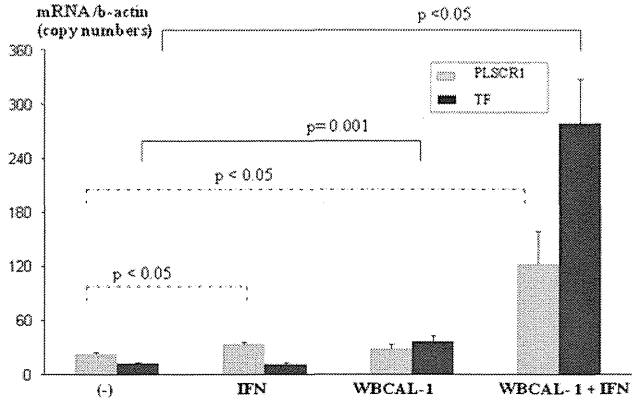

Fig. 3 Phospholipid scramblase 1 (PLSCR1) messenger RNA (mRNA) induction by monoclonal antiphospholipid antibody. RAW 267.4 cells were incubated in the presence or absence of interferon (IFN)-α2a, followed by β2-glycoprotein (GPI)-dependent monoclonal anticardiolipin antibody (WBCAL-1). PLSCR1 and tissue factor (TF) gene expression were evaluated by real-time polymerase chain reaction (PCR). Values are expressed as copy numbers of PLSCR1 or TF/β-actin mRNA. Data represent the mean ± standard error of three independent experiments. *P* values *above the dotted line* refer to PLSCR1 and *above the solid line* to TF

mechanisms have been related to the aPL-mediated thrombotic complications, including inhibition of natural anticoagulant systems, impairment of fibrinolytic activity, and the direct effect of aPL on cell functions, but the precise mechanism of thrombosis production in APS is not yet clear. The interaction between aPL and procoagulant cells is necessary for the onset of thrombosis, and the exposition of PS on the cell surface is essential for this binding.

In normal quiescent cells, the distribution of phospholipids over the two halves of the cellular membrane is asymmetric with neutral/polar-phospholipids, phosphatidylcholine and sphingomyelin confined to the outer monolayer, and amino-phospholipids, with PS and phosphatidylethanolamine being almost exclusively present in the inner monolayer. This asymmetric distribution of phospholipids is maintained by lipid transporters termed "flippases," which transport lipids from the outer to the inner leaflet of membranes, and "floppases," which catalyze the outward transport of phospholipids. However, injury and/or cell activation leads to a rapid redistribution of phospholipids in both directions that is catalyzed by scramblase and results in cell-surface exposition of PS and phosphatidylethanolamine [18, 19]. Externalization of PS has been associated with pathological phenomena, including hemostasis and thrombosis [20]. PLSCR1 is a member of the scramblase family of lipid transporters and has been detected in a variety of cells and tissues [8].

In this study, we found elevated levels of PLSCR1 mRNA in monocytes from patients with APS in the absence of acute thrombosis. We previously reported increased levels of PLSCR1 mRNA in circulating monocytes in SLE patients with a prothrombotic state, suggesting that PLSCR1 up-regulation was related to the thrombophilic state [9]. APS was originally described in patients with SLE and is recognized as a systemic disease, rather than merely a thrombotic disorder, which has several common clinical manifestations with SLE. On the other hand, thrombotic events are frequent manifestations in SLE. Patients with SLE and/or APS have a thrombophilic state related not only to the presence of aPL but also to other thrombotic risk factors and some predisposing conditions. In both clinical conditions, the increased levels of PLSCR1 may contribute to the prothrombotic tendency.

There are two aspects of APS: vascular manifestations and pregnancy complications. They have substantial differences in the aPL profile and clinical features. The obstetric complications in APS cannot be explained solely by thrombosis, and additional pathogenic mechanisms such as acute inflammatory-mediated tissue damage and complement activation have been reported [21]. In our APS patients, we observed higher levels of PLSCR1 mRNA in patients with pregnancy morbidity, as the only clinical feature of APS, compared with those with thrombosis. Mechanisms involved in the pathogenesis of obstetric and thrombotic complications in APS are partly different, and up-regulation of PLSCR1 may play a major role in the obstetric subgroup. Another possible explanation of this finding may be related to the difference in antithrombotic treatments. APS patients with thrombotic manifestations received anticoagulation combined with antiplatelet drugs, whereas obstetric APS patients did not. However, the small number of women with pregnancy complications only does not allow definitive conclusions.

PLSCR1 mRNA levels did not correlate with titers of aCL, anti-β2-GPI antibodies, aPS/PT, or D-dimer plasma levels in patients with APS, implying that PLSCR1 up-regulation is due to the total biological alteration in APS.

In our previous study in SLE patients [9], PS externalization was relatively increased in the surface of monocytes in patients compared with healthy controls. In the study reported here, we failed to demonstrate statistically significant differences in PS exposure in monocytes between patients with APS and healthy controls. PLSCR1 is not the sole determinant of PS externalization. The appearance of PS on the cell surface is related to multiple mechanisms, such as inhibition of lipid transporters involved in maintaining integrity of the membrane in quiescent cells. Recently, the transmembrane protein 16 F (TMEM16F) was reported to be an essential component for calcium-dependent scramblase activity for PS. A mutation at a splice acceptor site of the gene encoding TMEM16F was found in a patient with Scott syndrome, which results from a defect in phospholipid scrambling activity [22].

