the incidence and severity of infusion-related reactions (IRR)[11]. Second, as a humanized mAb, OCR may have lower immunogenicity than RTX, a chimeric mAb.

A 6-month, double-blind, phase I/II study of OCR (the ACTION study) was undertaken in the United States, enrolling patients with RA with an inadequate response to disease-modifying antirheumatic drugs (DMARD). The results of the ACTION study confirmed the clinical usefulness of OCR in combination with methotrexate (MTX)[12]. To investigate the dose-responsive effects of OCR in Japanese patients with RA, we conducted a 24-week, placebo-controlled, double-blind, phase II study of OCR with concomitant MTX treatment in Japanese patients with RA whose response to MTX had proved inadequate.

MATERIALS AND METHODS

Patients. Our study was conducted at 37 sites in Japan with approval from the Institutional Review Board at each participating site. Written informed consent was obtained from each patient participating in the trial. Our study was conducted in accord with the Declaration of Helsinki and the Good Clinical Practice guidelines, and was registered at ClinicalTrials.gov, NCT00779220.

Patients selected were ≥ 20 years old, fulfilled the American College of Rheumatology (ACR) 1987 revised criteria for RA[13], were rheumatoid factor (RF)-positive (> 20 IU/ml), showed an inadequate response to MTX at a dosage of 6–8 mg/week (maximum approved dose in Japan at that time: 8 mg/wk) for at least 12 weeks with a stable dose for the last 4 weeks before study treatment, had not used tocilizumab, infliximab, adalimumab, or leflunomide for at least 8 weeks before study treatment, and had used no other DMARD except MTX for at least 4 weeks before study treatment. Active disease was defined as swollen joint count ≥ 8 (66-joint count), tender joint count ≥ 8 (68-joint count), and either serum C-reactive protein (CRP) ≥ 1.5 mg/dl or erythrocyte sedimentation rate (ESR) ≥ 28 mm/h. Key exclusion criteria were additional autoimmune disorders, previous treatment with cell-depleting agents, neutrophil count < 1500/μl, platelet count < 100,000/μl, IgG or IgM less than the lower limit of normal (LLN), or hemoglobin < 8.5 g/dl.

Study design. This was a placebo-controlled, double-blind, multicenter, phase II study. The overall study design is illustrated in Figure 1. The subjects were randomly allocated into 4 groups, the OCR 50, 200, or 500 mg group, or the placebo group, in equal numbers and then given an infusion of their assigned investigational product on Days 1 and 15. Methylprednisolone 100 mg was given intravenously as premedication 30 min before administration of each investigational product. The use of oral antihistamine and acetaminophen 30 to 60 min before administration of investigational product was also permitted. Patients who were withdrawn from the double-blind period entered the safety followup period and were followed for at least 48 weeks from the first infusion of investigational product. This report includes the initial 24-week results.

All patients received uninterrupted stable dosages of MTX (6–8 mg/wk) and folate (≥ 5 mg/wk) from at least 4 weeks before the initiation of study treatment to the end of the study period. Concomitant use of a stable dosage of oral corticosteroid (prednisolone equivalent dose ≤ 10 mg/day) was permitted if the dosage was unchanged in the last 4 weeks before the study, and the concomitant use of nonsteroidal antiinflammatory drugs was also permitted if the dosage had not been changed within the last 2 weeks. Concomitant use of biological or nonbiological DMARD other than MTX was prohibited. The following rescue treatments were allowed from Week 8 at the investigator's discretion if control of disease activity was judged inadequate: increased MTX up to 8 mg/week, use of nonbiological DMARD, increase of oral corticosteroid, intraarticular administration of corticosteroid, intraarticular administration of hyaluronic acid preparation, and the use of 1 biological DMARD (excluding RTX).

Evaluation. Safety and efficacy were evaluated on Days 1 and 15, and every 4 weeks thereafter from Week 4 to Week 24 in the double-blind treatment period. During the safety followup period, safety and efficacy were evaluated every 12 weeks. The primary efficacy endpoint was the ACR 20% (ACR20) response rate at Week 24[14]. The ACR50 and ACR70 response rates and a reduction in the Disease Activity Score (DAS28-ESR) values[15] and European League Against Rheumatism (EULAR) response rates[16] over time up to Week 24 were calculated as secondary endpoints. The percentage of patients achieving DAS28-ESR remission (DAS28-ESR < 2.6) by Week 24 was investigated as exploratory analyses.

To evaluate safety, all adverse events (AE) that occurred during the study were recorded; their severity was judged using the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) Version 3.0. Serious AE (SAE) were defined using criteria from the International Conference on Harmonization. Serious infections (SI), defined as SAE infections or infections requiring intravenous antibiotic injection, were tabulated. Human anti-human antibody (HAHA) and serum immunoglobulin (IgG, IgM, and IgA) concentrations were also measured. To evaluate pharmacokinetics,

