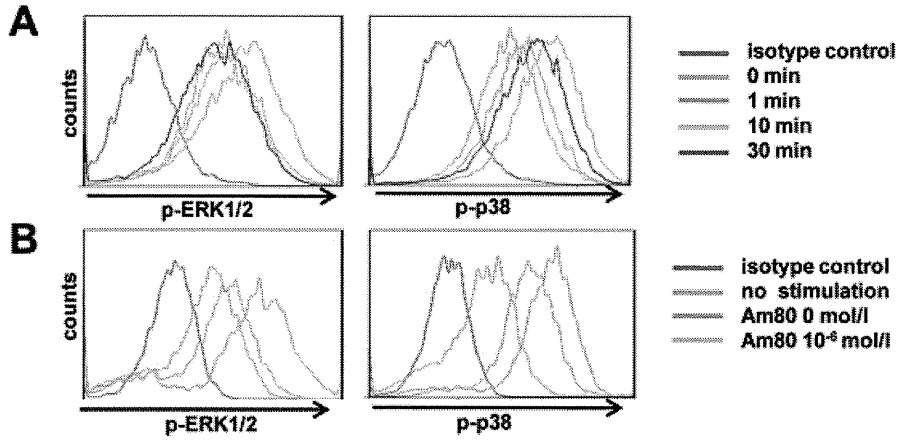

Figure 5. Am80-induced inhibition of MAPK signals. **A,** Purified human peripheral blood neutrophils were stimulated with fMLP (1 μM) and lipopolysaccharide (LPS) (1 μg/ml) for 1, 10, or 30 minutes. Phosphorylation of ERK-1/2 and p38 was analyzed by flow cytometry. **B,** Neutrophils were treated with Am80 (10^{-6} moles/liter) and stimulated with fMLP and LPS for 1 minute to analyze phosphorylation of ERK-1/2 and for 10 minutes to analyze phosphorylation of p38. Results shown are representative of at least 3 independent experiments.

0131), and the path of each cell was observed (Figure 3D). We examined the speed of movement and direction of each cell. The migration speed of Am80-treated neutrophils was slower than that of untreated neutrophils (Figure 3E). Am80 also significantly reduced the directionality of the chemotaxis (Figure 3F).

Reduced ROS production from neutrophils after Am80 treatment. We also assessed the effect of Am80 on ROS production from human peripheral blood neutrophils, based on the fluorescence intensity of rhodamine 123. After incubation with Am80 in various concentrations, the cells were stimulated with PMA, Pam_3CSK_4, or LPS. ROS production was measured by flow cytometry. Incubation with Am80 significantly inhibited production of ROS by the stimulated cells (Figures 4A and B).

Effects of Am80 on elastase release from neutrophils. To determine the effect of Am80 on elastase release by human peripheral blood neutrophils, we treated the neutrophils with cytochalasin B and fMLP after culture for 5 hours with Am80. The concentration of elastase in the culture supernatant was analyzed by ELISA. Incubation with Am80 inhibited elastase release by the neutrophils in a dose-dependent manner (Figure 4C).

Inhibitory effects of Am80 on MAPK signaling. Retinoids are reported to inhibit MAPK signaling (30–33). Accordingly, we analyzed phosphorylation of ERK-1/2 and p38 MAPK in neutrophils stimulated with LPS plus fMLP, using intracellular flow cytometry. Phosphorylation of ERK-1/2 and p38 was increased to the greatest extent after 1 minute and 10 minutes of stimulation, respectively (Figure 5A). Am80 attenuated the increased phosphorylation of ERK-1/2 and p38 in stimulated neutrophils (Figure 5B).

DISCUSSION

In the present study we demonstrated that treatment with Am80, a synthetic retinoid, ameliorated murine vasculitis induced by CAWS and also inhibited neutrophil migration into inflamed vessels. Am80 treatment resulted in marked suppression of chemotaxis, ROS production, and elastase release in human peripheral blood neutrophils in vitro. Neutrophils mainly infiltrated the site of vasculitis, and depletion of neutrophils suppressed murine vasculitis. Collectively, the results suggest that Am80 suppresses CAWS-induced vasculitis through inhibition of neutrophil migration and activation.

Neutrophils infiltrate the affected vessels in patients with certain vasculitides, such as microscopic polyangiitis and polyarteritis nodosa (34), and there has been ample evidence implicating neutrophils in the pathogenesis of vasculitis (35). In our vasculitis model mice, abundant neutrophils infiltrated the inflamed vessel wall, and neutrophil depletion by rabbit anti-mouse neutrophil antibody–positive serum as a prophylactic treatment ameliorated the vasculitis, suggesting that neutrophils play an important role in this model. However, the rabbit anti-mouse neutrophil antibody–positive serum depleted peripheral blood neutrophils by only 57%. This insufficient depletion of neutrophils may have

resulted in the inefficacy of the therapeutic administration.

Accumulated neutrophils can injure vessels by producing ROS, inflammatory cytokines, and elastase. Previous studies have suggested that neutrophils have important roles in the pathogenesis of vasculitis and that retinoid directly inhibits the production of superoxide anion and release of proteolytic enzymes by human and rat neutrophils (14,15,36). However, the effects on neutrophils vary among the different retinoids (15). In the present study, we showed that Am80 suppressed neutrophil migration into inflamed vessels in vivo, and fMLP-induced chemotaxis in vitro. In the in vivo chemotaxis assay, the recipient mice were administered Am80 only 3 times during 2 days, and the Am80 treatment did not significantly reduce the vasculitis score. It is believed that neutrophils may survive for 1–2 days after infiltrating tissue (37). Specifically, neutrophils that migrate to sites of inflamed tissue have a prolonged lifespan and become resistant to apoptosis (38). Therefore, neutrophils that have already infiltrated the vessels could remain in the tissue during the 2 days of Am80 treatment. Moreover, Am80 also reduced ROS production and elastase release by neutrophils. Thus, Am80 appears to inhibit CAWS-induced vasculitis via inhibition of neutrophil migration and activation.

It was previously shown that retinoid inhibited the MKK-6/p38 and MEK/ERK signaling pathways (39). In the present study, we observed that Am80 suppressed the activation of ERK-1/2 and p38 MAPKs in human peripheral blood neutrophils. Since ERK-1/2 and p38 MAPK are implicated in the respiratory burst, adherence, exocytosis, and cell motility in neutrophils (40,41), Am80 may hamper neutrophil activation via interference with those signaling pathways.