PLSCR1 expression is induced by IFN [23, 24] or by various growth factors [25–27]. We observed induction of PLSCR1 mRNA by IFN-α in cultured monocyte cell lines and in human PBMC. INF-targeted genes have been associated with the pathogenesis of autoimmune diseases, and IFN-α up-regulation may be also linked to thrombophilia through the overexpression of PLSCR1. Expression of type 1 IFN-induced genes, including PLSCR1, was increased in PBMC in patients with APS [28].

Evidence has supported the role of the TF pathway in the pathogenesis of aPL-related thrombosis [29], and we demonstrated TF up-regulation by aPL [5]. In the study reported here, we showed that IFN-α markedly increased TF expression mediated by β2-GPI-dependent monoclonal aCL antibody in RAW 264.7 cells. The effect of WBCAL-1 on PLSCR1 expression was also evaluated on the THP-1 cell line. However, THP-1 cells showed a lower response to WBCAL-1 with regard to TF induction compared with RAW 264.7 cells. Therefore, the effect of IFN-α2a/WBCAL-1 combination on PLSCR1 induction could not be fully evaluated in THP-1 cells.

Increased PLSCR1 expression in APS may be related to IFN-α up-regulation and represent one of the contributing factors in the prothrombotic tendency in APS. Although the regulation of PLSCR1 and PS exposure may be strong drivers toward thrombosis, patients do not develop thrombosis unless an additional trigger is present.

In conclusion, our findings demonstrated PLSCR1 up-regulation in patients with APS. Additional studies will increase our understanding of the molecular effects of this protein.

Acknowledgments The authors thank Miki Aoto for technical support. This work was supported by the Japanese Ministry of Health, Labour and Welfare, the Japanese Ministry of Education, Culture, Sports, Science and Technology (MTX), and the Japanese Society for the promotion of the Science (JSPS). Olga Amengual is funded by JSPS/MEXT (project number 21-40106).

Conflict of interest None.

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Mod Rheumatol (2013) 23:284–296
DOI 10.1007/s10165-012-0657-2

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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Received: 16 January 2012 / Accepted: 19 April 2012 / Published online: 17 May 2012

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

Electronic supplementary material The online version of this article (doi:10.1007/s10165-012-0657-2) contains supplementary material, which is available to authorized users.

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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

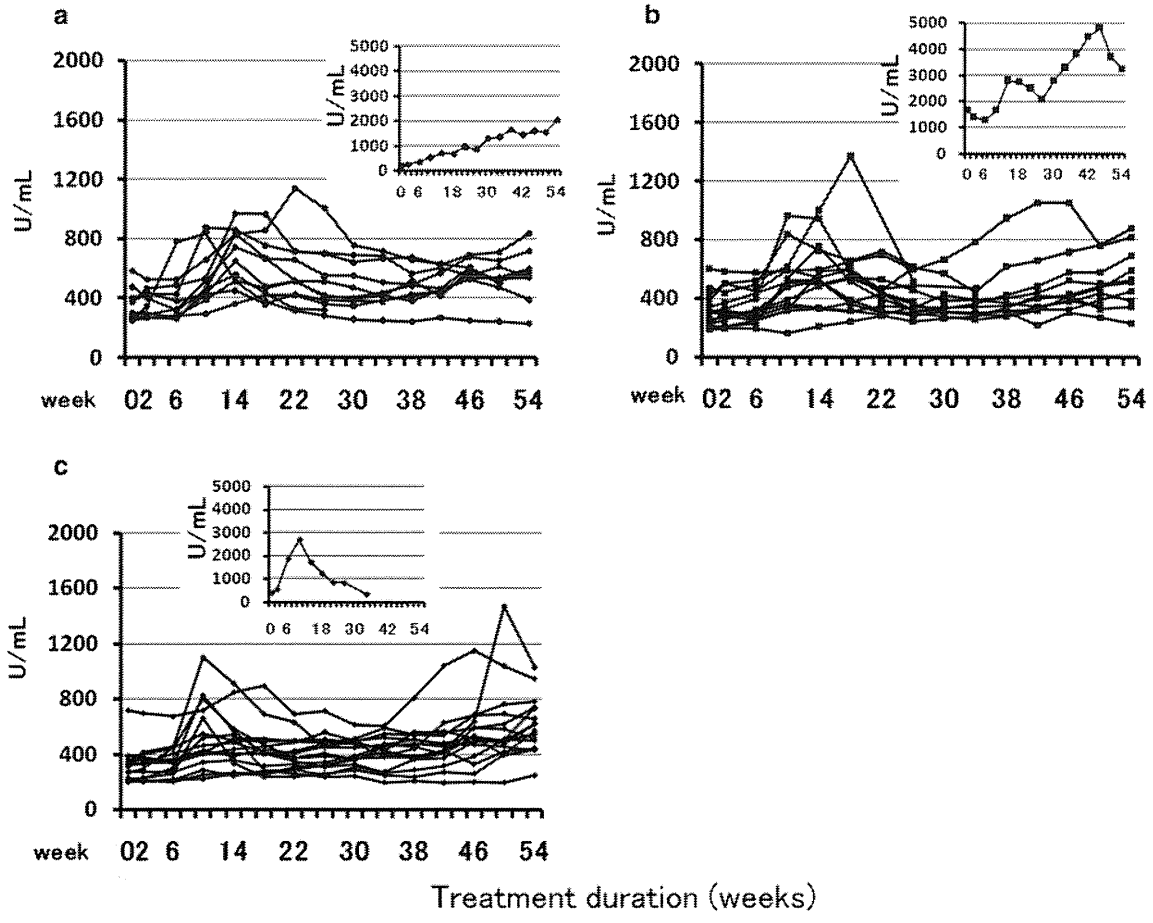

Fig. 1 Changes in serum Krebs von den Lungen-6 (KL-6) antigen levels over time in 51 rheumatoid arthritis (RA) patients who met criterion B at least one time by week 54 in the RISING study. Data from the infliximab (IFX) 3 mg/kg group ($n = 14$) and from patients who did not enter the double-blind (DB) 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