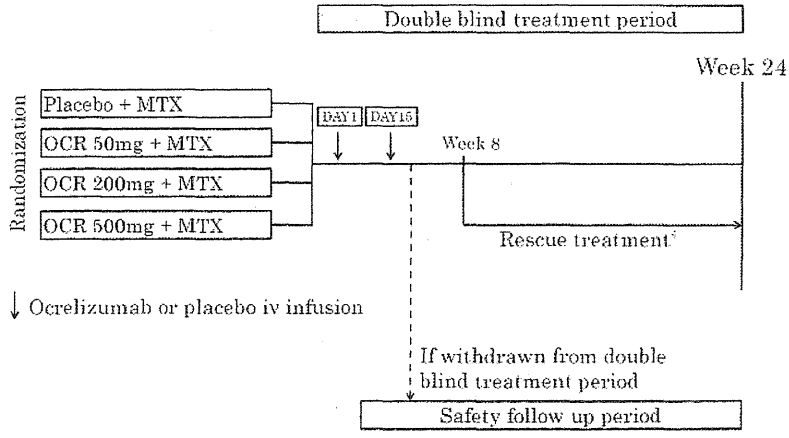

Figure 1. The study design. †Use of additional treatments for RA was permitted after Week 8 if control of disease activity was inadequate, at the discretion of the investigators or subinvestigators. OCR: ocrelizumab; MTX: methotrexate.

OCR concentration in serum was measured, and the number of CD19-positive cells in peripheral blood was measured using flow cytometry.

Statistical analyses. The target sample size was calculated based on the ACR20 response rate in the ACTION study. Using an allocation ratio of 1:2 (placebo vs combined OCR 200 and 500 mg groups), the ACR20 response rate in the combined OCR group of 48.7% and the placebo group of 24.3%, a 2-tailed significance level of 5%, and a power of 80%, the required sample size was calculated by the chi-square test to be 46 patients per group. Allowing for untreated patients, the target group size was set at 50 patients, giving a total target sample size of 200 patients. Calculation of the sample size was performed using nQuery Advisor Version 5.0 (Statistical Solutions Ltd., Farmer's Cross, Ireland).

While our study was in progress, an increased incidence of SI, including opportunistic infections, was reported in multinational clinical trials of OCR that were being conducted at the same time. Based on these safety reports, the enrollment of new patients and the administration of the investigational product in our study were halted, resulting in administration of investigational product to only 151 patients. The double-blind period was prematurely terminated in January 2010 and all patients entered a safety followup period.

The analysis of efficacy was performed using 145 patients (36 patients in the placebo group, 37 in the OCR 50 mg, 36 in the OCR 200 mg, and 36 in the OCR 500 mg), excluding 1 patient in the placebo group, 2 in the OCR 50 mg, and 3 in the OCR 200 mg group who did not receive the second infusion of investigational product because the study was stopped. We recalculated the statistical power and confirmed that it decreased from 80% to 69% with the same assumptions except for the number of patients. The analysis of safety was performed using 151 patients who received investigational products at least once. Safety data were evaluated up to 24 weeks from the first infusion of investigational product regardless of whether patients completed the double-blind period.

The ACR20 response rate at Week 24 (primary endpoint) and ACR50 and ACR70 response rates at Week 24 (secondary endpoints) in each OCR group were compared with the placebo group using the Cochran-Mantel-Haenszel test, accepting a 2-sided significance level of 5%. Based on the predefined analysis plan, descriptive statistics were calculated for the remaining endpoints, but no intergroup comparisons were performed. Adjusted mean changes in DAS28-ESR were based on the analysis of covariance using the baseline value as a covariate.

Efficacy data obtained after the day of rescue treatment or after the day when the decision to withdraw was made were handled as follows: categorical data (ACR responses, EULAR response rates, DAS28-ESR remission) were treated as "no response," continuous data (DAS28-ESR) as "missing data," and the last observation was carried forward.

RESULTS

Baseline characteristics and patient distribution. The mean RA disease duration of the patients in each group was 6.7–10.0 years. The patients had high RA disease activity with a mean DAS28-ESR of 6.3–6.5, a mean serum CRP level of 1.8–3.0 mg/dl, a mean ESR of 53.1–57.0 mm/h, and functional disabilities shown by a mean J-HAQ of 1.3–1.4. The mean MTX dosage was 7.3–7.6 mg/week. In each group, 25.6%–38.9% of the patients had previously received a biological DMARD (Table 1).

Including withdrawals because of the halt in administration of the investigational product, the patients who withdrew from the study before Week 24 numbered 6 in the placebo group, 10 in the OCR 50 mg, 11 in the OCR 200 mg, and 6 in the OCR 500 mg groups. The number of patients who withdrew because of insufficient response was 3 in the placebo group and none in the OCR groups. The proportion of patients receiving rescue treatments up to Week 24 was 32.4% in the placebo group, but lower in the

Table 1. Rheumatoid arthritis (RA) patient demographics and baseline disease characteristics (n = 151).

