A Followup Study of Asymptomatic Carriers of *Pneumocystis jiroveci* During Immunosuppressive Therapy for Rheumatoid Arthritis

SHUNSUKE MORI, ISAMU CHO, and MINEHARU SUGIMOTO

ABSTRACT. ***Objective.*** To examine the preventive effects of prophylaxis against *Pneumocystis jiroveci*-induced pneumonia (PCP) in patients receiving immunosuppressive therapy for rheumatoid arthritis (RA) who are colonized by this organism.

Methods. We performed molecular testing by polymerase chain reaction (PCR) for *P. jiroveci* on induced sputum or bronchoalveolar lavage fluids of 82 patients with RA. During primary prophylaxis, asymptomatic carriers of this organism were examined by high-resolution computed tomography and PCR every 2 weeks. RA patients who had developed PCP received PCR tests every week. Once negative results were obtained, PCR testing was scheduled at Months 1, 3, and 6, followed by reexaminations every 6 months.

Results. We found 9 cases of asymptomatic carriage of *P. jiroveci*. All the carriers had received low doses of methotrexate. Upon introduction of PCP prophylaxis, 5 cases tested negative for PCR within 1 month. Three carriers developed PCP before starting prophylaxis, but these tested negative for PCR after short periods (1–2 weeks) of PCP treatment. Once *P. jiroveci* was eradicated, all cases maintained negative PCR results during followup without prophylactic intervention, even after resuming immunosuppressive therapy. One patient refused PCP prophylaxis, but no PCP developed.

Conclusion. RA patients with asymptomatic carriage of *P. jiroveci* benefited from short-term prophylaxis against PCP. Positive PCR results appeared to be predictive of future development of PCP in RA patients. Identification of *P. jiroveci* carriers will encourage prompt introduction of PCP prophylaxis when rheumatologists consider immunosuppressive therapy for RA. (J Rheumatol First Release June 15 2009; doi:10.3899/jrheum.081270)

Key Indexing Terms:
PNEUMOCYSTIS JIROVECI RHEUMATOID ARTHRITIS COLONIZATION
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Pneumocystis jiroveci pneumonia (PCP) has been recognized as a rare opportunistic infection exclusively occurring in immunocompromised hosts, such as patients with acquired immunodeficiency syndrome. However, a growing number of studies have reported PCP occurring in patients treated with cytotoxic or immunosuppressive drugs for malignancy, organ transplant, or other disorders[1-5]. Indeed, it has been reported in Japan that the use of methotrexate (MTX) or prednisolone for patients with connective tissue disease (CTD) is related to susceptibility to PCP[6-9]. A postmarketing surveillance report by the Japan College of Rheumatology also indicated a high incidence of PCP in patients with rheumatoid arthritis (RA) receiving infliximab, an anti-tumor necrosis factor-α (anti-TNF-α) agent, in combination with MTX[10].

Molecular testing by polymerase chain reaction (PCR) on induced sputum has proven to be a sensitive, specific, and noninvasive technique for detecting very low copies of *P. jiroveci* in the lungs, in quantities that are undetectable by conventional microscopic examination[9,11]. Recently, we performed PCR examinations on 55 patients with RA and found that 6 (10.9%) had asymptomatic carriage of *P. jiroveci*[8]. One month after the detection of *P. jiroveci*, one patient developed PCP during treatment for RA with low-dose MTX and prednisolone. These findings may suggest that asymptomatic carriers of this fungus are at a high risk of developing PCP when treated with immunosuppressive agents.

Despite the high mortality rate of patients with rheumat-

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ic disease who develop PCP, in clinical practice, these cases do not routinely receive prophylaxis against PCP, even when clinicians consider combination therapy with prednisolone, MTX, and biological agents. To examine the efficacy of prophylactic use of antibiotics for the prevention of PCP development in patients with RA who are colonized by this organism, we followed up 9 cases of asymptomatic carriage of *P. jiroveci* who were receiving immunosuppressive therapy for RA, using molecular testing with PCR.

MATERIALS AND METHODS

Patients. In our previous study, we performed PCR examinations using induced sputum from 47 patients with RA who had no complaints of respiratory symptoms. We also used bronchoalveolar lavage (BAL) fluids from 8 patients with RA who required diagnostic bronchoscopy for their pulmonary symptoms and whose symptoms were ultimately not diagnosed as PCP[8]. Six of the 55 patients were asymptomatic carriers of *P. jiroveci* (Cases 1–4, 6, and 9 in Table 1). We examined another 27 patients with RA by PCR of induced sputum; of these, 3 cases were found to be colonized by *P. jiroveci* (Cases 5, 7, and 8 in Table 1). Patients who had shown respiratory distress or radiological findings suggestive of development of PCP at the time of enrollment were excluded. In summary, the specimens were collected consecutively from a total of 82 patients with RA who visited our outpatient clinic between March 2005 and September 2008 and voluntarily participated in this study (BAL fluids from 8 patients and induced sputum from 74 patients). All participants fulfilled the 1987 American College of Rheumatology (ACR) criteria for diagnosis of RA. Regardless of whether they had a possible risk factor for *P. jiroveci* infection such as advanced age, preexisting pulmonary disease, or administration of immunosuppressive drugs, patients were enrolled consecutively. Further, patients were not selected based on their reasons for visit, including regular checkups, treatment for RA, RA complications, or other reason. Patients were excluded from this followup study if they had developed PCP at the study enrollment or they were suspected of developing PCP at that time. No patient was known to be HIV-positive. The ethics committee of our hospital approved the protocol for the followup study of RA patients with *P. jiroveci* carriage, and written consent was obtained from all patients.