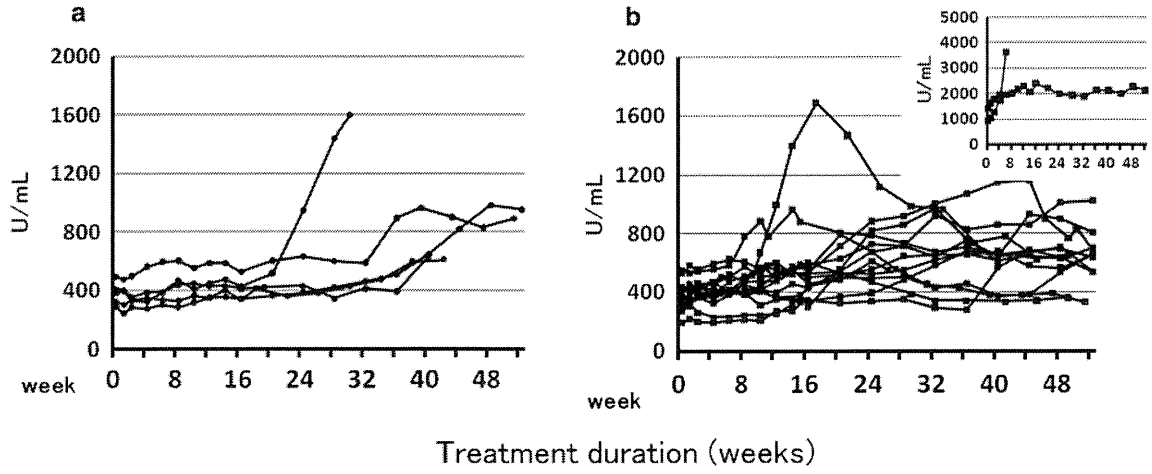

Fig. 2 Changes in serum KL-6 levels over time in 21 RA patients given certolizumab pegol (CZP) who met criterion B at least one time by week 52 in the HIKARI study. Data from the placebo group ($n = 6$) (**a**) and the CZP 200 mg group ($n = 15$) (**b**) are shown separately. The treatment for each patient 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