The pathogenic mechanisms of CAWS-induced murine vasculitis are still unclear. Arteritis could be induced by *C albicans*–derived substances (42,43). Investigators in our group have found that CAWS is a useful compound to induce murine vasculitis and have extensively studied this model of the disease (3–8). CAWS is primarily composed of a complex of mannoproteins, such α-mannan, and β-glucan (5) that can bind to Toll-like receptors and/or C-type lectin receptors on neutrophils, macrophages, and dendritic cells (44). Injection of CAWS increases peripheral blood neutrophil and monocyte numbers and promotes activation of neutrophils and complement, which in turn release ROS, proinflammatory cytokines, and myeloperoxidase from neutrophils and soluble intercellular adhesion molecule 1 from endothelial cells in mice (8). These processes of neutrophil and endothelial cell activation may be involved in the development of CAWS-induced vasculitis.

It has been reported that retinoids regulate differentiation and activation of T cells and macrophages (11,12), and these effects of Am80 on T cells and macrophages might also contribute to the inhibition of vasculitis. However, in this study we did not investigate the effects of Am80 on these cells in CAWS-induced vasculitis. Further studies should be carried out to investigate inhibitory influences of Am80 on T cells and macrophages.

Am80, in addition to all-*trans*-retinoic acid, has been approved in Japan as a therapeutic agent for acute promyelocytic leukemia (21,22). We and other groups have shown that Am80 ameliorates experimental autoimmune myositis (45), experimental autoimmune encephalitis (46), collagen-induced arthritis (47,48), and murine chronic graft-versus-host disease (49). In the present study we have demonstrated for the first time that retinoids, especially Am80, ameliorate experimental vasculitis, suggesting that Am80 can be potentially useful for the treatment of vasculitis. In further studies to examine the potential effects of Am80 on different vasculitides, the inhibitory effects of Am80 on neutrophils from vasculitis patients must be analyzed, and the effects compared among different vasculitides.

In conclusion, Am80 significantly inhibits vasculitis in a murine model, presumably through suppression of neutrophil migration and activation. This agent may have potential as a new mode of treatment in vasculitis.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Nanki had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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ORIGINAL ARTICLE

Pneumocystis jirovecii pneumonia associated with etanercept treatment in patients with rheumatoid arthritis: a retrospective review of 15 cases and analysis of risk factors

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Abstract
Objectives The association of anti-tumor necrosis factor therapy with opportunistic infections in rheumatoid arthritis (RA) patients has been reported. The goal of this study was to clarify the clinical characteristics and the risk factors of RA patients who developed *Pneumocystis jirovecii* pneumonia (PCP) during etanercept therapy.
Methods We conducted a multicenter, case–control study in which 15 RA patients who developed PCP were compared with 74 RA patients who did not develop PCP during etanercept therapy.
Results PCP developed within 26 weeks following the first injection of etanercept in 86.7% of the patients. All PCP patients presented with a rapid and severe clinical course and the overall mortality was 6.7%. Independent risk factors were identified using multivariate analysis and included age ≥65 years [hazard ratio (HR) 3.35, $p = 0.037$], coexisting lung disease (HR 4.48, $p = 0.009$), and concomitant methotrexate treatment (HR 4.68, $p = 0.005$).

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In patients having a larger number of risk factors, the cumulative probability of developing PCP was significantly higher ($p < 0.001$ for patients with two or more risk factors vs. those with no risk factor, and $p = 0.001$ for patients with one risk factor vs. those with no risk factor).
Conclusion Physicians must consider the possibility of PCP developing during etanercept therapy in RA patients, particularly if one or more risk factors are present.

Keywords *Pneumocystis jirovecii* pneumonia · Rheumatoid arthritis · Etanercept · Anti-TNF therapy · Opportunistic infection

Introduction

Tumor necrosis factor (TNF) plays an important role in the pathological mechanism of rheumatoid arthritis (RA) [1]. The excellent efficacy of TNF inhibitors for RA seen in various clinical trials has established TNF as a major pathogenic cytokine in RA [2–4]. TNF is one of the key molecules protecting the human body against microorganisms in vivo. The blockade of TNF with TNF inhibitors in RA patients has been associated with increased risks of opportunistic and serious infections [5–8].

In Japan, mandatory post-marketing surveillance (PMS) programs have been implemented, requiring registration and 6-month tracking of all RA patients who have received TNF inhibitors. Of 5,000 patients treated with infliximab, 13,894 patients treated with etanercept, and 3,000 patients treated with adalimumab tracked by these programs, 22 patients (0.4%) receiving infliximab, 25 patients (0.18%) treated with etanercept, and 9 patients (0.3%) treated with adalimumab developed *Pneumocystis jirovecii* pneumonia (PCP) [9–11], a rare opportunistic infectious disease seen in patients with human immunodeficiency virus (HIV) infection [12] and other immune-compromised states [13]. The incidence rate of PCP in the PMS programs in Japan was notably higher than that found in corresponding studies in the United States [14, 15].

The diagnosis of PCP in immunosuppressed patients without acquired immunodeficiency syndrome (AIDS) is challenging because of lower numbers of the organism in the lung [13]. In order to overcome this problem and to achieve prompt clinical diagnosis of PCP in non-AIDS patients who have a lower burden of *P. jirovecii* [16], several molecular techniques, such as the polymerase chain reaction (PCR) [17] and the use of serum markers, such as 1,3-β-D-glucan (BDG) [18, 19], have been developed. We, and other investigators, have performed several clinical studies of PCP using diagnostic criteria that involved these new diagnostic tools [9, 20–22].

Although the etanercept PMS program in Japan identified 25 patients with PCP, the diagnoses were based on reports from attending physicians and detailed analyses have not been implemented. Independent from the etanercept PMS program, we conducted a multicenter case–control study of PCP in RA patients treated with etanercept to delineate the clinical characteristics of PCP and identify risk factors in this population.

