Table 2 Clinical characteristics and diagnostic indicators in rheumatoid arthritis patients at the onset of *Pneumocystis jirovecii* pneumonia (PCP)

Pt	Clinical manifestations	PaO_2 (Torr) [O_2 (l/min)][a]	CT findings	Response to treatments	PCR test	Serum β-D-glucan (pg/ml)
1	Fever/DOE	56 [3]	A	+	+	134[b]
2	Fever/cough/DOE	60.6 [0]	B	+	+	13.5[c]
3	Fever	SpO_2 86% [0]	B	+	+	14.6[c]
4	Fever/cough/DOE	SpO_2 86% [0]	B	+	+	21[c]
5	Fever/cough/DOE	57.6 [0]	A	+	+	<3.6[c]
6	Fever/cough/DOE	67.4 [0]	A	+	NA	14.2[c]
7	Fever/DOE	83.1 [0]	B	+	+	49.2[c]
8	Fever/DOE	68.5 [1]	C	+	+	20.2[c]
9	Fever/cough/DOE	66.3 [0]	A	+	+	27.4[c]
10	Fever/DOE	50 [0]	A	+	+	14.8[c]
11	Fever/DOE	64.8 [4]	B	+	NA	181[b]
12	Fever/DOE	49.4[10]	B	+	+	7.5[b]
13	Fever/DOE	SpO_2 90% [0]	A	+	+	43.3[c]
14	Fever/cough/DOE	55.6 [0]	B	+	NA[d]	187[c]
15	Fever/cough/DOE	61.7 [3]	B	+	NA	18.6[c]

Pt patient, *PaO_2* oxygen partial pressure in arterial blood, *cough* dry cough, *DOE* dyspnea on effort, *CT* thoracic computed tomography, *PCR test* polymerase chain reaction test for *P. jirovecii*, *NA* not assessed

[a] Oxygen therapy during the measurement of PaO_2 or oxygen saturation (SpO_2). SpO_2 was measured with a pulse oximeter

[b] Upper limit of normal (ULN) <20 pg/ml

[c] ULN <11 pg/ml

[d] *P. jirovecii* was detected microscopically as the cystic form in the bronchoalveolar lavage fluid

Although 14 patients responded well to these treatments and survived, one patient (patient 8) died. Patient 8 initially showed clinical and radiographic improvement arising from treatment for PCP with TMP/SMX and mPSL pulse therapy, but he later developed bacterial and fungal infections and finally died due to pulmonary hemorrhage 8 weeks after his admission.

While 13 patients were empirically treated with antibiotics and 4 patients were empirically treated with antifungal agents, cultures of respiratory samples from these patients before the commencement of these therapies revealed no causative bacteria, mycobacterium, or fungi. Anti-*Mycoplasma pneumoniae* antibody was positive in one of the five patients tested. Testing for urinary *Legionella* antigen was conducted in five patients and testing for serum *Aspergillus* antigen was conducted in eight patients; all results were negative. Detection of *Candida* antigen in the serum was positive at a low titer in two of the seven patients who were examined, but *Candida* species were not detected in sputum cultures from these two patients. Five patients were empirically treated with ganciclovir, but the *Cytomegalovirus* antigenemia assay was negative for all of them. These data, combined with other clinical and laboratory data and the GGO on the thoracic CT, suggested a low possibility of other infectious diseases in the PCP group patients.

Case–control study

To more precisely characterize the PCP group, we compared demographics, comorbidities, concomitant drugs, and laboratory data between the PCP and the non-PCP groups at the time of initiation of treatment with etanercept (Table 4). On univariate analysis, the PCP group was significantly older ($p < 0.001$), and had a significantly lower percentage of females ($p = 0.049$) and a significantly higher percentage of patients with lung diseases ($p = 0.002$) than the non-PCP group. Also, the PCP group was treated with significantly higher dosages of concomitant PSL ($p = 0.045$) and MTX ($p = 0.007$) than the non-PCP group.

Based on the results of the univariate analysis, we identified independent risk factors for PCP in RA patients treated with etanercept using Cox proportional hazard models. The results showed that the development of PCP was significantly associated with age (≥65 vs. <65 years) [hazard ratio (HR) 3.35, 95% confidence interval (CI) 1.01–10.42, $p = 0.037$], the coexistence of lung disease

(yes vs. no) (HR 4.48, 95% CI 1.46–13.72, $p = 0.009$), and the concomitant use of MTX (yes vs. no) (HR 4.68, 95% CI 1.59–13.81, $p = 0.005$).