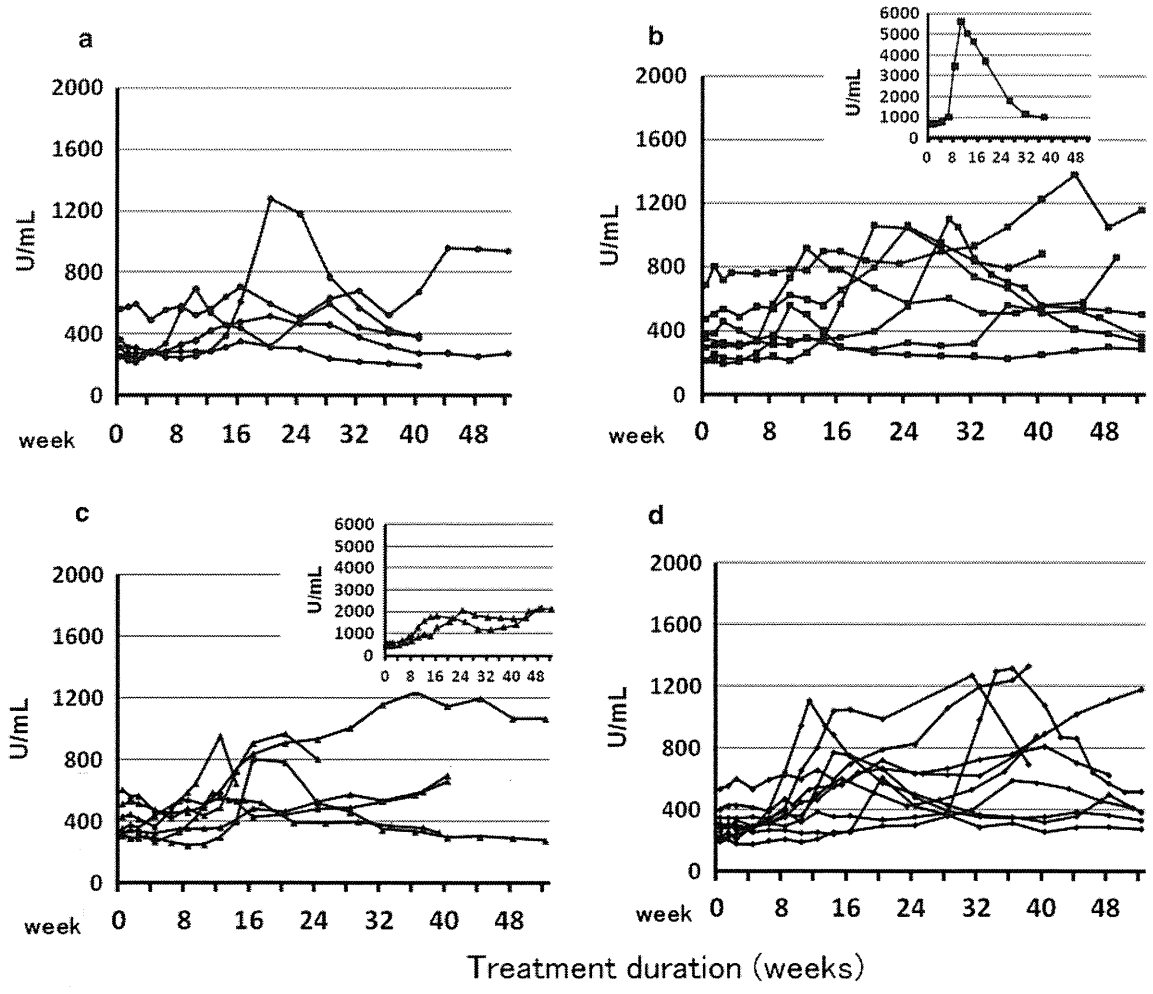

Fig. 3 Changes in serum KL-6 levels over time in 32 RA patients given CZP who met criterion B at least one time by week 52 in the J-RAPID study. Data from the methotrexate (MTX) + placebo group ($n = 5$) (**a**), MTX + CZP 100 mg group ($n = 8$) (**b**), MTX + CZP 200 mg group ($n = 9$) (**c**), and 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

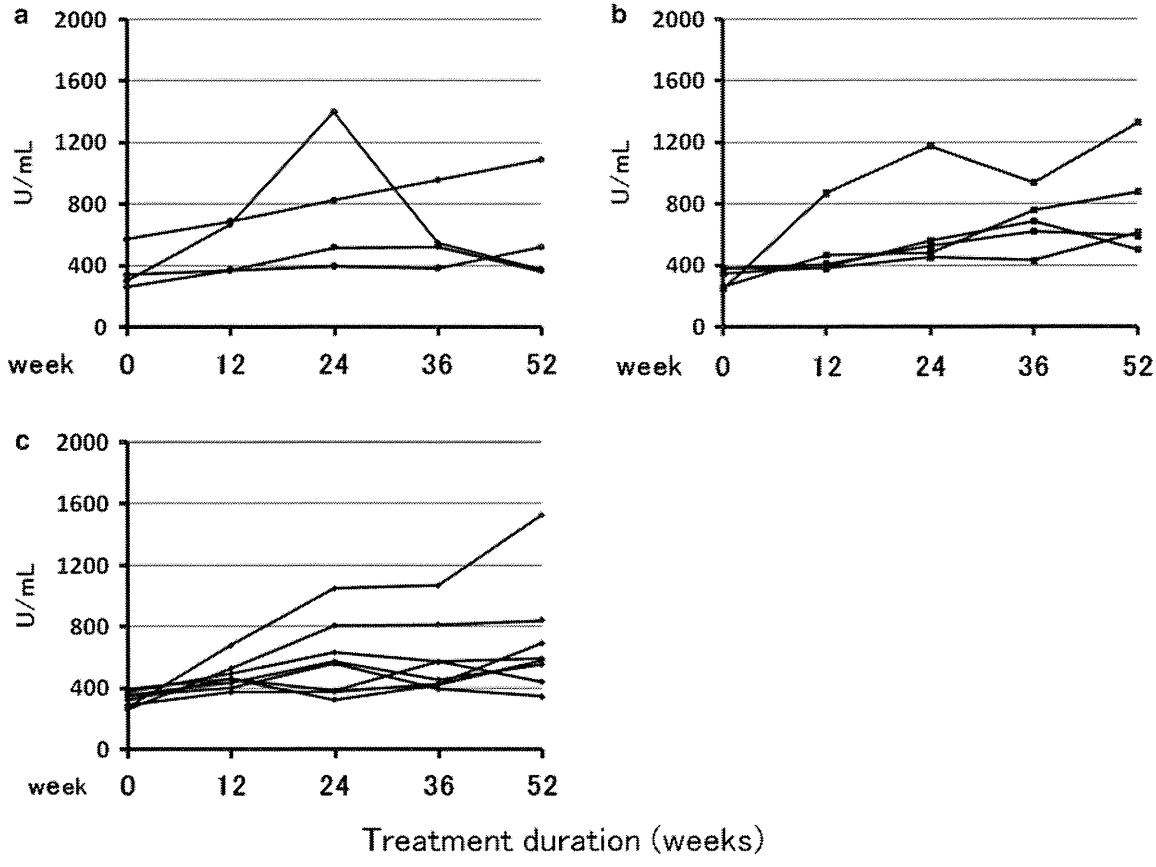

Fig. 4 Changes in serum KL-6 levels over time in 17 RA patients given golimumab (GLM) who met criterion B at least one time by week 52 in the GO-MONO study. Data from the placebo group ($n = 4$) (**a**), GLM 50 mg group ($n = 5$) (**b**), and GLM 100 mg group ($n = 8$) (**c**) are shown separately. The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