	Placebo, n = 37	OCR 50 mg, n = 39	OCR 200 mg, n = 39	OCR 500 mg, n = 36
Age, mean (SD), yrs	55.0 (12.1)	54.3 (10.9)	53.1 (10.9)	53.4 (10.3)
No. female (%)	27 (73.0)	30 (76.9)	33 (84.6)	29 (80.6)
RA duration, mean (SD), yrs	8.9 (7.8)	6.7 (7.1)	9.7 (8.1)	10.0 (9.3)
Steinbrocker stage, I/II/III/IV	5/8/9/15	3/15/10/11	3/10/4/22	6/6/13/11
Swollen joint counts (66 joints), mean (SD)	15.2 (6.1)	18.2 (9.7)	15.6 (8.8)	17.4 (9.7)
Tender joint counts (68 joints), mean (SD)	22.2 (11.6)	21.5 (12.3)	19.8 (9.7)	19.0 (9.9)
J-HAQ score, mean (SD)	1.4 (0.6)	1.4 (0.7)	1.3 (0.6)	1.3 (0.7)
CRP, mg/dl, mean (SD)	2.7 (2.7)	2.4 (2.7)	1.8 (1.5)	3.0 (2.8)
ESR, mm/h, mean (SD)	53.1 (29.0)	57.0 (29.0)	54.0 (26.6)	54.7 (31.3)
DAS28-ESR, mean (SD)	6.3 (0.9)	6.5 (0.8)	6.3 (0.8)	6.4 (0.9)
Anti-CCP antibody-positive, no. (%)	35 (94.6)	34 (87.2)	37 (94.9)	32 (88.9)
RF-positive, no. (%)	37 (100)	39 (100)	39 (100)	36 (100)
Corticosteroid use, no. (%)	23 (62.2)	23 (60.0)	28 (71.8)	18 (50.0)
Corticosteroid dose, mg/day, mean (SD)	5.4 (2.2)	5.1 (2.8)	5.2 (2.2)	6.3 (2.4)
MTX dose, mg/wk, mean (SD)	7.4 (0.9)	7.6 (0.8)	7.3 (1.0)	7.6 (0.8)
Previous use of biologics, no. (%)	11 (29.7)	10 (25.6)	11 (28.2)	14 (38.9)
Anti-TNF agent	10 (27.0)	10 (25.6)	10 (25.6)	13 (36.1)
Tocilizumab	2 (5.4)	0 (0.0)	1 (2.6)	3 (8.3)
Abatacept	0 (0.0)	0 (0.0)	1 (2.6)	0 (0.0)
Previous nonbiological DMARD (except for MTX), mean (SD)	1.8 (1.4)	1.4 (1.1)	1.8 (1.6)	1.7 (1.7)

OCR: ocrelizumab; RA: rheumatoid arthritis; TNF: tumor necrosis factor; DMARD: disease-modifying antirheumatic drug; J-HAQ: Japanese version of the Health Assessment Questionnaire; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS28: Disease Activity Score (28 joint count); CCP: cyclic citrullinated protein; RF: rheumatoid factor; MTX: methotrexate.

OCR groups: 12.8% in the OCR 50 mg, 7.7% in the OCR 200 mg, and 16.7% in the OCR 500 mg groups (Figure 2).

Safety. During the 24-week observation period, the incidence of AE was 59.5% in the placebo group and 79.5% in the OCR 50 mg, 79.5% in the OCR 200 mg, and 61.1% in the OCR 500 mg groups (Table 2). The majority of AE were infections and IRR. An IRR was defined as an AE occurring during or within 24 hours after administration of investigational product.

The proportion of subjects experiencing at least 1 infection was 18.9% (7/37) in the placebo group and 37.7% (43/114) in the OCR groups combined. There were no patients with SI in the placebo group and 7 in the OCR groups combined. There were 4 SI in 2 patients in the OCR 50 mg group, consisting of 1 incident each of herpes zoster, pneumonia, sepsis, and septic shock. Six SI occurred in 4 patients in the OCR 200 mg group, 2 incidents of *Pneumocystis jirovecii* pneumonia (PCP), and 1 each of sepsis, herpes simplex, bacterial pneumonia, and febrile neutropenia. There was 1 SI (epididymitis) in 1 patient in the OCR 500 mg group.

Two incidents of malignant tumors (uterine cancer and ovarian cancer) in 1 patient in the OCR 500 mg group were reported, which were diagnosed 153 days after the first infusion of OCR. There were no intergroup differences in the incidences of other AE.

There were 2 deaths during our study. One was a 61-year-old man who had concurrent depression and hypertension, and a history of cerebral infarction and cerebral hemorrhage. He developed pneumonia and sepsis 67 days after administration of OCR 500 mg followed by septic shock, disseminated intravascular coagulation, and multiorgan failure and died the following day. The other death was a 64-year-old man in the placebo group; he died of acute respiratory failure after withdrawal from the study because of insufficient response. No definitive diagnosis was made and an autopsy was not performed.

The increase in incidences of IRR following the first administration (Day 1) of investigational product was dose-dependent: 0% in the placebo group and 15.4% in the OCR 50 mg, 20.5% in the OCR 200 mg, and 25.0% in the OCR 500 mg groups. Following the second administration (Day 15) of investigational product, the incidence of IRR was markedly decreased in all 3 OCR groups (Table 2, Figure 3), 2.9% in the OCR 50 mg, 6.1% in the OCR 200 mg and 8.8% in the OCR 500 mg groups. All patients, except for 1 in the OCR 500 mg group, who experienced an IRR at the second administration also had an IRR at the first administration. Of the 26 IRR, 4 were moderate (NCI CTC Grade 2) and 22 were mild (NCI CTC Grade 1). One patient in each of the OCR 200 mg and the OCR 500 mg groups withdrew from the study because of IRR.