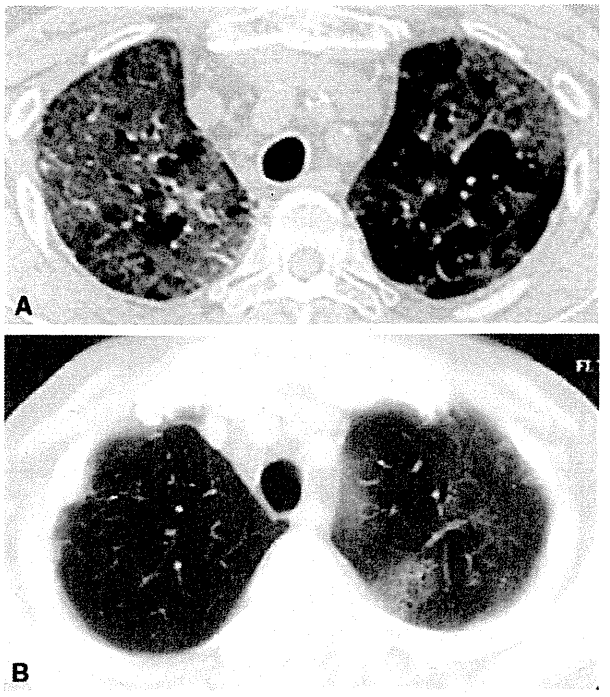

Fig. 1 Thoracic computed tomography findings of rheumatoid arthritis patients who developed *Pneumocystis jirovecii* pneumonia while receiving etanercept. **a** Ground-glass opacity (GGO) with sharp demarcation by interlobular septa and geographic pattern. **b** GGO without interlobular septal boundaries

Accumulation of risk factors and development of PCP

We calculated the cumulative probability for developing PCP in patient groups stratified by the number of coexisting risk factors. When all patients ($n = 89$) were stratified by the number of risk factors, including age (≥65 years, yes/no), coexistence of lung disease, and use of MTX, the cumulative probability for the occurrence of PCP was significantly higher in patients with one risk factor compared to patients with no risk factor ($p = 0.015$); as well, the cumulative probability for the occurrence of PCP was significantly higher in patients with two or three risk factors compared to patients with no risk factor ($p < 0.001$) or compared to patients with one risk factor ($p = 0.001$) (Fig. 2).

Discussion

The highest available number of patients with RA who developed PCP during treatment with etanercept was located and the clinical, laboratory, and radiographic characteristics of these 15 patients were described. Independent risk factors for the development of PCP in these patients were also identified.

This study clarified important characteristics of PCP in RA patients receiving etanercept: (1) rapid development with a severe clinical course; (2) relatively low levels of plasma BDG and a low microscopic detection rate for *P. jirovecii*; and (3) infection occurring even in patients with normal peripheral lymphocyte counts and normal serum IgG levels. Of note, PCP in non-AIDS patients develops

Table 3 Laboratory findings in rheumatoid arthritis patients at the onset of *Pneumocystis jirovecii* pneumonia (PCP)

Pt	WBC (/μl)	Lymphocytes (/μl)	CRP (mg/dl)	Serum Alb (g/dl)	Serum IgG (mg/dl)	KL-6 (U/ml)
1	2,900	397	22.8	2.2	NA	821
2	7,200	1,900	7.92	4.1	NA	385
3	8,540	1,836	2.59	3.8	NA	NA
4	14,300	686	3.2	3.65	NA	487
5	7,000	1,260	4.81	3.4	2,230	1,516
6	5,000	860	9.49	3.4	1,645	687
7	8,700	1,305	23.7	3.5	1,405	162
8	10,300	309	19.1	2.2	707	820
9	5,700	627	6.0	3.1	1,120	864
10	7,600	1,547	21	4.2	1,090	485
11	6,340	628	9.86	2.44	1,341	666
12	20,730	2,094	22.41	2.57	NA	779
13	9,200	1,435	9.53	2.6	1,714	197
14	8,900	462	5.0	2.4	NA	420
15	13,260	2,386	18.05	2.3	665	NA
Median (IQR)	8,540 (6,670–9,750)	1,260 (628–1,692)	9.5 (5.5–20.1)	3.1 (2.4–3.6)	1,341 (1,090–1,645)	666 (420–820)