Patients, materials, and methods

Patients

In this study, we collected data from 21 hospitals on 28 RA patients suspected of having PCP; data were collected either through the PMS etanercept program or from voluntary case reports at scientific meetings, or from the relevant pharmaceutical company. Among these 28 patients, we identified one definitive PCP patient (patient 14) and 14 presumptive PCP patients (patients 1–13 and 15) based on the predefined criteria presented below. These 15 patients did not have other risk factors for PCP, such as malignancy, post-transplantation status, or other immunodeficiency states. We did not examine antibody for HIV because this laboratory test was not routinely conducted in clinical practice in Japan. These 15 patients were classified as the 'PCP group' in this study. The other 13 patients were not diagnosed with PCP because their data were incompatible with the diagnostic criteria for PCP and diagnoses including other infectious diseases or rheumatoid lung were considered more appropriate, and these patients were

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excluded from further evaluation. The data of 74 RA patients who did not develop PCP within 12 months after the beginning of etanercept treatment were collected and these patients were termed the 'non-PCP' group. These patients' were randomly extracted from consecutive RA patients receiving etanercept at hospitals that participated in this study. All patients in this study fulfilled the 1987 American College of Rheumatology (formerly the American Rheumatism Association) diagnostic criteria for RA [23].

Diagnostic criteria for PCP

For this study, we used previously established diagnostic criteria for PCP [22, 24]. A diagnosis of PCP was considered definitive if: (1) *P. jirovecii* was found on microscopic analysis of respiratory samples from patients with concurrent clinical manifestations (fever, dry cough, or dyspnea), (2) the patients presented with hypoxemia, and (3) radiographic findings were indicative of PCP. The diagnosis of PCP was considered presumptive if a patient fulfilled the clinical and radiographic conditions [i.e., criteria (2) and (3)] in the absence of evidence of other infectious diseases and in the presence of either a positive PCR test for *P. jirovecii* DNA (qualitative PCR analysis by SRL, Tokyo, Japan, or Mitsubishi Chemical Medicine Corporation, Tokyo, Japan) or increased serum BDG levels above the upper limit of normal (ULN) (Fungitec *G* test MK; Seikagaku, Tokyo, Japan, or Wako β-D-glucan test; Wako Pure Chemical Industries, Tokyo, Japan) and responded to standard treatments for PCP with trimethoprim/sulfamethoxazole (TMP/SMX) or pentamidine isethionate. Both the PCR test for *P. jirovecii* DNA and the serum BDG test 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

Data were collected from medical records using a standardized format including demographic information, comorbidities, concomitant drugs, laboratory data, radiographic data, treatments, and outcomes. Chest radiographs and computed tomography (CT) scans of the thorax were evaluated by a radiologist (F.S.) and a pulmonologist (H.S.).

Ethics

The guidelines of the Helsinki Declaration and the ethical 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 (#545 in 2008). The ethical guidelines for epidemiological studies in Japan required notifying eligible RA patients of this study and allowed us to implement this study without obtaining individual written informed consent. Patients were notified of this study by leaflets or posters at the outpatients clinics of each participating institution 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 their unwillingness to participate in this study.

Statistical analyses

Fisher's exact test was used for categorical variables and the Mann–Whitney *U*-test was used for continuous variables, with the Bonferroni correction for multiple pair comparisons. To identify risk factors for PCP, the Cox proportional-hazards regression model was used. The cumulative probability of PCP was calculated using the Kaplan–Meier method and the comparison among groups was performed using the log-rank test. All analyses were performed using SPSS software, version 17.0 (SPSS Japan, Tokyo, Japan).

Results

Demographics and treatment of RA patients who developed PCP

The demographics and treatment of RA patients at the onset of PCP are summarized in Table 1. The mean age of the PCP group was 66 years. The median interval between the first injection of etanercept and the onset of PCP was 14 weeks. Thirteen patients (86.7%) developed PCP within 26 weeks after the first injection of etanercept. All patients were treated with 50 mg/week of etanercept, except for patient 14, who was given 25 mg/week. At the onset of PCP, ten patients (66.7%) were receiving concomitant methotrexate (MTX) and 12 patients (80%) were receiving concomitant corticosteroids with etanercept. The median dosage of MTX was 8 mg/week and the median dosage of prednisolone (PSL) was 5 mg/day. Patient 8 received concomitant cyclophosphamide. None of the patients received chemoprophylaxis for PCP. Seven patients had pulmonary comorbidities, including interstitial pneumonia (IP) ($n = 4$), prior pleuritis ($n = 1$), pneumoconiosis ($n = 1$), and prior pulmonary tuberculosis ($n = 1$). Three patients had diabetes mellitus.

Table 1 Demographics and treatment of rheumatoid arthritis patients at the onset of *Pneumocystis jirovecii* pneumonia (PCP)

Pt	Age (years)	Number of injections[a]	Duration of ETN (weeks)[b]	MTX (mg/week)	PSL (mg/day)	Lung disease	Diabetes mellitus
1	66	38	21	8	3	–	–
2	32	8	7	12	0	–	+
3	74	55	27	8	0	–	–
4	61	35	19	6	8	–	–
5	79	51	27	0	2.5	IP	–
6	74	80	43	10	1	IP	+
7	72	28	13	0	10	Old pleuritis	–
8	73	25	14	0	30	Pneumoconiosis	–
9	72	29	13	8	5	IP	–
10	61	13	10	10	5	–	–
11	63	7	3	0	25	IP	–
12	72	12	11	0	4	Prior tbc	–
13	61	33	9	10.5	7.5	–	–
14	79	17	17	4	17.5	–	+
15	58	6	3	10	0	–	–
Median (IQR)	72 (61–73)	28 (12.5–36.5)	14 (9.5–20)	8 (0–10)	5 (1.75–9)		

Pt patient, *M* male, *F* female, *ETN* etanercept, *MTX* methotrexate, *PSL* prednisolone, *IP* interstitial pneumonia, *tbc* tuberculosis, *IQR* interquartile range

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

[b] Duration of treatment with etanercept before the onset of PCP

Clinical characteristics of RA patients who developed PCP

The clinical characteristics of each patient at the onset of PCP are summarized in Table 2. All had fever, 14 patients (93%) showed dyspnea on effort, and seven patients (46.7%) had a dry cough. Hypoxemia was observed in all patients at the onset of PCP; most had either severe hypoxemia with oxygen partial pressure in arterial blood (PaO_2) <60 mmHg on room air or required immediate oxygen therapy. Chest radiographs and CT scans were performed for all patients. On CT scans, ground-glass opacity (GGO) was observed in all patients. Six patients had GGO with sharp demarcation by interlobular septa (type A), while eight patients had GGO without interlobular septal boundaries (type B) (Fig. 1). One patient showed a combination of consolidation and GGO without interlobular septal boundaries. These thoracic CT findings in RA patients receiving etanercept who developed PCP were essentially the same as those in RA patients receiving infliximab who developed PCP [22, 24].