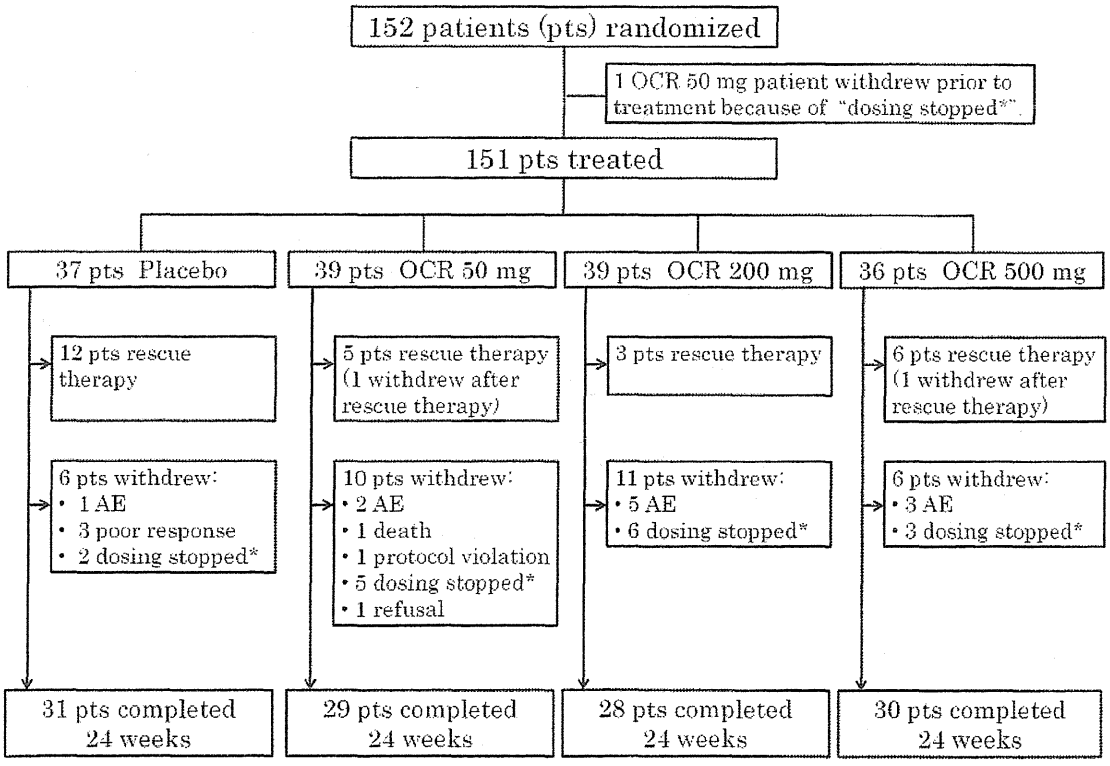

Figure 2. Disposition of patients with RA at Week 24. *Dosing of investigational product was stopped, patients were withdrawn from the study, and enrollment of new patients was halted because of the increased incidence of serious infections, including opportunistic infections, reported in other multinational clinical studies of ocrelizumab (OCR). AE: adverse event.

Table 2. Summary of adverse events (AE) in the safety analysis population of patients with rheumatoid arthritis (n = 151) during the 24-week observation period. Values are the number (%) of patients.

	Placebo, n = 37	OCR 50 mg, n = 39	OCR 200 mg, n = 39	OCR 500 mg, n = 36
Any AE	22 (59.5)	31 (79.5)	31 (79.5)	22 (61.1)
Serious AE	3 (8.1)	2 (5.1)	7 (17.9)	5 (13.9)
AE leading to withdrawal	1 (2.7)	2 (5.1)	5 (12.8)	3 (8.3)
Infection	7 (18.9)	16 (41.0)	16 (41.0)	11 (30.6)
Serious infection	—	2 (5.1)	4 (10.3)	1 (2.8)
Infusion-related reactions	2 (5.4)	6 (15.4)	8 (20.5)	10 (27.8)
Serious infusion-related reactions	—	—	—	1 (2.8)
All AEs affecting ≥ 5% of patients				
Pharyngitis	1 (2.7)	3 (7.7)	1 (2.6)	1 (2.8)
Nasopharyngitis	2 (5.4)	1 (2.6)	3 (7.7)	0
Bronchitis	0	2 (5.1)	3 (7.7)	0
Upper respiratory tract infection	0	1 (2.6)	2 (5.1)	1 (2.8)
Herpes zoster	0	2 (5.1)	1 (2.6)	1 (2.8)
Cystitis	0	0	2 (5.1)	1 (2.8)
P. jirovecii pneumonia	0	0	2 (5.1)	0
Infusion-related reaction	2 (5.4)	6 (15.4)	8 (20.5)	10 (27.8)
Pyrexia	1 (2.7)	2 (5.1)	0	0
Hepatic function abnormal	1 (2.7)	2 (5.1)	2 (5.1)	4 (11.1)
Constipation	1 (2.7)	2 (5.1)	1 (2.6)	0
Stomatitis	2 (5.4)	0	0	0
Upper abdominal pain	0	0	0	2 (5.6)
Urticaria	0	2 (5.1)	0	1 (2.8)
Drug eruption	0	0	2 (5.1)	0
Headache	0	0	1 (2.6)	3 (8.3)
Conjunctivitis	0	2 (5.1)	0	0

OCR: ocrelizumab.