Pt patient, *WBC* white blood cells, *CRP* C-reactive protein, *Alb* albumin, *NA* not assessed, *KL-6* a serum marker for interstitial pneumonia and PCP, *IQR* interquartile range

Table 4 Clinical characteristics of rheumatoid arthritis patients treated with etanercept at initiation of therapy

Characteristics	PCP group ($n = 15$)	Non-PCP group ($n = 74$)	p value
Age (years)[a]	66.4 ± 11.7	54.7 ± 13.5	<0.001†
Age (≥65 years, %)	60	17.6	0.001‡
Female (%)	53.3	78.4	0.049‡
Disease duration (months)[a]	120.2 ± 102.5	114.4 ± 88.1	0.908†
Coexistence of lung disease (%)[b]	46.7	9.5	0.002‡
Coexistence of diabetes mellitus (%)	20.0	4.1	0.057‡
Concomitant use of MTX (%)	66.7	31.1	0.009‡
Dosage of MTX (mg/week)[a]	5.5 ± 4.6	2.5 ± 4.1	0.007†
Concomitant use of PSL (%)	80.0	64.9	0.204‡
Dosage of PSL (mg/day)[a]	11.4 ± 16.3	3.7 ± 3.4	0.045†
Dosage of PSL (≥5 mg/day, %)	53.3	28.4	0.06‡
Concomitant use of immunosuppressants, except for MTX (%)	6.7	20.3	0.193‡
White blood cells (/μl)[a]	8,279 ± 3,352	8,603 ± 3,021	0.587†
Lymphocytes(/μl)[a]	1,591 ± 810	1,379 ± 591	0.254†
Serum albumin (g/dl)[a]	3.4 ± 0.7	3.8 ± 0.4	0.06†
Serum IgG (mg/dl)[a]	1,447 ± 430	1,568 ± 570	0.557†

After the Bonferroni's correction, only the differences in age and pulmonary diseases retained statistical significance

p Values were calculated using the Mann–Whitney U-test test (†) or the χ^2 test (‡)

PCP Pneumocystis jirovecii pneumonia, *MTX* methotrexate, *PSL* prednisolone

[a] Mean ± SD

[b] Four interstitial pneumonia cases, one old pleuritis, one pneumoconiosis, and one prior tuberculosis

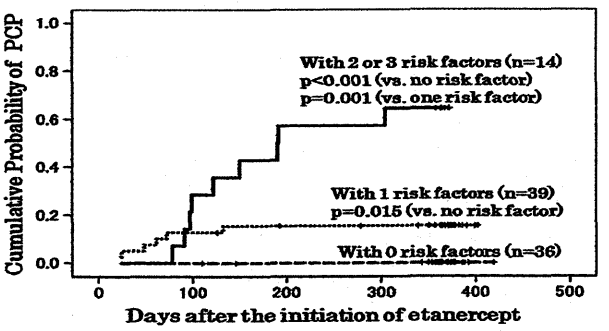

Fig. 2 Cumulative probability of developing *Pneumocystis jirovecii* pneumonia (*PCP*) in rheumatoid arthritis patients with associated risk factors when treated with etanercept. The patients were stratified by the number of risk factors, including age ≥65 years, coexistence of lung disease, and concomitant use of methotrexate (MTX). The cumulative probability for developing PCP according to the number of risk factors was calculated using the Kaplan–Meier method and comparison between the groups was performed using the log rank test

more rapidly and is more severe with a poorer prognosis than PCP in AIDS patients [13, 26–31]. We also have reported that in RA patients treated with infliximab [22] PCP developed rapidly and progressed to severe respiratory failure. In agreement with these previous reports, we found that all 15 patients who received etanercept in the present study showed acute-onset PCP and severe hypoxemia or requirement for oxygen therapy. While some studies suggest that low peripheral blood lymphocyte counts are associated with the development and severity of PCP in patients with rheumatic diseases [32–34], the immunological status of the patients with PCP in the present study, as judged from conventional laboratory tests, was not seriously impaired; peripheral blood lymphocyte counts at the onset of PCP were more than 500 cells/μl in 12 patients (80%) and serum IgG levels were normal in 7 of 9 patients (77.8%).