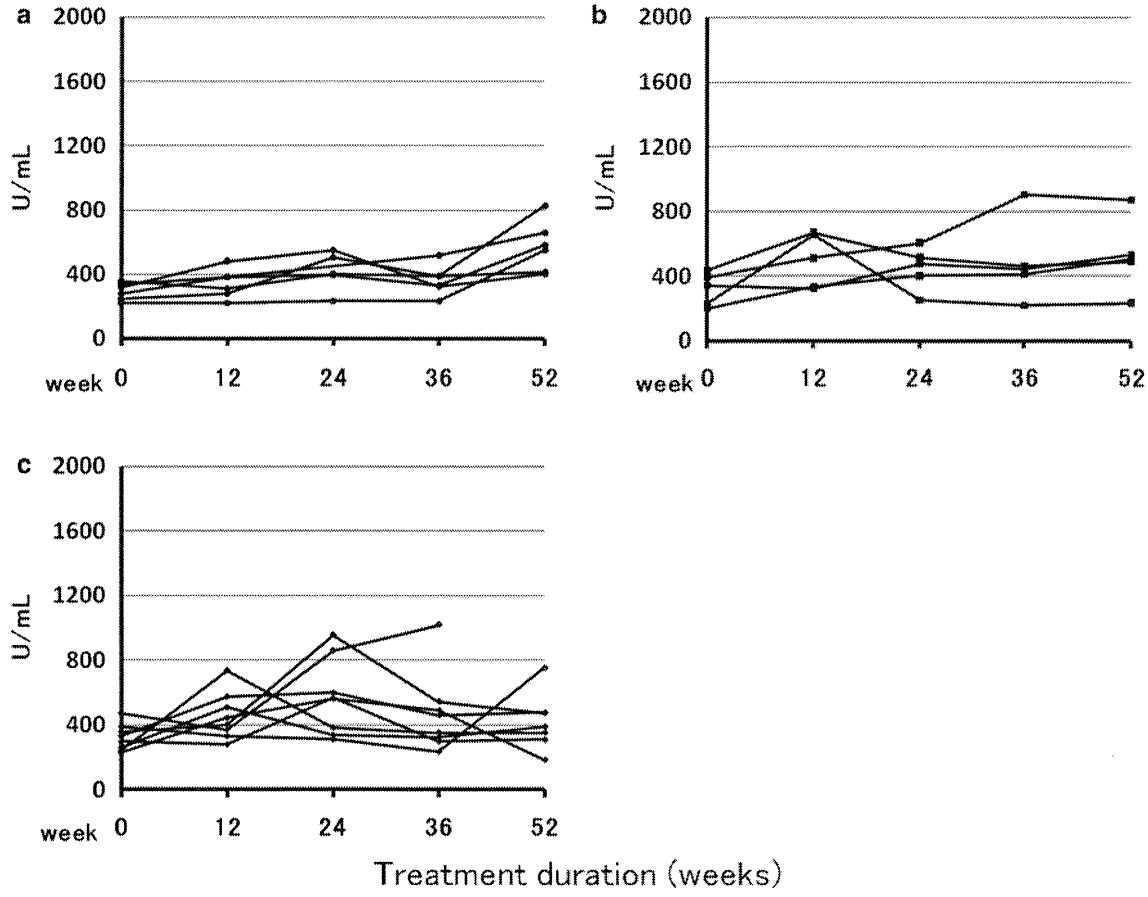

Fig. 5 Changes in serum KL-6 levels over time in 19 RA patients given GLM who met criterion B at least one time by week 52 in the GO-FORTH study are shown. Data from the MTX + placebo group ($n = 6$) (**a**), MTX + GLM 50 mg group ($n = 5$) (**b**), and MTX + GLM 100 mg group ($n = 8$) (**c**) are shown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

in only two (9.5 %) patients (peak values 14.4 and 32.7 pg/ml, respectively). The patient with a peak value of 14.4 pg/ml developed PCP with the simultaneous elevation of serum KL-6 and BDG levels. In J-RAPID, of 32 patients who met criterion B by week 52, only 3 (9.4 %) patients (peak values 11.5, 12.3, and 18.1 pg/ml, respectively) showed abnormal, but modest, elevations of serum BDG levels that were observed in parallel with an elevation of serum KL-6 levels. These data and the favorable clinical courses of these patients indicate that the possibility of subclinical PCP was quite low in the majority of patients meeting criterion B.

Some important clinical questions arise from our findings. First, do we have to stop treatment with TNF inhibitors in RA patients with elevated serum KL-6 levels? The answer is no. We should search for reasons for the elevation, such as PCP, IP, and malignancy, as the first step, but when these adverse events are not identified, continuing treatment with TNF inhibitors under careful observation is a reasonable option for RA patients who have shown good response to the treatment. Second, is it worthwhile to monitor KL-6 every 4 weeks during treatment with TNF inhibitors? When we used criterion B to define the elevation of serum KL-6 levels, KL-6 had low positive predictive values for PCP and IP in RISING (5.9 %), HIKARI (14.3 %), and J-RAPID (0 %) and high negative predictive values (99–100 %) in all three trials. However, in these studies, there were only three PCP and three IP patients among those who met criterion B and three IP patients among those who did not met criterion B; there is, therefore, a possibility that more stringent criteria would have better predictive abilities for these adverse events. To clarify the usefulness of monitoring KL-6 serum levels during treatment with TNF inhibitors, a specifically designed clinical study is required. Therefore, we cannot provide a definite answer for the second question from our present analysis.

The potential contribution of concomitant MTX to the elevation of serum KL-6 levels in RA patients given TNF inhibitors should be mentioned. When we combined the MTX + placebo groups from J-RAPID and GO-FORTH, 3 (2.0 %) of 149 patients given MTX + placebo met criterion B by week 24 or 28 without associated pulmonary events. In our retrospective study [22], 5 (10.6 %) of 47 RA patients given MTX without biological DMARDs met criterion B, and 4 of these (8.5 %) did not have any clinical reasons for the elevation of serum KL-6 levels. These data indicate that we should consider a potential contribution of MTX to the elevation of serum KL-6 levels during treatment with TNF inhibitor + MTX.

The mechanisms of the elevation of serum KL-6 levels in RA patients given TNF inhibitors remain to be determined. Little is known about the molecular mechanisms of KL-6 expression and its transport mechanism through the alveolar–capillary barrier. Further studies are required to clarify the roles of TNF in these processes in both physiological and pathological conditions.