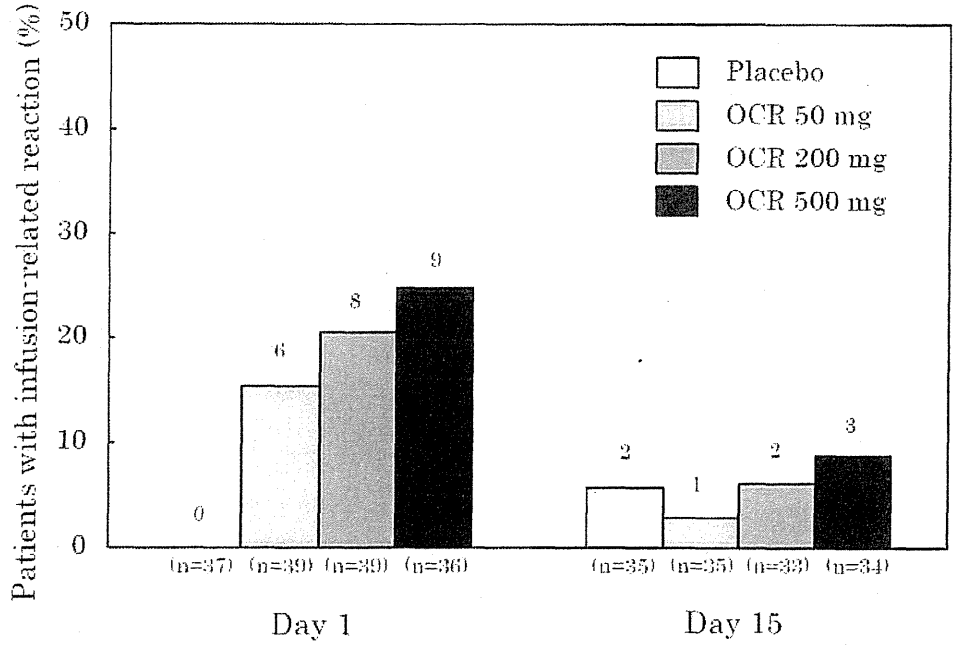

Figure 3. Incidence of infusion-related reactions at Days 1 and 15. Patient numbers shown here represent patients in each group at Days 1 and 15. OCR: ocrelizumab.

No placebo group patients became HAHA-positive during the study, but 2 patients in the OCR 50 mg group and 1 each in the OCR 200 mg and OCR 500 mg groups were HAHA-positive. An IRR occurred in 1 of the 4 patients who became HAHA-positive; this patient developed the IRR at the first administration prior to the expression of HAHA. No serious AE occurred in any HAHA-positive patient. Two of the 4 HAHA-positive patients achieved ACR20 at Week 24, and 1 achieved ACR70. A comparison of HAHA-positive and HAHA-negative patients showed no consistent difference in serum OCR concentration during the study period. The presence of HAHA did not appear to influence either efficacy or safety outcomes.

Efficacy. The ACR20 response rates at Week 24, the primary endpoint, in the OCR groups were significantly higher than the 25.0% of the placebo group [OCR 50 mg: 54.1% ($p = 0.0080$), OCR 200 mg: 55.6% ($p = 0.0056$), OCR 500 mg: 47.2% ($p = 0.044$)]. The ACR50 responses at Week 24 were 16.7% for the placebo group, 37.8% for the OCR 50 mg, 38.9% for the OCR 200 mg, and 30.6% for the OCR 500 mg groups. The ACR50 response rates in the OCR 50 mg and OCR 200 mg groups were significantly higher than those in the placebo group ($p = 0.038$, $p = 0.031$, respectively). The ACR20, ACR50, and ACR70 response rates over time are shown in Figure 4 A-C. The adjusted means (± SE) of the ΔDAS28-ESR, the good responses rates using the EULAR response criteria, and the DAS28-ESR clinical remission rates (DAS28-ESR < 2.6) of the OCR groups at Week 24 were better than those of the placebo groups (Figure 4D, 4E).

Pharmacodynamics. Although the number of CD19-positive cells increased transiently in the placebo group following intravenous administration of methylprednisolone as premedication on Days 1 and 15, the number remained stable through Week 24. In all 3 OCR-treated groups, the number of CD19-positive cells in peripheral blood decreased rapidly after the first administration of OCR; that effect was maintained throughout the 24-week study period (Figure 5). The proportion of patients in whom the number of CD19-positive cells had recovered to at least LLN (80 cells/μl) or the baseline value, whichever was lower, by Week 24 was 80.6% in the placebo group and 6.9% in the OCR 50 mg, 3.4% in the OCR 200 mg, and 0% in the OCR 500 mg groups.

DISCUSSION

Our double-blind placebo-controlled study demonstrated the safety profile of OCR in Japanese patients with RA. The OCR clinical development program in patients with RA was terminated because the risk of SI outweighed the clinical benefits observed in patients with RA, based on the data from our trial and multinational clinical trials of OCR.

