 5$) (**a**), MTX + CZP 100 mg group ($n = 8$) (**b**), MTX + CZP 200 mg group ($n = 9$) (**c**), and MTX + CZP 400 mg group ($n = 10$) (**d**) are shown separately. The treatment for each patient was changed as described in ESM Fig. S2-B. Data from patients whose maximum serum KL-6 level surpassed 2,000 U/ml are shown in the *insets* of the figures

depicted in Fig. 3. Three patients from the MTX + placebo group met criterion B while they were receiving placebo and 2 patients met criterion B after their treatments were changed to 200 mg of CZP. In 19 (59.4 %) of the 32 patients who met criterion B, serum KL-6 levels spontaneously decreased to meet criterion R by week 52. In these 32 RA patients, 27 had serum KL-6 data available after reaching their maximum level of whom 19 (70.4 %) met criterion R by week 52.

Elevation of serum KL-6 levels in GO-MONO

In GO-MONO, study blindness was maintained until week 16 and there was no EE. Patients from the placebo group started 50 mg GLM on and after week 16 (ESM Fig. S3). By week 16, 1 (1.3 %) patient in the GLM 50 mg group, 2 (2.2 %) patients in the GLM 100 mg group, 3 (1.8 %) patients in the GLM groups combined, and 1 (1.3 %) patient in the placebo group met criterion B (Table 5). No significant difference between the GLM groups combined and the placebo group was found. By week 52, of the 250 patients, 8.0 % (8.8/100 PY) met criterion A, 6.8 % (7.5/100 PY) met criterion B, and 0.8 % (0.9/100 PY) met criterion C. For this 52-week analysis, the exposure period of patients who were initially assigned to the placebo group was counted only for the period when they received GLM and the exposure period of patients who were assigned to the GLM groups was counted from weeks 0 to 52. The exposure period of patients who were withdrawn from the clinical trial before week 52 included only the period before withdrawal.

We analyzed the association between elevated serum KL-6 levels and the pulmonary events described in "Patients and methods". Among the 17 cases meeting

Table 5 Percentage and incidence rate/100 PY of patients who met the criteria for elevated serum KL-6 levels at least one time by week 16 in GO-MONO

Treatment group[a]	Number of patients[b]	Percentage and incidence rate		
		Criterion A	Criterion B	Criterion C
GLM (50 mg)	79	1.3 % (4.1/100 PY)	1.3 % (4.1/100 PY)	0.0 % (0.0/100 PY)
GLM (100 mg)	91	2.2 % (7.1/100 PY)	2.2 % (7.1/100 PY)	0.0 % (0.0/100 PY)
GLM (combined)	170	1.8 % (5.7/100 PY)	1.8 % (5.7/100 PY)	0.0 % (0.0/100 PY)
Placebo	80	1.3 % (4.1/100 PY)	1.3 % (4.1/100 PY)	0.0 % (0.0/100 PY)

Percentages of the patients meeting the three criteria in the GLM groups combined did not differ significantly from the placebo group by the Fisher's exact probability test. Criteria A, B, and C for elevation of serum KL-6 levels are described in Table 1. The exposure period of patients who were withdrawn from the trial before week 16 was counted only for the period until the withdrawal. Lengths of exposure were 24.5 PY for the GLM 50 mg group, 28.2 PY for the GLM 100 mg group, and 24.7 PY for the placebo group

GLM Golimumab

[a] GLM (combined) refers to the total of all GLM treatment group patients

[b] Number of patients who gave consent to measure serum KL-6 levels and had available data