In summary, the transient elevation of serum KL-6 levels in patients meeting criterion B, without accompanying specific clinical events, was observed in 6.8–15.6 % of RA patients treated with TNF inhibitors by year 1 in five clinical trials. Continuing treatment with TNF inhibitors under careful observation is a clinically reasonable option when serum KL-6 levels rise.

Acknowledgments This work was supported by a grant-in-aid from the Ministry of Health, Labor and Welfare, Japan (H23-meneki-sitei-016 to M. Harigai and N. Miyasaka), by a grant-in-aid for scientific research from the Japan Society for the Promotion of Science (no. 23590171 to M. Harigai and N. Miyasaka) and the grant from the Japanese Ministry of Education, Global Center of Excellence (GCOE) Program, International Research Center for Molecular Science in Tooth and Bone Diseases (to N. Miyasaka). We would like to thank Mitsubishi Tanabe Pharma Corporation, Otsuka Pharmaceutical, UCB Japan, and Janssen Pharmaceutical for providing data and replying to queries from the Ad Hoc Committee for Safety of Biological DMARDs of the Japan College of Rheumatology. We also thank Ms. Ryoko Sakai and Ms. Marie Yajima for their contributions to the statistical analyses and manuscript preparation, respectively.

Conflict of interest The Ad Hoc Committee for Safety of Biological DMARDs of the Japan College of Rheumatology did not receive any financial support from industries and independently investigated and discussed the issues and prepared this manuscript. M.H. has received research grants from Abbott, Bristol Myers Squibb, Chugai Pharmaceutical, Eisai Pharmaceutical, Janssen Pharmaceutical, Mitsubishi Tanabe Pharma Corporation, Takeda Pharmaceutical, and Pfizer and received consultant fees from Abbott, Bristol Myers Squibb, Chugai Pharmaceutical, and Janssen Pharmaceutical. T.A. has received research grants from Chugai Pharmaceutical and Takeda Pharmaceutical. H.K. has received honoraria from Abbott, Mitsubishi Tanabe Pharma Corporation, and Pfizer. Y.S. has received consultant fee from Abbott. T.K. has received consultancies, speaking fees and honoraria from Abbott, Bristol Myers Squibb, Chugai Pharmaceutical, Eisai Pharmaceutical, Mitsubishi Tanabe Pharma Corporation, Takeda Pharmaceutical, and Pfizer, Otsuka Pharmaceutical. N.M. has received research grants from Abbott, Astellas Pharmaceutical Banyu Pharmaceutical, Chugai Pharmaceutical, Daiichi Sankyo Pharmaceutical, Eisai Pharmaceutical, Janssen Pharmaceutical, Mitsubishi Tanabe Pharma Corporation, Takeda Pharmaceutical, and Teijin Pharmaceutical.

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Mod Rheumatol (2013) 23:297–303
DOI 10.1007/s10165-012-0658-1

ORIGINAL ARTICLE

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

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Received: 16 January 2012 / Accepted: 19 April 2012 / Published online: 10 May 2012

Abstract
Objective We investigated associations between treatment with methotrexate (MTX) or biological disease-modifying antirheumatic drugs (DMARDs) and elevation of serum Krebs von den Lungen-6 (KL-6) levels in Japanese patients with rheumatoid arthritis (RA).
Methods Using a standardized form, data were collected retrospectively from medical records and analyzed descriptively.
Results Of a total of 198 RA patients with KL-6 serum levels measured at initiation of treatment (month 0) and two or more times by month 12, 27 (17.9 %) of 151 RA patients treated with biological DMARDs, including infliximab, etanercept, adalimumab, and tocilizumab (the biological DMARDs group), and 5 (10.6 %) of 47 patients treated without biological DMARDs but with MTX (MTX group), met criterion B (max. KL-6 $\geq$500 U/ml and >1.5-fold from baseline) by 12 months. The majority of patients ($n = 28$) meeting criterion B had no apparent interstitial lung disease or malignancy. Of these 28 patients, 21 had serum KL-6 levels available after reaching their maximum level, and 13 (61.9 %) of the 21 then met criterion R [decrease to less than 500 U/ml or to less than (baseline + 0.5 × (maximum − baseline))] by month 12.
Conclusion Serum KL-6 levels may increase during treatment with MTX or these biological DMARDs without significant clinical events.

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Keywords Rheumatoid arthritis · Biological disease-modifying antirheumatic drug · KL-6

Introduction

Six biological disease-modifying antirheumatic drugs (DMARDs), infliximab (IFX), etanercept (ETN), adalimumab (ADA), tocilizumab (TCZ), abatacept, and golimumab, have been approved in Japan. Today, these biological DMARDs are widely used for treatment of rheumatoid arthritis (RA). Pulmonary diseases with interstitial lesion, including rheumatoid lung, drug-induced pulmonary injury, or *Pneumocystis jirovecii* pneumonia (PCP), sometimes develop during treatment of RA with biological DMARDs [1–4]. Better prognosis for affected patients would be provided by prompt diagnosis of these diseases [5–8].

Krebs von den Lungen-6 (KL-6) is a circulating high-molecular-weight glycoprotein recently classified in humans as a cluster 9 mucin-1 (MUC1) [9]. The serum level of KL-6 was reported to be elevated in patients with idiopathic interstitial pneumonia (IIP), interstitial pneumonia (IP) associated with collagen diseases, IP associated with drug allergy, PCP, other interstitial lung diseases, and malignancy [10–15]. Measurement of serum KL-6 levels is an officially approved laboratory test in Japan, widely used as an adjunctive diagnostic or monitoring tool for patients with interstitial lung diseases and patients with RA treated with MTX or biological DMARDs.