Serum levels of BDG, a reliable serum marker for PCP [18], were elevated above the ULN in 10 patients, with marked elevation (BDG >100 pg/ml) observed in 3 patients (Table 2). The PCR test for detection of *P. jirovecii* was utilized for 11 patients, using either induced sputum (nine patients) or bronchoalveolar lavage (BAL) fluid (two patients). All test results were positive. *P. jirovecii* was microscopically identified in BAL samples from patient 14 (Table 2).

Laboratory test results for PCP patients

Laboratory data from each patient at the onset of PCP are summarized in Table 3. Severe lymphopenia (<500 cells/μl) was observed in only 3 patients, while 4 patients had 500–1,000 cells/μl, and 8 patients had >1,000 cells/μl. The median serum level of C-reactive protein (CRP) was 9.5 mg/dl ($n = 15$); that of IgG was 1,341 mg/dl ($n = 9$); that of albumin (Alb) was 3.1 g/dl ($n = 15$); and that of the KL-6 antigen was 666 U/ml ($n = 13$). The KL-6 antigen is produced by type II alveolar epithelial cells and is reported to be elevated in patients with active IP [25], as well as in those with PCP [26].

Clinical course of PCP in RA patients treated with etanercept

All patients developed PCP rapidly and were hospitalized 3 or 4 days after the appearance of the clinical manifestations. Three patients required mechanical ventilation immediately upon admission because of progressive respiratory failure. Disease- modifying anti-rheumatic drugs (DMARDs), immunosuppressive drugs, and etanercept were discontinued in all patients. All patients received therapeutic doses of TMP/SMX immediately after the laboratory and radiographic examinations. Treatment with TMP/SMX was changed to pentamidine isethionate in three patients who had adverse drug reactions. Eight patients were treated with methylprednisolone (mPSL) pulse therapy, three with high-dose PSL, and five with increasing dosages of PSL within a few days after admission.

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, $p = 0.005$).

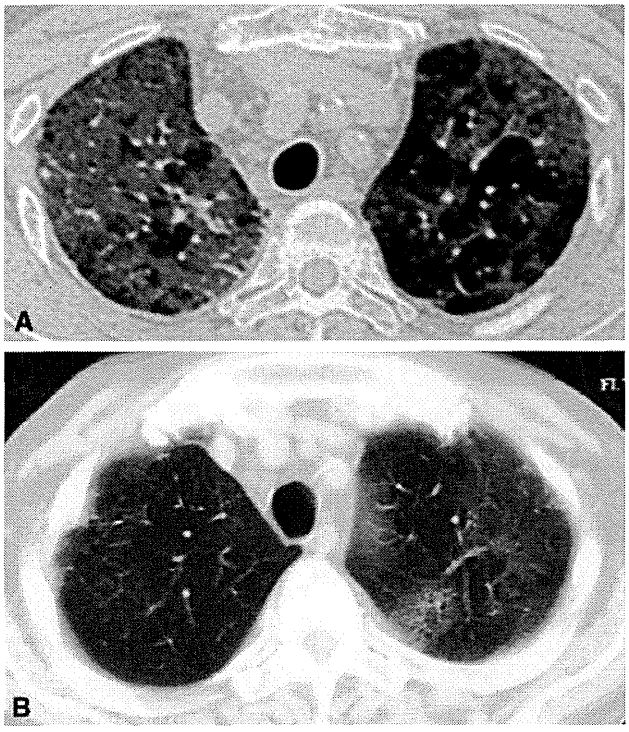

Fig. 1 Thoracic computed tomography findings of rheumatoid arthritis patients who developed *Pneumocystis jirovecii* pneumonia while receiving etanercept. **a** Ground-glass opacity (GGO) with sharp demarcation by interlobular septa and geographic pattern. **b** GGO without interlobular septal boundaries

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

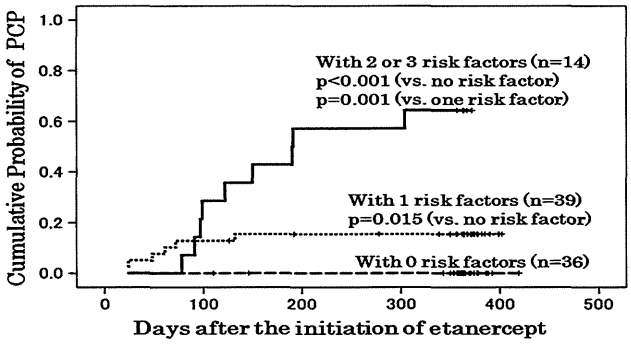

Fig. 2 Cumulative probability of developing *Pneumocystis jirovecii* pneumonia (*PCP*) in rheumatoid arthritis patients with associated risk factors when treated with etanercept. The patients were stratified by the number of risk factors, including age ≥65 years, coexistence of lung disease, and concomitant 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.

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EXTENDED REPORT

Drug retention rates and relevant risk factors for drug discontinuation due to adverse events in rheumatoid arthritis patients receiving anticytokine therapy with different target molecules

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► Additional supplementary data are published online only. To view these files please visit the journal online (http://ard.bmj.com/content/early/recent)

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ABSTRACT

Objective To compare reasons for discontinuation and drug retention rates per reason among anticytokine therapies, infliximab, etanercept and tocilizumab, and the risk of discontinuation of biological agents due to adverse events (AE) in patients with rheumatoid arthritis (RA).

Method This prospective cohort study included Japanese RA patients who started infliximab (n=412, 636.0 patient-years (PY)), etanercept (n=442, 765.3 PY), or tocilizumab (n=168, 206.5 PY) as the first biological therapy after their enrolment in the Registry of Japanese Rheumatoid Arthritis Patients for Long-term Safety (REAL) database. Drug retention rates were calculated using the Kaplan–Meier method. To compare risks of drug discontinuation due to AE for patients treated with these biological agents, the Cox proportional hazard model was applied.