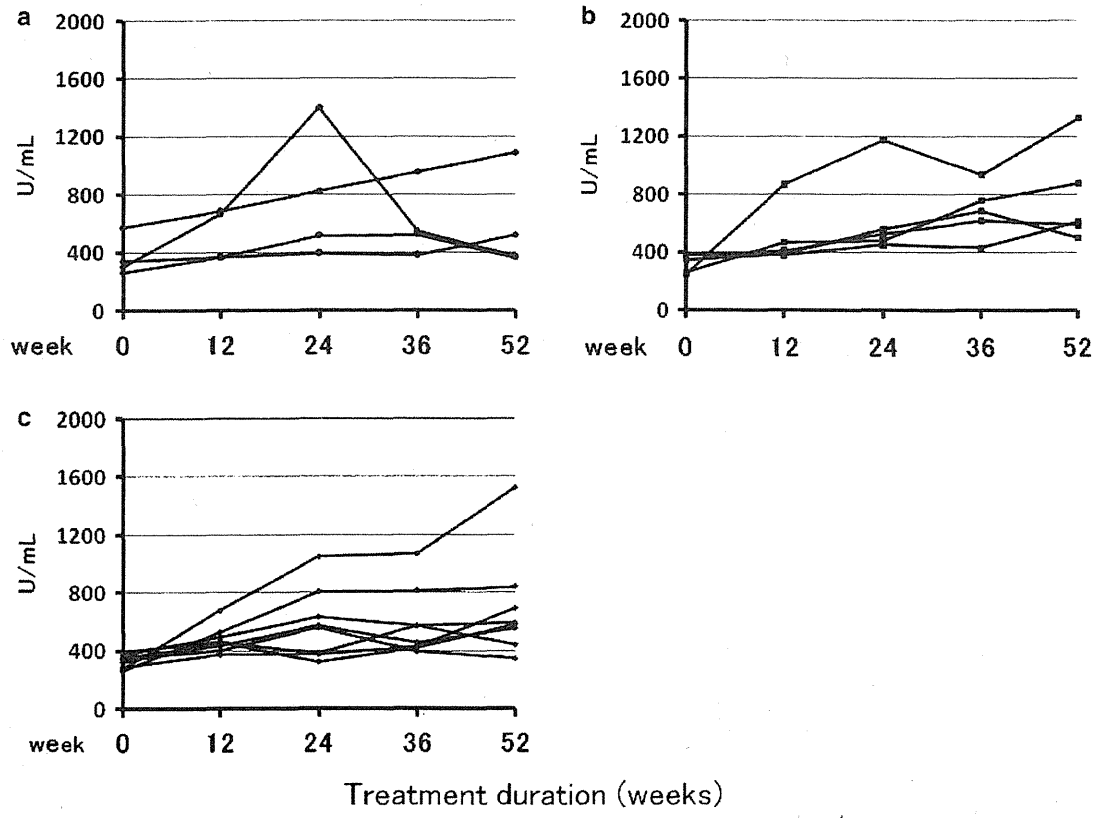

Fig. 4 Changes in serum KL-6 levels over time in 17 RA patients given golimumab (GLM) who met criterion B at least one time by week 52 in the GO-MONO study. Data from the placebo group ($n = 4$) (**a**), GLM 50 mg group ($n = 5$) (**b**), and GLM 100 mg group ($n = 8$) (**c**) are shown separately. The treatment for each patient was changed as described in ESM Fig. S3

criterion B by week 52, no patients developed the predefined pulmonary events, but one case of organizing pneumonia was reported by week 52. We could not identify other specific reasons, including malignancy, for the elevation of KL-6 serum levels in these 17 patients.

Changes in serum KL-6 levels over time in these 17 RA patients meeting criterion B in the placebo group (4 patients), the GLM 50 mg group (5), and the GLM 100 mg group (8) are shown in Fig. 4. One patient from the placebo group met criterion B when receiving placebo, while 3

patients met criterion B after their treatments were changed to 50 mg of GLM. Serum KL-6 levels spontaneously decreased to meet criterion R by week 52 in 6 (35.3 %) of the 17 RA patients. Of these 17 RA patients, 7 had serum KL-6 data available after reaching their maximum level of whom 6 (85.7 %) met criterion R by week 52.

Elevation of serum KL-6 levels in GO-FORTH

Patients in GO-FORTH who entered EE at week 16 from the MTX + placebo group received 50 mg of GLM with MTX and the MTX + GLM 50 mg group received 100 mg. All patients in the MTX + placebo group who did not enter EE received 50 mg of GLM at week 24 (ESM Fig. S3). We therefore performed on-drug analysis for weeks 0–24. The exposure period of patients who entered EE at week 16 included only the first 16 weeks in their originally allocated treatment group. The exposure period of patients who did not enter EE was 24 weeks or until withdrawal from the trial, whichever came first. Between weeks 0 and 24, 3 (4.4 %) patients from the MTX + GLM 50 mg group, 7 (9.7 %) patients from the MTX + GLM 100 mg group, and 10 (7.1 %) patients from MTX + GLM groups combined satisfied criterion B, while no patients from the MTX + placebo group met criterion B ($p = 0.017$ for GLM groups combined and $p = 0.013$ for GLM 100 mg group using the Fisher's exact probability test) (Table 6). By week 52, of the 212 patients, 9.4 % (10.9/100 PY) met criterion A, 9.0 % (10.4/100 PY) met criterion B, and 0 % (0/100 PY) met criterion C. For this 52-week analysis, the exposure periods were the same as those described for GO-MONO.