In the Impact on Radiographic and clinical response of Infliximab therapy concomitant with methotrexate in rheumatoid arthritis patients by the trough Serum level in the dose-escalatING (RISING) study [16], abnormal elevation of serum KL-6 levels in RA patients treated with IFX with no pulmonary diseases was seen. Considering the prevalent use of serum KL-6 levels as a laboratory test for RA patients in Japan, the Japan College of Rheumatology (JCR) convened the Ad Hoc Committee for Safety of Biological DMARDs to investigate the abnormal elevation of serum KL-6 levels in RA patients during treatment with biological DMARDs. The committee implemented two studies, one using clinical trial data and one clinical practice data. Here, we report the results from a retrospective analysis of clinical practice data.

Patients and methods

Data source

Criteria for admission to this study included those patients (1) meeting the 1987 American College of Rheumatology criteria for RA [17], (2) ≥20 years old, (3) willing to provide informed consent, (4) starting treatment with biological DMARDs (IFX, ETN, ADA, and TCZ) or MTX, and (5) having serum KL-6 levels measured at start of treatment (month 0) and two or more times by month 12. Exclusion criteria included those patients (1) withdrawing consent to join the study, or (2) found to be unsuitable for the study at the discretion of the attending physician. Data, including age, gender, comorbidities, past history, disease duration, laboratory data [KL-6, surfactant protein-D (SP-D), beta-D-glucan, white blood cell counts, lymphocyte cell counts, lactate dehydrogenase, C-reactive protein, and erythrocyte sedimentation rate] at months 0, 6, and 12, number of tender and swollen joints at months 0, 6, and 12, the patients' global assessments at months 0, 6, and 12, and treatments during the 12 months, were collected from medical records using a standardized case report form. We also collected data on pulmonary events, including PCP, IP, and others, and any malignancies during the 12 months. Data on pulmonary events included diagnosis, date of diagnosis and prognosis of pulmonary events, and laboratory data, results of imaging analyses, and treatments at onset of pulmonary events.

Measurement of serum KL-6 levels and the criteria for increase and decrease

Serum KL-6 levels were measured using Picolumi KL-6 (Eidia Co., Ltd., Tokyo, Japan) or Lumipulse Presto KL-6 (Eidia Co., Ltd., Tokyo, Japan) by in-house laboratories or outsourced, depending on the institution. Baseline serum KL-6 levels were measured within 1 month from initiation of treatment (month 0). We defined elevation of serum KL-6 levels as follows: criterion A (max. KL-6 ≥500 U/ml and >1.25-fold from baseline), criterion B (max. KL-6 ≥ 500 U/ml and >1.5-fold from baseline), and criterion C (max. KL-6 ≥1000 U/ml and >3.0-fold from baseline). Reduction of serum KL-6 levels was defined as a decrease to less than 500 U/ml or to less than [baseline + 0.5 × (maximum − baseline)] after meeting criterion B and achieving the maximum level of an individual patient (criterion R).

Statistical analysis

In consideration of the retrospective nature of our study and unavoidable biases in selection of enrolled patients, we restricted the statistics to descriptive analysis. The chi-square test was used to compare categorical variables.

Ethics

The guidelines of the Helsinki Declaration and the ethics guidelines for epidemiological research in Japan were

followed. The study protocol was approved by the Institutional Ethics Committee of the Tokyo Medical and Dental University Hospital (protocol #853 in 2010). The ethics guideline for epidemiological research in Japan requires notifying eligible RA patients of this study and allows implementation of this study without obtaining individual written informed consent. This study was publicized by leaflets or posters in outpatient clinics of each participating institute and on the website of the Department of Pharmacovigilance of the Tokyo Medical and Dental University. Patients were excluded from the study when they expressed unwillingness to participate in this study.

Results

Of the 198 patients enrolled in the study, 151 received biological DMARDs (IFX, 44; ETN, 50; ADA, 33; TCZ, 24) (biological DMARDs group) and 47 received MTX without biological DMARDs (MTX group). Baseline characteristics of the biological DMARDs and MTX group are given in Table 1. Patients from the MTX group were numerically older, had shorter disease duration for RA, had lower serum KL-6 levels at baseline, and used lower doses of MTX. The mean observation period was 11.2 months, and 87.9 % patients were observed for 12 months.

Overall, 41 of 198 patients met criterion A, 32 criterion B, and 8 criterion C, for elevation of serum KL-6 levels at least once by month 12. Percentages (incidence rate/100 PY) of 151 patients in the biological DMARDs group patients who met the criteria by month 12 were 21.9 % (23.1/100PY) for criterion A, 7.9 % (18.9/100PY) for criterion B, and 3.3 % (3.5/100PY) for criterion C. The percentages of patients who met criterion A or B in the biological DMARDs group were higher than those for the MTX group (21.9 versus 17.0 % for criterion A, 17.9 versus 10.6 % for criterion B, respectively), but lower for criterion C (3.3 versus 6.4 %, respectively) (Table 2). In the biological DMARDs group, patients treated with TCZ showed lower incidence of elevation of serum KL-6 levels compared with tumor necrosis factor (TNF) inhibitors (8.3 % in the TCZ group versus 24.4 % in the TNF inhibitor group for criterion A, 8.3 versus 19.7 % for criterion B, 0 versus 3.0 % for criterion C) (Table 3).