Results The authors found significant differences among the three therapeutic groups in demography, clinical status, comorbidities and usage of concomitant drugs. Development of AE was the most frequent reason for discontinuation of biological agents in the etanercept and tocilizumab groups, and the second most frequent reason in the infliximab group. Discontinuation due to good control was observed most frequently in the infliximab group. Compared with etanercept, the use of infliximab (HR 1.69; 95% CI 1.14 to 2.51) and tocilizumab (HR 1.98; 95% CI 1.04 to 3.76) was significantly associated with a higher risk of discontinuation of biological agents due to AE.

Conclusions Reasons for discontinuation are significantly different among biological agents. The use of infliximab and tocilizumab was significantly associated with treatment discontinuation due to AE compared with etanercept.

Biological disease-modifying antirheumatic drugs (biological agents) are a standard treatment for rheumatoid arthritis (RA).[1 2] A number of clinical trials have demonstrated that biological agents significantly improve signs and symptoms of RA patients with both early and established disease, and that remission of RA can be achieved with biological agents not only in early RA patients, but also in established RA patients who have shown inadequate responses to conventional non-biological disease-modifying antirheumatic drugs (DMARD).

In Japan, six biological agents have been approved for the treatment of RA, infliximab in 2002, etanercept in 2005, tocilizumab and adalimumab in 2008, abatacept in 2010 and golimumab in 2011. These drugs are widely used in clinical practice according to treatment guidelines for biological agents by the Japan College of Rheumatology[3 4] and Japanese drug package inserts. Postmarketing surveillance and some clinical studies have shown short-term effectiveness and safety of these biological agents for Japanese RA patients.[5–8] The European League Against Rheumatism recommendations for the management of RA state that a tumour necrosis factor (TNF) antagonist should be administered as the first biological DMARD for patients who fail to respond to non-biological DMARD, including methotrexate,[9] whereas Japanese guidelines do not clearly specify the precedence of biological agents.

Some RA patients treated with biological agents are compelled to stop the administration of these drugs because of lack of efficacy (LOE), adverse events (AE), or financial reasons. In addition, some RA patients discontinue biological agents in the hope of a biological-free remission or biological-free low disease activity status.[10–12] In general, drugs with high retention rates have a good balance between long-term effectiveness and tolerability, reflecting the satisfaction of patients and doctors with the treatment. Because treatment for RA continues for many years or is life-long in the majority of patients, the examination of long-term drug retention rates using a prospective cohort study is important for the evaluation of biological agents.

To establish better treatment strategies for RA, it is important to identify reasons and risk factors causing the discontinuation of a drug, especially for biological agents. Several studies have shown that

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Table 1 Characteristics of RA patients treated with infliximab, etanercept or tocilizumab at the start of the observation period

	Infliximab group (n=412)	Etanercept group (n=442)	Tocilizumab group (n=168)	p Value
Age, years	53.6±13.5	58.5±13.0	59.8±13.4	<0.001
Female, %	85.9	78.1	80.4	0.011
Disease duration, years	7.9±7.8	10.3±8.9	10.3±9.6	<0.001
Steinbrocker's class (3 or 4), %	24.8	31.2	27.4	0.108
Steinbrocker's stage (III or IV), %	43.9	57.0	46.4	<0.001
DAS28 (3/CRP)	4.5±1.2 (n=411)	4.5±1.3 (n=440)	5.1±3.4 (n=167)	0.056
Use of ≥3 previous non-biological DMARD, %	41.0	54.5	31.5	<0.001
Biological-naive, %	96.4	83.9	46.4	<0.001
Methotrexate use, %	99.3	44.6	44.0	<0.001
Methotrexate dose, mg/week	8.0±2.1	7.0±2.0	8.2±2.9	<0.001
Use of immunosuppressive drugs, except for methotrexate, %	1.9	5.7	14.9	<0.001
Oral corticosteroid use, %	68.9	73.1	60.1	0.008
Prednisolone-equivalent dose of corticosteroids (mg/day)	5.4±2.6	6.1±3.3	4.9±2.2	<0.001
Chronic pulmonary disease, %	22.6	36.7	40.5	<0.001
Diabetes mellitus, %	8.5	14.9	12.5	0.015

CRP, C-reactive protein; DAS28, disease activity score including 28-joint count; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis

a frequent reason for the discontinuation of biological agents is the development of AE.[5–7 13–16] Mid to long-term tolerability of TNF inhibitors[6 13 14 16–24] and tocilizumab[7 15 25] has been reported, and some studies have directly compared drug retention rates among TNF inhibitors or between TNF inhibitors and other biological agents.[14 16 17 25–27] To summarise, infliximab had the lowest overall retention rate among infliximab, etanercept and adalimumab[14 16 17] and among infliximab, etanercept and anakinra.[26] A recent report from the CORRONA registry demonstrated the highest retention rate of infliximab compared with etanercept and adalimumab.[27] However, drug retention rates have not been compared between TNF inhibitors and the interleukin-6 receptor inhibitor, tocilizumab, in the real world. In addition, the risk factors causing drug discontinuation due to AE for patients given these biological agents have not been thoroughly evaluated.

The purpose of this study was to compare drug retention rates and reasons for discontinuation of infliximab, etanercept and tocilizumab among Japanese RA patients, and to investigate the association of the use of these biological agents and other clinical characteristics with drug discontinuation due to AE.

PATIENTS AND METHODS

Database

The Registry of Japanese Rheumatoid Arthritis Patients for Long-term Safety (REAL) is an ongoing prospective cohort established to investigate the long-term safety of biological agents in RA patients. Twenty-seven institutions participate, including 16 university hospitals and 11 referring hospitals. Details of REAL have previously been described.[28 29] Briefly, the criteria for enrolment in REAL include patients meeting the 1987 American College of Rheumatology criteria for RA, written informed consent, and starting or switching treatment with biological agents or starting, adding or switching non-biological DMARD at the time of enrolment in the study. Enrolment in the REAL database was started in June 2005 and closed in January 2012. To facilitate enrolment to the REAL registry, participating physicians were asked to enrol their patients already registered in postmarketing surveillance programmes previously implemented by pharmaceutical companies for biological agents.[5 8] In addition, our investigators were also encouraged to enrol as many patients as possible who fulfilled the inclusion criteria.[29]

Data were retrieved from the REAL database on 4 April 2011 for this study. The REAL study was approved by the ethics committees of the Tokyo Medical and Dental University Hospital and other participating institutions.