In our study, the majority of AE were IRR and infections, and the incidence of IRR was consistent with results reported for anti-CD20 antibodies[7,8,12]. Characteristic IRR symptoms in the OCR group were hypertension in 7 patients (6.1%), headache in 5 (4.4%), pyrexia in 4 (3.5%), and pruritus in 4 (3.5%); these results did not differ from previous studies of OCR or RTX.

By Week 24, SI had occurred only in the OCR group. In the OCR groups combined, the 7 patients who developed SI and the 107 patients who did not develop SI had comparable baseline white blood cell (WBC), neutrophil, and lymphocyte counts and immunoglobulin (IgG, IgM, and IgA) levels. The WBC, neutrophil, and immunoglobulin levels did not fall below LLN [WBC < 3900/μl; neutrophils < 1500/μl; IgG < 870 mg/dl; IgM < 33 mg/dl (males), < 46 mg/dl (females); IgA < 10 mg/dl] in any of the 7 patients with SI during our study, but the lymphocyte count did fall below 500/μl during the study period in 2 patients with SI. In the OCR groups combined, 2 of the 9 patients (22%) whose lymphocyte counts fell below 500/μl developed SI, while 5 of the 105 patients (4.8%) with lymphocyte count > 500/μl developed SI. Among the SI, PCP occurred in 2 patients in the OCR 200 mg group. At the onset of PCP, both patients exhibited pyrexia, hypoxemia, pulmonary ground-glass opacity, and increased serum ß-D-glucan levels, and 1 patient was positive on the polymerase chain reaction test for *P. jirovecii*. Both patients recovered with methylprednisolone pulse therapy and trimethoprim-sulfamethoxazole. Advanced age, concurrent lung disease, and concomitant corticosteroid use have been reported as risk factors for bacterial pneumonia or SI including PCP during treatment of Japanese RA patients with tumor necrosis factor (TNF) inhibitors[17,18,19,20,21]. In our study, 6 of the 7 patients with SI were using prednisolone concomitantly, but only 3 of the 7 patients with SI were over 60 years of age (3 were in their 40s and 1 in her 20s) and none had concurrent lung disease. Similarly, both patients who developed PCP in our study were taking 7.5 mg/day oral prednisolone, but both were 42 years old and did not have concurrent lung disease. Further, their lymphocyte counts at onset of PCP were decreased to 651 and 890/μl, respectively. These results suggest that risk factors for SI, including PCP, during OCR treatment may differ from those during treatment with TNF inhibitors.

Although there were differences of sample size, patient background, and observation period, the incidence of SI in our study (6.1%) was comparable to the results of other Japanese clinical trials of biologic agents: 7.6% in a 52-week study of tocilizumab in patients with inadequate response to DMARD (SAMURAI study)[22]; 3.3% in a 24-week study of tocilizumab in patients with inadequate response to MTX (SATORI)[23]; 5.2% in a 54-week study of infliximab in patients with inadequate response to MTX (RISING)[24]; and 4.9% in a 24-week study of adalimumab in patients with inadequate response to DMARD (CHANGE)[25]. PCP was observed in 2 patients (1.75%) in our study, but in the SAMURAI, SATORI, and CHANGE studies, no PCP was observed. Further, the incidence of PCP in our study was