Baseline characteristics of the patients in the biological DMARDs group who did and did not meet criterion B are compared in Table 4. Those who met criterion B were numerically older, had higher percentages of past illnesses

Table 1 Characteristics of the enrolled rheumatoid arthritis patients

	Biological DMARDs group ($n = 151$)	MTX group ($n = 47$)
Gender (female) (%)	75.5	70.2
Mean age (years)	59.1 ± 13.3	63.3 ± 11.3
Mean disease duration for RA (months)	99.2 ± 110.2	40.6 ± 68.0
Comorbidity		
Interstitial pneumonia (%)	30.5	19.1
Other pulmonary disease (%)	9.9	21.3
Past illness		
PCP (%)	0.0	0.0
Malignancy (%)	6.6	8.5
Drug-induced pulmonary disease (%)	2.0	2.1
Others (%)	25.2	38.3
Clinical characteristics		
Baseline KL-6 (U/ml)	375.1 ± 346.8	276.9 ± 141.7
MTX use at month 0 (%)	64.2	100
Mean dose of MTX at month 0 (mg/week)	8.5 ± 2.5	5.6 ± 1.3
Mean dose of MTX at month 12 (mg/week)[a]	8.4 ± 2.4	8.2 ± 2.3
Corticosteroid use at month 0 (%)	51.0	40.4
Mean dose of corticosteroid at month 0 (mg/day) (prednisolone equivalent)	6.2 ± 3.4	7.9 ± 8.2
DMARDs other than MTX use at month 0 (%)	25.2	17.0

Values are mean ± SD, unless otherwise stated

RA rheumatoid arthritis, *PCP Pneumocystis jirovecii* pneumonia, *MTX* methotrexate, *DMARDs* disease-modifying antirheumatic drugs, *SD* standard deviation

[a] At month 12 for patients followed up for 12 months and at last observation for patients followed up for less than 12 months

Table 2 Number and percentage of rheumatoid arthritis patients meeting the criteria for elevation of serum KL-6 levels at least once by month 12

	Biological DMARDs group ($n = 151$)		MTX group ($n = 47$)	
	n	%	*n*	%
Criterion A	33	21.9	8	17.0
Criterion B	27	17.9	5	10.6
Criterion C	5	3.3	3	6.4

Criteria A, B, and C for elevation of serum KL-6 levels are defined in "Patients and methods" section

MTX methotrexate, *DMARDs* disease-modifying antirheumatic drugs

Table 3 Number and percentage of rheumatoid arthritis patients in the biological DMARDs group meeting the criteria for elevation of serum KL-6 levels at least once by month 12

	IFX ($n = 44$)		ETN ($n = 50$)		ADA ($n = 33$)		TNF inhibitors[a] Total ($n = 127$)		TCZ ($n = 24$)	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Criterion A	10	22.7	11	22.0	10	30.3	31	24.4	2	8.3
Criterion B	8	18.0	8	16.0	9	27.3	25	19.7	2	8.3
Criterion C	4	9.1	1	2.0	0	0	5	3.0	0	0

Criteria A, B, and C for elevation of serum KL-6 levels are defined in "Patients and methods" section

TCZ tocilizumab

[a] TNF inhibitors include infliximab (IFX), etanercept (ETN), and adalimumab (ADA)

other than PCP, IP, and drug-induced lung injury, and had a higher percentage of biological DMARDs-naïve patients at baseline.

We analyzed the association between elevation of serum KL-6 levels and pulmonary events. A total of 11 pulmonary events in 10 patients, including one PCP, two IP, and eight other pulmonary events such as bacterial pneumonia, pneumonia [not otherwise specified (NOS)], pulmonary tuberculosis, interstitial pulmonary shadow (ground-glass opacity and small nodules, NOS), patchy pulmonary shadow in right S10 (NOS), *Mycobacterium avium* complex, drug-induced pneumonia, and pleural effusion (NOS), were reported by month 12; however, no malignancies were reported. In two patients with bacterial pneumonia or interstitial pulmonary shadow, pulmonary lesions were depicted by chest X-ray. In the remaining eight patients, thoracic computed tomography identified the pulmonary lesions.

Five patients in the biological DMARDs group and one patient in the MTX group met both criteria A and B with one or two of these pulmonary events. In these cases, serum KL-6 levels were elevated around 1 month or less before or after the onset of the pulmonary events. When we restricted the pulmonary events to IP, PCP, and interstitial pulmonary shadow, to which elevation of serum KL-6 levels has been attributed in the literature, three patients in the biological DMARDs group and one patient in the MTX group met both criteria A and B with these pulmonary events. However, we could not identify any apparent reasons for elevation of serum KL-6 levels to the criterion B level in 24 patients (15.9 %) in the biological DMARDs group and four patients (8.5 %) in the MTX group.

Changes in serum KL-6 levels in patients who met criterion B without developing IP, PCP, or interstitial pulmonary shadow from the biological DMARDs group ($n = 24$) and MTX group ($n = 4$) are shown in Fig. 1. Of the 24 RA patients in the biological DMARDs group, 10 met criterion R by month 12 (Fig. 1a), 7 did not (Fig. 1b), and 7 lacked available data on serum KL-6 levels after meeting criterion B and reaching their maximum levels (Fig. 1c). Of the 4 RA patients in the MTX group, 3 met criterion R and 1 did not by month 12 (Fig. 1d).

Discussion

Our retrospective analysis of clinical practice data demonstrated that serum KL-6 levels increased without apparent clinical events in a substantial percentage of RA patients during treatment with MTX and/or biological DMARDs. These observations are in agreement with the report from our committee on data derived from clinical trials that were conducted in Japan.

We investigated whether the elevation of serum KL-6 levels in the DMARDs group was induced by biological DMARDs alone, or additively or synergistically by the