Fourteen of the 15 patients had presumptive diagnoses of PCP without microscopic detection of *P. jirovecii*. Because it has been reported that PCP in patients without AIDS presented with fewer numbers of the pathogen in the lung [13], we, and other investigators who have studied PCP in RA patients, included patients who did not have microscopic detection of the organism but who were positive for the PCR test or had an elevated serum BDG level. Recently, Kameda et al. [20] conducted a retrospective, multicenter study of acute-onset diffuse interstitial lung disease in patients with RA receiving biological agents. They defined 'definite PCP' as microscopically positive, or double-positive for the PCR test and serum BDG level, and 'probable PCP' as positive for either the PCR test or serum BDG level. They found that the two groups (i.e., definitive and probable PCP) were clinically

and radiologically indistinguishable. Because our criteria for presumptive PCP were not stringent by definition, it was mandatory to exclude other infectious diseases, as far as possible, by means of bacteriological examinations, laboratory tests, and radiological characteristics. As mentioned in the "Results" section, in our PCP patients there were no definitive data for other infectious lung diseases. Based on these data and discussion, we included presumptive PCP patients in the present study for analysis, in addition to the microscopically diagnosed PCP patients.

The efficacy of the use of corticosteroids for the treatment of PCP that develops in patients with rheumatic diseases is controversial [33, 35]. Pareja et al. [33] and Tokuda et al. [21] reported good clinical outcomes in PCP patients without HIV infection who received concomitant high-dose corticosteroids with TMP/SMX. In our study, 9 of the 15 patients received high-dose corticosteroids concomitant with TMP/SMX. In our previous study of PCP in RA patients during infliximab therapy, 19 of 21 patients received high-dose corticosteroids concomitantly with TMP/SMX [22, 24]. The mortality of the patients with PCP receiving infliximab (0%) or etanercept (6.7%) is considerably lower than the mortality found in previous studies of PCP in patients without HIV infection (32–45.7%) [34, 36]. Our diagnostic criteria included good response to standard treatment for PCP with TMP/SMX or pentamidine isethionate; concomitant corticosteroid therapy with TMP/SMX might also have contributed to the lower mortality seen in our study.

The risk factors for the development of PCP were similar for both RA patients receiving infliximab and for those given etanercept, the risk factors in common being age of $\geq$65 years and the coexistence of lung disease [24]. The concomitant use of MTX was another risk factor for PCP in RA patients receiving etanercept. An association between MTX therapy and increased risk of infection or serious infection in RA patients remains controversial [7, 37, 38]. It seems possible that the association between MTX and PCP is specific to the ethnic group studied or the concomitant drug used (i.e., etanercept). Because the number of patients in our study was small, further investigations of more patients are needed to answer these questions.

In our study, no patients received chemoprophylaxis for PCP. In HIV-infected patients, primary prophylaxis for PCP is recommended when the CD4+ lymphocyte count is <200 cells/μl or when a patient has a history of oropharyngeal candidiasis [39]. However, the peripheral blood lymphocyte counts of most patients with PCP in the present study were higher than 500 cells/μl. Based on the results of our Kaplan–Meier analysis (Fig. 2), chemoprophylaxis for PCP might be considered when a patient has all of the risk factors at the initiation of etanercept therapy.

There are definite limitations to our study. First is the inclusion of presumptive cases. The traditional diagnosis of PCP, the microscopic detection of *P. jirovecii*, was made in only one of the 15 patients. The other 14 patients, however, had clinical, laboratory, and radiological characteristics compatible with PCP, but did not have evidence for other pulmonary infectious diseases. The interpretation of the results of our study should take our diagnostic criteria into account. Second, because our criteria included the presenting characteristics of the patients, we cannot exclude the possibility that milder PCP cases were missed; however, such cases are less clinically relevant than those of the patients included in this study. Third, the p value for age from the Cox proportional hazard analysis for risk factors for PCP was 0.037 and the lower limit of the 95% CI of the risk factors was about 1.0. Although this value has limited statistical significance, older age has been recognized as an important risk factor for infections in RA patients [40] and it is safest to assume this risk factor for PCP is real for RA patients receiving etanercept.