We analyzed the association between elevated serum KL-6 levels and pulmonary events as defined in "Patients and methods". Among the 19 cases meeting criterion B by week 52, no patients developed the predefined pulmonary events. We could not identify other specific reasons, including malignancy, for the elevation of KL-6 serum levels in these patients.

Changes in serum KL-6 levels over time in these 19 RA patients meeting criterion B in the MTX + placebo (6 patients), the MTX + GLM 50 mg group (5), and the MTX + GLM 100 mg group (8) are depicted in Fig. 5. All patients from the MTX + placebo group met criterion B after their treatments were changed to 50 mg of GLM with MTX. Serum KL-6 levels spontaneously decreased to meet criterion R by week 52 in ten (52.6 %) of these 19 RA patients. Of these 19 RA patients, 11 had serum KL-6 data available after reaching their maximum level of whom 10 (90.9 %) met criterion R by week 52.

Discussion

The major findings of our study are that: (1) the use of TNF inhibitors was significantly associated with elevated serum KL-6 levels compared to placebo in two of the four clinical trials studied; (2) 8.0–18.6 % of RA patients given TNF inhibitors met criterion A, 6.8–15.3 % met criterion B, and 0–2.6 % met criterion C by year 1; (3) 134 (95.7 %) of 140 patients who met criterion B did not have any other specific clinical reasons for the elevation of serum KL-6 levels and the serum marker spontaneously decreased in the majority of these patients.

While we have presented data for serum KL-6 levels during treatment with TNF inhibitors from five clinical trials in a similar manner in our attempt to compare these trials, it should be noted that the frequency of the

Table 6 Percentage and incidence rate/100 PY of patients who met the criteria for elevated serum KL-6 levels at least one time by week 24 in GO-FORTH

Treatment group[a]	Number of patients[b]	Percentage and incidence rate		
		Criterion A	Criterion B	Criterion C
GLM (50 mg)	68	4.4 % (9.8/100 PY)	4.4 % (9.8/100 PY)	0.0 % (0.0/100 PY)
GLM (100 mg)	72	9.7 % (20.9/100 PY)	9.7 % (20.9/100 PY)**	0.0 % (0.0/100 PY)
GLM (combined)	140	7.1 % (15.6/100 PY)	7.1 % (15.6/100 PY)*	0.0 % (0.0/100 PY)
Placebo	72	1.4 % (3.4/100 PY)	0.0 % (0.0/100 PY)	0.0 % (0.0/100 PY)

Criteria A, B, and C for elevation of serum KL-6 levels are defined in Table 1. The exposure period of patients who entered EE at week 16 was considered to be 16 weeks. The exposure period of patients who did not enter EE was considered to be 24 weeks or until withdrawal from the trial. Lengths of exposure were 30.7 PY for the MTX + GLM 50 mg group, 33.5 PY for the MTX + GLM 100 mg group, and 29.8 PY for the MTX + placebo group

Significance * $p = 0.017$ (the GLM groups combined vs. placebo group), ** $p = 0.013$ (the GLM 100 mg vs. placebo group) by the Fisher's exact probability test

[a] In GO-FORTH, patients received placebo, GLM 50 mg, or GLM 100 mg with concomitant MTX. GLM (combined) refers to the total of all GLM treatment group patients

[b] Number of patients who gave consent to measure serum KL-6 levels and for whom data were available