Data collection

Each patient's recorded baseline data included demography, disease activity, physical disability, comorbidities, treatments and laboratory data at the beginning of the observation period. A follow-up form was submitted by the site investigators every 6 months to the REAL data centre at the Department of Pharmacovigilance of Tokyo Medical and Dental University to report the occurrence of serious AE, current RA disease activity, treatments and clinical laboratory data.[28 29] We collected the Steinbrocker class[30] as the baseline measurement for each patient's physical disability, instead of the health assessment questionnaire disability index.[31] The investigators in each hospital confirmed the accuracy of their data submitted to the REAL data centre. The centre examined all the data sent by site investigators and sent queries if necessary to verify the accuracy of the data.

Patients

By April 2011, 2067 RA patients were registered in REAL, of these 1044 patients started treatment with infliximab, etanercept or tocilizumab at the time of enrolment or after enrolment in REAL. Four patients were excluded from this study because the reason for discontinuation of the initial biological agents was not identified. Eighteen patients who were enrolled in another clinical study requiring the discontinuation of infliximab were also excluded. We did not include patients who used adalimumab, abatacept or golimumab as the first biological agent in REAL because we did not have sufficient numbers of patients on adalimumab in the database (n=98) compared with infliximab and etanercept and had no patients given abatacept or golimumab in the database at the time our data were compiled. Our analysis included 412 patients who started infliximab, 442 patients who started etanercept and 168 patients who started tocilizumab.

Follow-up

For patients who initiated biological agents (infliximab, etanercept, or tocilizumab) at enrolment in REAL, the start date

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Table 2 Reasons for drug discontinuation in RA patients treated with infliximab, etanercept or tocilizumab*

Reason for discontinuation	Infliximab (n=157)†	Etanercept (n=130)†	Tocilizumab (n=51)†
Adverse events	57 Cases (36.3%)	57 Cases (43.8%)	23 Cases (45.1%)
Infection	20 Cases (12.7%)	22 Cases (16.9%)	8 Cases (15.7%)
Pulmonary diseases except infection‡	7 Cases (4.5%)	7 Cases (4%)	3 Cases (5.9%)
Infusion reaction	6 Cases (3.8%)	NA	0 Case (0%)
Allergy except infusion reaction	7 Cases (4.5%)	12 Cases (9.2%)	6 Cases (11.8%)
Malignancy	6 Cases (3.8%)	3 Cases (2.3%)	1 Case (2%)
Cardiovascular system disease	2 Cases (1.3%)	2 Cases (1.5%)	2 Cases (3.9%)
Others	9 Cases (5.7%)	11 Cases (8.5%)	3 Cases (5.9%)
Lack of efficacy	68 Cases (43.3%)	47 Cases (36.2%)	23 Cases (45.1%)
Good control	21 Cases (13.4%)	7 Cases (5.4%)	2 Cases (3.9%)
Miscellaneous§	11 Cases (7.0%)	19 Cases (14.6%) §	3 Cases (5.9%)

The χ^2 test was applied to assess differences in the proportion of causes for discontinuation (ie, adverse event, lack of efficacy, good control and miscellaneous), and the adjusted residuals were calculated. A significant difference among the three groups (p=0.026) was observed. The adjusted residuals indicated that significantly higher percentages of patients in the infliximab group stopped the treatment due to good disease control compared with the other two groups (p<0.05).
*Values are the number (percentage) of patients who discontinued use because of each reason.
†Number of patients who discontinued their first biological DMARD for any reason.
‡Pulmonary diseases except for infection included interstitial pneumonia (three cases for infliximab, five for etanercept, two for tocilizumab) and other pulmonary diseases (four for infliximab, two for etanercept, one for tocilizumab).
§*Miscellaneous reasons for drug discontinuation include patients' preference, financial reasons, and pregnancy.*
DMARD, disease-modifying antirheumatic drugs; RA, rheumatoid arthritis.
NA, not applicable

of the observation period was the date these agents were first administered. For patients who started non-biological DMARD at the time of entry in REAL and who later started treatment with biological agents, the start of the observation period was the date of the first administration of biological agents in REAL. Observation was stopped either at 2.5 years after the start of the observation period, on the date of death of a patient, loss to follow up, enrolment in clinical trials, or when therapy was stopped with the first biological agent in REAL for more than 90 days, or on 4 April 2011, whichever came first. The period following switching to a second biological agent was excluded from this study. We defined termination of treatment with biological agents as stopping treatment with the agent for more than 90 days. The date of the last administration of each biological DMARD was retrieved from medical records and reported by the site investigators. Reasons for drug discontinuation were obtained from case report forms of REAL supplemented by medical records, if necessary, and classified into AE, good control, LOE or miscellaneous. We did not discriminate between a primary and secondary LOE. Note that we collected only serious AE in REAL, but also collected AE in this study if it was the main reason for the discontinuation of a biological agent. When a patient had two or more reasons for drug discontinuation, site investigators assigned precedence and we used the primary reason contributing to drug discontinuation for that patient.

Statistical analysis

The primary outcome of this study was the investigation of the association of the use of infliximab, etanercept and tocilizumab with drug discontinuation due to AE. We also sought to identify other risk factors for drug discontinuation due to AE. Drug retention rates were calculated by the Kaplan–Meier method and compared using the log-rank test among groups. For univariate analysis, the χ^2 test was used for comparison of categorical variables and the Kruskal–Wallis test was used for continuous variables among the three agents. For multivariate analysis, the Cox regression hazard model with the forced entry method was employed to compare risks for drug discontinuation due to AE. The validity of the proportional hazards assumption was confirmed by the log-minus-log survival function. We followed the STROBE statement[32] for clear reporting except for 'the number and reasons for non-participation' in this study.

These statistical analyses were conducted using SPSS (version 16.0Illinois,). All p values were two-tailed and p<0.05 was considered statistically significant.

RESULTS

Baseline characteristics of the patients

This analysis included 412 patients in the infliximab group (636.0 patient-years (PY)), 442 in the etanercept group (765.3 PY) and 168 in the tocilizumab group (206.5 PY). Table 1 shows the baseline characteristics of the groups. There were significant differences in age, gender, disease duration and clinical status of the patients. The etanercept and tocilizumab groups had longer disease duration (p<0.001) and higher percentages of comorbidities than the infliximab group (p<0.001 for chronic pulmonary disease, p=0.011 for diabetes mellitus). The rates of biological-naive patients (96.4% for the infliximab group, 83.9% for the etanercept group and 46.4% for the tocilizumab group) (p<0.001) and of the use of three or more non-biological DMARD (p<0.001) in the tocilizumab group were the lowest among the three groups. The rate of the use (p=0.007) and dose (p<0.001) of oral corticosteroids of the etanercept group were higher than those for the other two groups. Disease activity did not differ significantly among the groups.