In conclusion, physicians must be alert to the possibility of PCP developing during etanercept therapy in RA patients, particularly if one or more risk factors are present, and physicians must also be vigilant for clinical manifestations, indicative laboratory tests, and radiological findings.

Acknowledgments We would like to thank Drs. Saburo Matsubara (Center for Arthritis and Clinical Rheumatology Matsubara Clinic), Kenichi Miyagi (Miyagi Clinic), Masao Sato (Nishimino Welfare Hospital), Kazuaki Katsumata (Nissei Hospital), Tetsu Oyama (Oyama Clinic), Tsuyoshi Kasama (Showa University Hospital), Masahito Koiwa (Shuwa General Hospital), Kazuhide Tanimura (Hokkaido Medical Center for Rheumatic Diseases), Yoshiko Sato (Yokkaichi Social Insurance Hospital), and Hideaki Oka (Yokohama City University Hospital), for their critical discussions during our study group meetings concerning all patients who had or were suspected to have PCP. We also thank Drs. Koichi Amano (Saitama Medical Center, Saitama Medical University), Masahiro Iwamoto (Jichi Medical University), and Noriyoshi Ogawa (Hamamatsu University School of Medicine), who were the members of the Japan College of Rheumatology (JCR) subcommittee for interstitial pneumonia and PCP during the post-marketing surveillance program of etanercept in Japan, for their discussion about some cases in the present study during the meeting of the subcommittee.

Conflict of interest Masayoshi Harigai and Nobuyuki Miyasaka have received research grants from Abbott Japan, Astellas, Bristol-Myers Squibb, Chugai Pharmaceutical Co. Ltd., Eisai Co. Ltd., Mitsubishi-Tanabe Pharma Corp., Novartis Pharma K.K., Takeda Pharmaceutical Co. Ltd., and Wyeth K.K. (now Pfizer). Tsutomu Takeuchi has received grants and consultant fees from Abbott Japan, Bristol-Myers Squibb, Chugai Pharmaceutical Co. Ltd., Janssen Pharmaceutical Co. Ltd., Mitsubishi-Tanabe Pharma Corp., Novartis Pharma K.K., Takeda Pharmaceutical Co. Ltd., and Wyeth K.K. (now Pfizer). Yoshiya Tanaka has received consultant fees from Abbott Japan, Astellas, Banyu Pharmaceutical Co. Ltd., Chugai Pharmaceutical Co. Ltd., Eisai Co. Ltd., Mitsubishi-Tanabe Pharma Corp., and Takeda Pharmaceutical Co. Ltd. There is no other competing

interest for the other authors regarding this article. This work was supported by Grants-in-Aid for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Science to R.K. (#19590530), M.H. (#20390158) and M.T. (#23590171), and by Grants-in-Aid from the Ministry of Health, Labour and Welfare, Japan (H19-meneki-ippan-009 to M.N. and M.H. and H22-meneki-ippann-001 to T.T. and M.H.). This work was also supported by the Global Center of Excellence (GCOE) program, 'International Research Center for Molecular Science in Tooth and Bone Diseases'.

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Mod Rheumatol (2013) 23:1085–1093
DOI 10.1007/s10165-012-0796-5

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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Received: 28 June 2012 / Accepted: 31 October 2012 / Published online: 5 December 2012