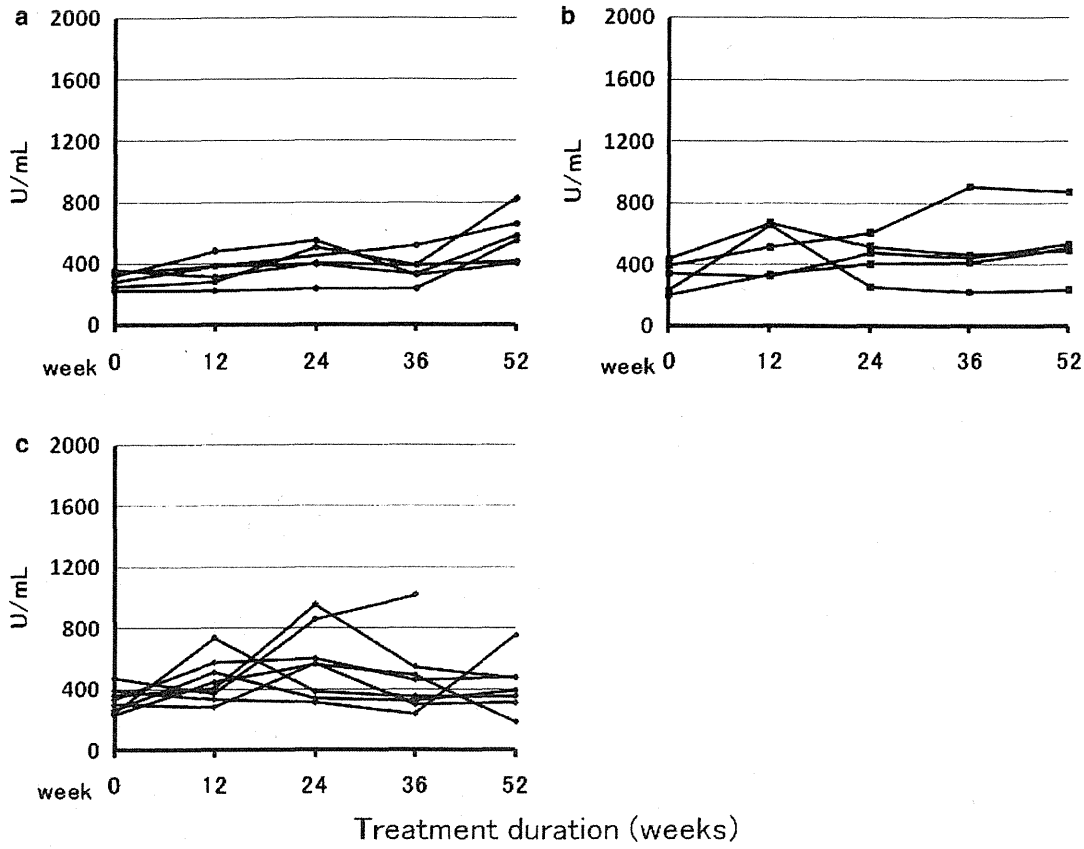

Fig. 5 Changes in serum KL-6 levels over time in 19 RA patients given GLM who met criterion B at least one time by week 52 in the GO-FORTH study are shown. Data from the MTX + placebo group ($n = 6$) (**a**), MTX + GLM 50 mg group ($n = 5$) (**b**), and MTX + GLM 100 mg group ($n = 8$) (**c**) are shown separately. The treatment for each patient was changed as described in ESM Fig. S3

measurement of serum KL-6 levels differed among these clinical trials and that the designs of the trials varied in terms of length of placebo-controlled and DB periods, EE, and treatment changes after the DB periods (ESM Figs. S1, S2, S3). The frequency of KL-6 measurement was highest in HIKARI and J-RAPID, followed by RISING, GO-MONO, and GO-FORTH. Because the spontaneous reduction of serum KL-6 levels was observed in all clinical trials, less frequent measurements may result in lower percentages of patients meeting the criteria for elevation of serum KL-6 levels. It should also be noted that the patient populations were different among the five clinical trials because of their mutually independent eligibility criteria. These differences should be considered when our findings are interpreted.

In HIKARI and J-RAPID, serum levels of pulmonary surfactant protein D (SP-D), another marker for interstitial lung disease [19], were retrospectively measured and visually compared with changes in serum KL-6 levels over time in some patients who met criterion B and had relatively high serum KL-6 levels. Both serum markers increased in parallel in about half of these patients (data not shown). Serum lactate dehydrogenase levels were also measured in these patients, but these did not correlate with serum KL-6 levels. These data indicate that the elevation of serum KL-6 levels in RA patients given TNF inhibitors was not a non-specific fluctuation, but may be associated with subclinical interstitial changes in the lung or that TNF may have a physiological role in regulatory pathways common to both serum markers.

In Japan, PCP is one of the most clinically important opportunistic infection in RA patients during treatment with TNF inhibitors [3, 4, 20]. Because serum KL-6 levels frequently increase in patients with PCP [10], the elevation of serum KL-6 levels in RA patients given TNF inhibitors may be explained by subclinical PCP. However, chest X-ray or thoracic computed tomography has not supported this hypothesis (data not shown). Serum levels of beta-D-glucan (BDG), a marker for PCP [21], were prospectively measured in HIKARI and J-RAPID. Of 21 patients who met criterion B by week 52 in HIKARI, an abnormal elevation of serum BDG levels (≥11.0 pg/ml) was observed