Occurrence of treatment termination

The median IQR of the observation period for each group was 1.50 (0.74–2.50) years for the infliximab group, 2.1 (0.98–2.50) years for the etanercept group and 1.0 (0.5–2.0) years for the tocilizumab group. The number of patients who discontinued biological agents for any reason during the observation period was 157 (38.1%) for the infliximab group, 130 (29.4%) for the etanercept group and 51 (30.4%) for the tocilizumab group (p=0.019 by χ^2). Table 2 shows the reasons for drug discontinuation for each group. A significant difference among the three groups (p=0.026 by χ^2) was seen in the proportions of reasons for discontinuation, and the adjusted residuals indicated that significantly higher percentages of patients in the infliximab group stopped treatment due to good disease control compared with the other two groups (p<0.05). The most frequently reported

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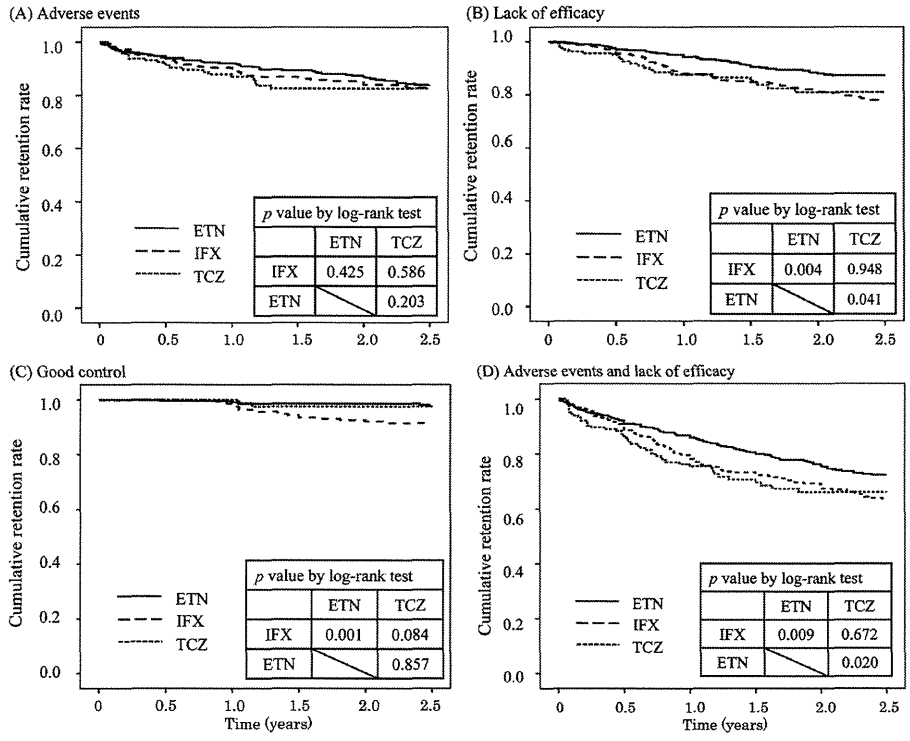

Figure 1 Kaplan-Meier curves for time to discontinuation for each biological agent (etanercept (ETN); infliximab (IFX); tocilizumab (TCZ)). Withdrawal for adverse events (A), lack of efficacy (B), good control (C), and adverse events and lack of efficacy (D) are presented separately. Drug retention rates are compared using the long-rank test among groups. The y axis shows the cumulative retention rates.

reason for discontinuation was LOE in the infliximab group, development of AE in the etanercept group and both in the tocilizumab group (table 2).

The retention rates of biological agents

Because the distribution of reasons for drug discontinuation was significantly different among these biological agents (table 2), we investigated drug retention rates per reason for discontinuation. Kaplan–Meier curves for time to discontinuation for each agent due to AE and LOE are shown in figure 1A,B, respectively. No significant differences existed among the three drugs for treatment discontinuation due to AE. The discontinuation rate due to LOE was significantly lower for etanercept compared with that of infliximab (p=0.004, log-rank test) and tocilizumab (p=0.041) (figure 1B), and the discontinuation rate for infliximab due to good control was significantly higher than that for etanercept (p=0.001, log-rank test) (figure 1C). We combined withdrawals due to AE and LOE to assess treatment failure; etanercept had a significantly lower discontinuation rate due to treatment failure compared with the other two agents (p=0.009 vs infliximab, p=0.020 vs tocilizumab, log-rank test) (figure 1D). To evaluate the possible effects of previous treatment with biological agents on drug discontinuation due to AE and LOE, we compared the retention rates per reason except for good control in the etanercept and tocilizumab groups between biological-naive and non-naive patients (see supplementary figures, available online only). In both groups, there was no significant difference in drug retention rates between biological-naive and non-naive patients. However, we found a numerically higher discontinuation rate of biological agent non-naive patients due to LOE in the tocilizumab group (see supplementary figure S3, available online only).

Multivariate analysis of the risk for discontinuation of biological agents due to AE

We compared patients who discontinued treatment with biological agents due to AE and remaining patients using a univariate analysis (see supplementary table S1, available online only) and used the same variables for the multivariate analysis of table 3. Although we found no significant difference in the use of infliximab and tocilizumab in the univariate analysis (table S1, available online only), the Cox regression hazard model revealed that the adjusted risk for discontinuation due to AE was significantly higher in patients using infliximab (HR 1.69; 95% CI 1.14 to 2.51) and tocilizumab (HR 1.98; 95% CI 1.04 to 3.76) compared with etanercept (table 3). Among the other variables, the risk of discontinuation due to AE was also significantly higher in patients with increasing age by decade (HR 1.64; 95% CI 1.38 to 1.97) and with the previous use of three or more non-biological DMARD (HR 1.86; 95% CI 1.30 to 2.67).