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

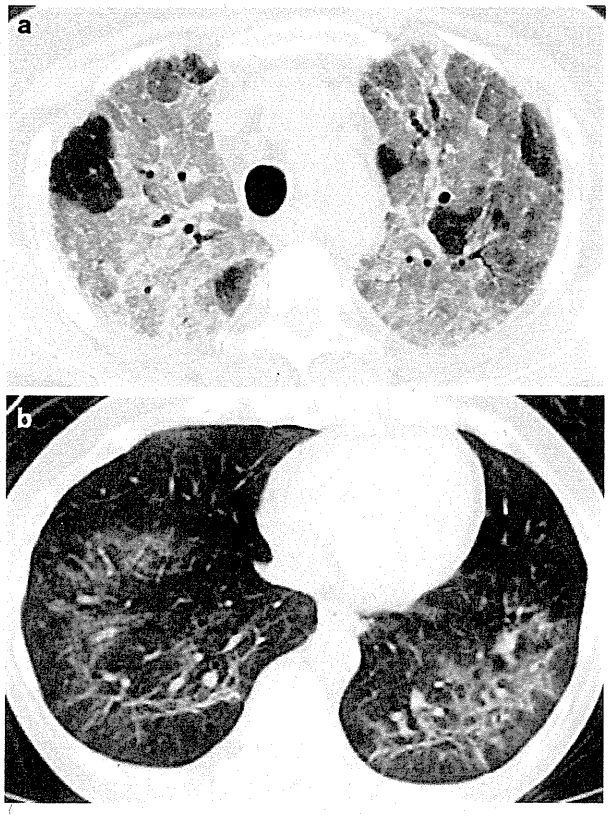

Fig. 1 Representative thoracic computed tomography findings of rheumatoid arthritis patients who developed *Pneumocystis jirovecii* pneumonia while receiving adalimumab. **a** Ground-glass opacity (GGO) with sharp demarcation by interlobular septa (type A) (patient 12). **b** Inhomogeneous GGO without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

Acknowledgments We would like to thank Drs. Yoshihisa Nojima (Gunma University Graduate School of Medicine), Takeo Sakurai (Inoue Hospital), Shoichi Ozaki (St. Marianna University School of Medicine), Kimihiro Kida (Nagara Orthopaedic Clinic), Tomio Shimizu (Chibune General Hospital), Toshio Tanaka (Osaka University Graduate School of Medicine), Motoaki Kin (Higashihiroshima Memorial Hospital), Atsuko Imai (Ichiban-cho Clinic for the Rheumatic Diseases), Masakazu Kondo (Kondo Clinic for Rheumatism and Orthopaedics), Eiichi Suematsu (National Hospital Organization Kyushu Medical Center), and Tamami Yoshitama (Yoshitama Rheumatoid Arthritis and Internal Medicine Clinic) for their contributions to the face-to-face meeting of this study.

Conflict of interest Fumikazu Sakai has received research funds form the Japanese Ministry of Labor, Health and Welfare, Japanese Ministry of Environment, Ministry of Education, Culture, Sports, Science and Technology in Japan, LTT Bio Ltd., Eisai Co. Ltd., Daiichi-Sankyo Co. Ltd., Kovidien Co., and Bayer Co. Ltd., and has received consulting fees form Takeda Pharmaceuticals, Chugai Pharmaceuticals, Rosche, MSD, Astra-Zeneca, Merk Serrono, Pfizer, Bayer, Jansen Pharma, and has received lecture fees form Daiichi Sankyo Co. Ltd., Eizai Co. Ltd., Kyorin Pharmaceuticals, Shionogi Pharmaceuticals. Yoshiya Tanaka has received consulting fees, speaking fees, and/or honoraria from Mitsubishi-Tanabe Pharma, Abbott Japan, Chugai Pharma, Janssen Pharma, Eisai Pharma, Santen Pharma, Pfizer, Astellas Pharma, Daiichi-Sankyo, GlaxoSmithKline, Astra-Zeneca, Otsuka Pharma, Actelion Pharma Japan, and Eli Lilly Japan, and has received research grant support from Bristol-Myers Squibb, MSD, Chugai Pharma, Mitsubishi-Tanabe Pharma, Astellas Pharma, Abbott Japan, Eisai Pharma, and Janssen Pharma. Masayoshi Harigai and Nobuyuki Miyasaka have received research grants from Abbott Japan, Astellas, Bristol-Myers Squibb, Chugai Pharmaceutical Co. Ltd., Eisai Co. Ltd., Mitsubishi-Tanabe Pharma Corp., Novartis Pharma K.K., Takeda Pharmaceutical Co. Ltd., and Wyeth K.K. (now Pfizer). There are no other competing interests for the other authors regarding this article. This work was supported by a grant-in-aid for scientific research (KAKENHI) from the Japan Society for the Promotion of Science to R.K. (#19590530), M.H. (#20390158) and M.T. (#23590171), and by a grant-in-aid from the Ministry of Health, Labor and Welfare, Japan (H19-meneki-ippan-009 to N.M. and M.H and 22-meneki-ippann-001 to M.H.). This work was also supported by the Global Center of Excellence (GCOE) program "International Research Center for Molecular Science in Tooth and Bone Diseases" (to N.M.).