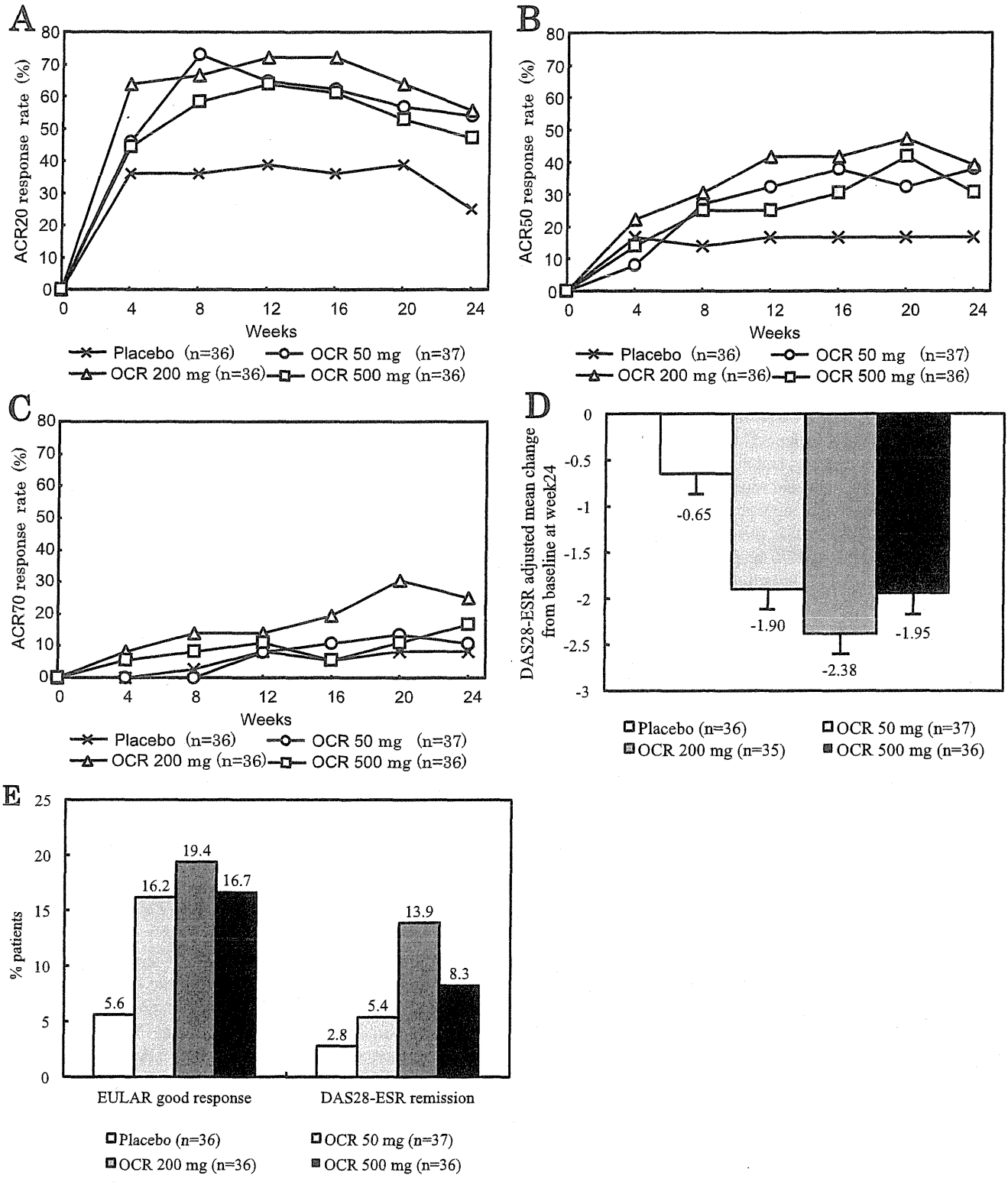

Figure 4. Clinical efficacy of ocrelizumab (OCR). A. ACR20 response rate over time. B. ACR50 response rate over time. C. ACR70 response rate over time. Patients receiving rescue therapy or withdrawing from the study were classified as nonresponders. D. DAS28-ESR mean changes from baseline at Week 24. Error bars represent standard error of the mean. E. The proportion of patients achieving a good response according to the EULAR criteria and remission according to DAS28-ESR. ACR: American College of Rheumatology; DAS28: Disease Activity Score (28 joint count); ESR: erythrocyte sedimentation rate; EULAR: European League Against Rheumatism.

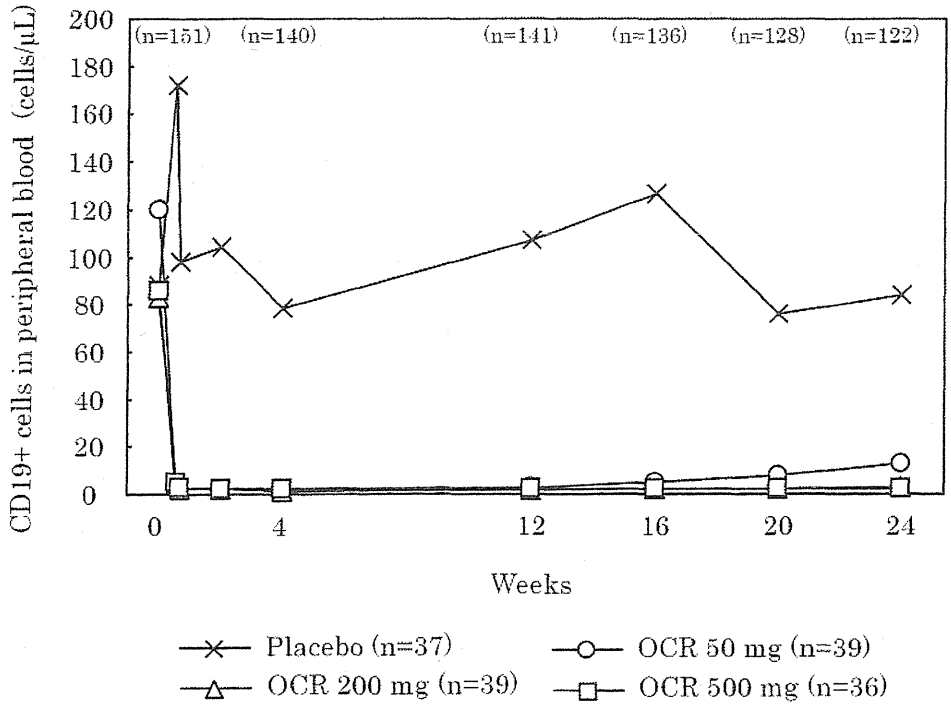

Figure 5. Median peripheral blood CD19-positive B cell counts over time. The lower limit of normal (LLN) was 80 cells/μl. The patient numbers shown with each investigational group represent the number of patients in that group at Time 0. Numbers shown above the different timepoints represent total number of patients in the study at that timepoint. OCR: ocrelizumab.

higher than those in the Japanese postmarketing surveillance data of biologic agents (tocilizumab, infliximab, and etanercept), that is, 0.2% to 0.4%[17,18,19,26]. These results suggest that treatment with OCR in Japanese patients with RA may have higher risk for PCP than treatment with the other biologic agents.