DISCUSSION

To our knowledge, this is the first report comparing drug retention rates among TNF inhibitors and tocilizumab and identifying risk factors causing drug discontinuation due to AE. The major findings of this study are: (1) the reasons for discontinuation were significantly different among the three biological agents studied; (2) the risk of discontinuation due to AE was significantly higher in patients using infliximab and tocilizumab compared with etanercept; and (3) other significant risk factors for the discontinuation due to AE were increasing age and the previous use of three or more non-biological DMARD.

There are some reports describing drug retention rates and reasons for drug discontinuations in patients treated with TNF

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Table 3 Multivariate analysis for drug discontinuation due to adverse events in RA patients treated with infliximab, etanercept or tocilizumab*

	HR (95% CI)	p Value
Infliximab (vs etanercept)	1.69 (1.14 to 2.51)	0.009
Tocilizumab (vs etanercept)	1.98 (1.04 to 3.76)	0.037
Age by decade	1.64 (1.38 to 1.97)	<0.001
Class 3 or 4 (vs class 1 or 2)	1.07 (0.74 to 1.54)	0.727
DAS28 (3/CRP) at baseline (per 1.0 increment)	1.03 (0.92 to 1.17)	0.585
Chronic pulmonary disease	1.19 (0.83 to 1.70)	0.336
Diabetes mellitus	0.95 (0.58 to 1.56)	0.841
Concomitant use of oral corticosteroids at baseline	1.15 (0.78 to 1.70)	0.489
Concomitant use of immunosuppressive drugs except for methotrexate at baseline	0.56 (0.20 to 1.55)	0.262
Previous use of three or more non-biological DMARD	1.86 (1.30 to 2.67)	0.001
Previous use of biological agents	1.05 (0.64 to 1.72)	0.842

*Cox regression hazard model analysis, adjusted for the variables included in the table, gender and calendar year.
Class, Steinbrocker's class; CRP, C-reactive protein;
DAS28, disease activity score including 28-joint count; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis.

inhibitors.[14 16–18 20 22 24 26 27 33–35] Among patients stopping treatment with TNF inhibitors due to any reason, approximately half of those discontinued due to AE, and the proportions of patients who discontinued the agents due to AE or LOE were similar in each group in the Swiss[14] and the French[16] registries. In this study, AE and LOE were the two major reported reasons for discontinuation, with similar percentages also for all three groups, but the discontinuation rate due to good control in the infliximab group was significantly higher than those in the other two groups. Several studies have shown successful discontinuation of treatment with infliximab[10 36–40] and tocilizumab[41] without flare of RA, but the reported percentage of patients who could discontinue infliximab was higher compared with tocilizumab. In contrast, there is no evidence of the successful discontinuation of treatment for etanercept to date. Therefore, our results might be influenced by physicians' expectations for successful discontinuation of biological agents based on previous reports.

We observed a significantly lower discontinuation rate due to LOE in the etanercept group compared with infliximab and tocilizumab (figure.1B), which can be explained by the following reasons. First, treatment with infliximab induces the formation of human antichimeric antibody in some patients, which may lead to LOE or adverse drug reactions.[42 43] The prevalence of antidrug antibodies in RA patients who were treated with infliximab is much higher compared with etanercept[44 45] and tocilizumab.[15 46] Second, the tocilizumab group had a significantly lower percentage of biological-naive patients, which may be associated with a less favourable response to treatment.[47 48] In the tocilizumab group, we confirmed that the discontinuation rate due to LOE was numerically lower in the biological-naive patients compared with biological agent non-naive patients (see supplementary figure S3, available online only).

In this study, we limited our multivariate analyses to the risk factors associated with discontinuation due to AE. Some previous studies identified risk factors for overall discontinuation in patients treated with TNF inhibitors.[6 17 26] Because treatments with biological agents are discontinued for various reasons, as shown in table 1, we postulated that it would not be appropriate to build a multivariate model for overall discontinuation from a medical point of view. In REAL, we did not collect measures of patients' disease activity, such as the disease activity score in 28 joints (DAS28), when patients stopped treatment with biological agents, and we could not define discontinuation due to LOE by using objective criteria. Therefore, we opted not to analyse risk factors for discontinuation due to LOE. The number of patients who discontinued the agents due to good control was too small to analyse associated factors using multivariate analysis.

Increasing age was also identified as a risk factor associated with the discontinuation of biological agents due to AE, data supported by a previous report.[16] In all three groups, infections were most frequent among AE leading to drug discontinuation (table 2). It is plausible that increasing age contributes to discontinuation because of an increasing risk of RA patients for infection[29 49] with age. Higher numbers of previous non-biological DMARD use suggests cases difficult to treat, with high disease activity or long-standing disease. Compatible with this possibility, patients who had been treated with three or more non-biological DMARD before enrolment in REAL had a significantly longer disease duration with more advanced disease stages and classes than those receiving less than three non-biological DMARD (data not shown). It has been reported that advanced stage or higher disease activity was reported as a risk for infections.[8 29 50]

Our study has limitations. First, we have to mention the possibility of selection bias in this study. However, because almost all patients who were registered from the participating hospitals of our study to the all-cases postmarketing surveillance programmes for each biological DMARD were enrolled in REAL, selection bias was substantially decreased. Second, we analysed the first biological agent administered to each patient at or after enrolment in REAL. However, these biological agents were not necessarily truly the first one used for each patient; rates of biological-naive patients were significantly different among the three groups (table 1), indicating the presence of channelling bias. Therefore, we adjusted for the previous use of biological agents in the multivariate analysis.

In conclusion, we have presented the first epidemiological data that directly compare TNF inhibitors and tocilizumab in a single cohort. We demonstrated that reasons for discontinuation were significantly different among the biological agents and that the use of infliximab and tocilizumab had a significantly higher risk of treatment discontinuation due to AE compared with etanercept after adjusting for various confounding factors.

Values are the mean±SD, unless otherwise stated. For univariate analysis, the χ^2 test for categorical variables and the Student's t test or Mann–Whitney test were used to compare continuous variables among groups.

Steinbrocker's classification[30] was used to definite RA disease stages and classes.

The immunosuppressive drugs used were tacrolimus, leflunomide, mizoribine and ciclosporin.

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The oral corticosteroid dose was converted to the equivalent prednisolone dosage. Methotrexate and corticosteroid doses are shown as the mean±SD among users of these drugs.

Chronic pulmonary diseases include interstitial pneumonia, chronic obstructive pulmonary disease, bronchial asthma, previous pulmonary tuberculosis and bronchiectasis.

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