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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 ≥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

Table 4 Baseline characteristics of rheumatoid arthritis patients in the biological DMARDs group who did or did not meet criterion B for elevation of serum KL-6 levels

	Meeting criterion B (n = 27)	Not meeting criterion B (n = 124)
Gender (female) (%)	70.4	76.6
Mean age (years)	63.5 ± 9.7	58.1 ± 13.8
Mean disease duration (months)	100.6 ± 120.3	98.9 ± 108.4
Comorbidity		
Interstitial pneumonia (%)	44.4	27.4
Other pulmonary disease (%)	7.4	10.5
Past illness		
PCP (%)	0.0	0.0
Malignancy (%)	0.0	8.1
Drug-induced pulmonary disease (%)	0.0	2.4
Others (%)	37.0	22.6
Clinical characteristics		
Baseline KL-6 (U/ml)	419.5 ± 180.1	365.4 ± 373.3
MTX use at month 0 (%)	63.0	64.5
Mean dose of MTX at month 0 (mg/week)	8.3 ± 3.0	8.6 ± 2.5
Corticosteroid use at month 0 (%)	51.9	50.8
Mean dose of corticosteroid at month 0 (mg/day) (prednisolone equivalent)	6.5 ± 2.0	6.1 ± 3.6
DMARDs other than MTX use at month 0 (%)	22.2	25.8
Biological DMARDs-naïve (%)	92.6	74.2

Criterion B for elevation of serum KL-6 levels is defined in "Patients and methods" section

Values are mean ± SD, unless otherwise stated

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

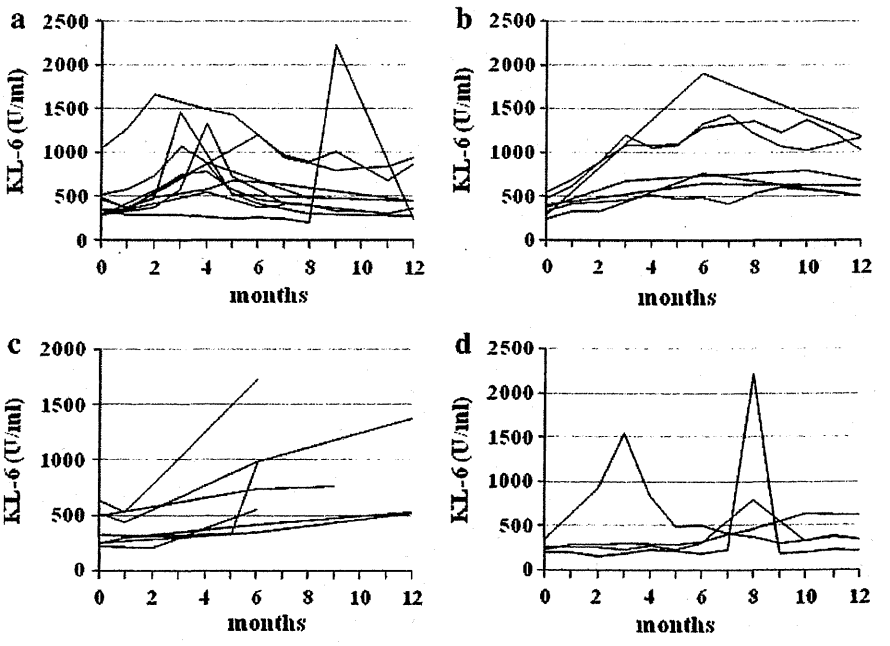

Fig. 1 Changes in serum KL-6 levels of rheumatoid arthritis patients meeting criterion B at least once during the observation period without apparent clinical events are shown. Data from the biological disease-modifying antirheumatic drugs (DMARDs) group: data from 10 patients meeting criterion R by month 12 (**a**), data from 7 patients not meeting criterion R by month 12 (**b**), and data from 7 patients without available data on serum KL-6 levels after meeting criterion B and reaching their maximum levels (**c**). **d** Data from the MTX group