A possible association of efficacy with B cell depletion was reported in patients with RA treated with OCR in the ACTION study[12], which ascertained that B cell depletion was maintained until Week 24 in groups that received ≥ 200 mg OCR, but was not maintained in groups with lower dosages. Significant improvements of signs and symptoms of RA shown by relatively stringent response criteria, including ACR70 response, DAS28-ESR clinical remission, and EULAR good response, were also obtained only in groups that received ≥ 200 mg OCR[12]. The clinical response to RTX has been reported to be determined by the level of B cell depletion rather than by the dose of the drug[27]. In our study, the percentages of patients with peripheral blood B cell count at Week 24 that was above LLN or baseline values were 6.9%, 3.4%, and 0% in the OCR 50, 200, and 500 mg groups, respectively. The OCR 200 mg group showed higher clinical responses than the other 2 OCR groups in every efficacy criterion used in our study. In addition, the peripheral B cell count recovered to at least the LLN (80 cells/μl) or the baseline value in 4 patients in the OCR groups combined, but these patients showed sustained efficacy through Week 24. It is difficult to draw firm conclusions because of the small number of patients with B cell recovery and the limited study period (24 weeks), but these results suggest that peripheral B cell count alone may not account for maintenance of efficacy in patients with RA treated with OCR.

As a limitation of our study, we note the dosage of MTX. The mean MTX dosage in each group was 7.3–7.6 mg/week, which was lower compared to clinical trials of OCR for RA conducted in some Western countries. The approved maximum dose of MTX was 8 mg/week in Japan when this trial was implemented and we had to design the trial under this restriction. This should be taken into account when interpreting our results.

Serious infections, including PCP, occurred only in the combined OCR groups in our study, possibly indicating an elevated risk for SI from OCR use in Japanese patients with RA. Treatment with OCR resulted in better clinical responses than treatment with the placebo in Japanese RA patients with an inadequate response to MTX, about 30% of whom had been previously treated with a biological DMARD. Although we should take into account the small sample size and the premature termination of the study, these results would suggest an inappropriate benefit-risk balance for OCR in this patient population. Because of the lack of

approval for RTX for RA in Japan and the recommended use of the drug for patients with RA who have failed TNF inhibitor therapy in Western countries, it will be necessary to investigate the safety and efficacy of other anti-B cell therapies in Japanese patients with RA.

ACKNOWLEDGMENT

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APPENDIX

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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, $p = 0.005$).

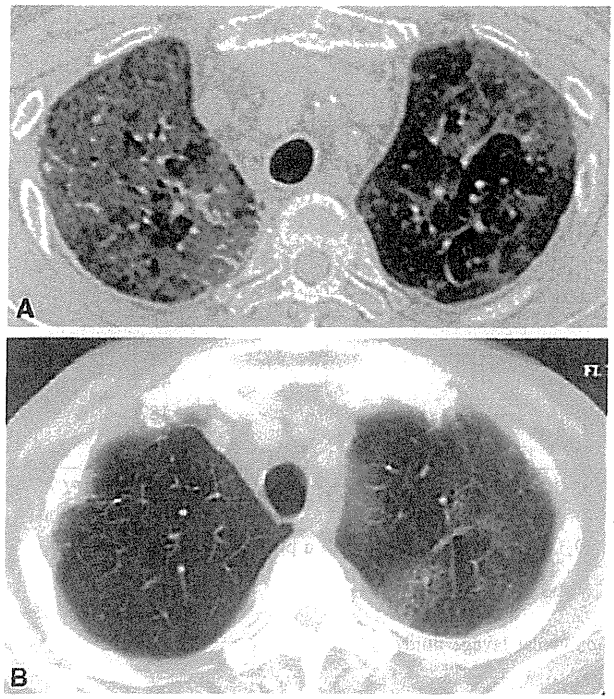

Fig. 1 Thoracic computed tomography findings of rheumatoid arthritis patients who developed *Pneumocystis jirovecii* pneumonia while receiving etanercept. **a** Ground-glass opacity (GGO) with sharp demarcation by interlobular septa and geographic pattern. **b** GGO without interlobular septal boundaries

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

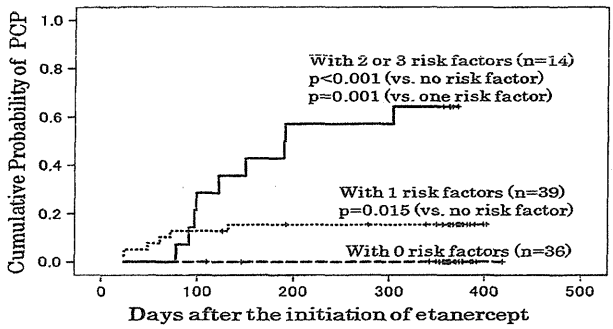

Fig. 2 Cumulative probability of developing *Pneumocystis jirovecii* pneumonia (*PCP*) in rheumatoid arthritis patients with associated risk factors when treated with etanercept. The patients were stratified by the number of risk factors, including age ≥65 years, coexistence of lung disease, and concomitant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

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

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

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

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