PCR for P. jiroveci. Sputum was induced by nebulization with 10 ml of distilled water and then collected for examinations[9]. DNA samples were extracted from induced sputum or BAL samples and subsequently subjected to PCR as templates. The PCR analysis for *P. jiroveci* was performed in 50 μl of an amplification reaction mixture, with denaturation at 94°C for 90 s, annealing at 50°C for 90 s, and extension at 72°C for 2 min (40 cycles). The following oligonucleotide primers were used at 100 pmol: 5'-GAT GGC TGT TTC CAA GCC CA-3' and 5'-GTG TAC GTT GCA AAG TAC TC-3'. DNA products with lengths of 376 bp were amplified from template DNA. This analysis was performed at SRL, Inc. (Tachikawa, Japan). The details of this method and its usefulness for detection of *P. jiroveci* have been described by Wakefield, *et al*[12,13].

Followup on asymptomatic carriers of P. jiroveci. During primary prophylaxis, patients with asymptomatic carriage of *P. jiroveci* were examined every 2 weeks: they were checked for pulmonary symptoms, their oxygen saturation was measured and other laboratory data were gathered, and PCR tests of induced sputum as well as high-resolution computed tomography (HRCT) scans were performed. Patients who had developed PCP were hospitalized and subjected to PCR tests of induced sputum and HRCT scans every week during treatment for this pneumonia. For a final diagnosis of PCP development, we performed PCR tests using BAL fluids. Once negative PCR results were obtained from a particular patient who had been colonized by *P. jiroveci* and/or had developed PCP, similar examinations were scheduled at Months 1, 3, and 6, followed by reexaminations every 6 months.

RESULTS

We performed PCR examinations of specimens from 82 patients with RA (BAL fluids from 8 patients and induced sputum from 74 patients). Enrolled patients were predominantly female (57 women and 25 men), with a mean age of

Table 1. Characteristics of asymptomatic carriers of *P. jiroveci* with RA who participated in the followup study.

Patient	Age/Sex	RA Duration, yrs	DAS28	Anti-RA Drugs	Treatment Duration, mo	Preexisting Lung Disease	Lymphocyte Count, μl	Complications
1	70 F	1	5.1	MTX 8 mg/wk	12	None	729	HT
2	78 M	2	3.5	MTX 10 mg/wk	12	None	1158	HT
				Tacrolimus 2 mg/day	3			ASO
3	80 M	3	6.7	MTX 8 mg/wk	8	NSIP	1060	HT
				PSL 5 mg/day	8			
4	80 F	8	3.4	MTX 6 mg/wk	39	Pulmonary hemorrhage	1303	HT
				PSL 5 mg/day	48			NIDDM*
				Tacrolimus 1 mg/day	1			
5	58 F	22	4.5	MTX 8 mg/wk	66	Bronchiolitis	799	HT
				Tacrolimus 1 mg/day	18			
6	76 F	7	4.1	MTX 8 mg/wk	12	None	901	None
				Tacrolimus 1 mg/day	3			
7	62 F	2 mo	5.2	MTX 8 mg/wk	2	Inflammatory scar	640	HT
				PSL 5 mg/day	2			Hypothyroidism
8	74 F	5	5.9	MTX 8 mg/wk	24	None	952	None
				PSL 5 mg/day	24			
9	66 M	1	2.9	MTX 8 mg/wk	12	None	586	NIDDM**
				PSL 5 mg/day	12			

All data were measured at the time of initial detection of *P. jiroveci*. * Case 4 was receiving insulin therapy, but HbA1c remained high (7.4). ** NIDDM in Case 9 had not been treated (HbA1c, 7.9). DAS28: Disease Activity Score for 28 joints; MTX: methotrexate; PSL: prednisolone; NSIP: nonspecific interstitial pneumonia; HT: hypertension; ASO: arteriosclerosis obliterans; NIDDM: non-insulin-dependent diabetes mellitus.

65.8 years (range 28–89 yrs) and a mean RA duration of 6.2 years (range 2 mo–30 yrs). Percentages of rheumatoid factor (RF)-positive and anti-cyclic citrullinated peptide antibody (anti-CCP)-positive patients were 86.6% and 93.9%, respectively. Patients who participated in this study were radiographically classified as follows: Steinbrocker stage I (22 patients), II (13), III (23), and IV (24). They were functionally grouped into the following classes: Steinbrocker class I (51 patients), II (27), III (4), and IV (0). On HRCT scans, 8 cases had a nonspecific interstitial pneumonia (NSIP) pattern and 5 cases showed a bronchiolitis pattern. Average counts of white blood cells and lymphocytes were 6996/μl and 1151/μl, respectively. All the patients were receiving at least 1 immunosuppressive drug. Numbers of patients taking MTX and prednisolone were 62 and 50, respectively. Twelve cases were taking anti-TNF-α agents.

The PCR tests revealed 9 cases (11.0%) of asymptomatic carriage of *P. jiroveci* (Table 1). Two positive results were obtained from PCR tests using BAL samples (Cases 3 and 4) and the other 7 from those with induced sputum (Cases 1, 2, and 5–9). There were no significant differences in demographic or clinical characteristics between patients with and those without detectable *P. jiroveci*. The mean age of the RA patients with positive PCR results was older than that of the negative patients, although this difference was not significant (71.6 vs 65.1 yrs; p = 0.12 Mann-Whitney U test). All the carriers had received low doses of MTX (6–10 mg/week), and 8 cases had been treated simultaneously with prednisolone (5 mg/day) and/or tacrolimus (1–2 mg/day). Serum levels of ß-D-glucan in 6 asymptomatic carriers were available (Cases 2, 4, 5, and 7–9). With a single exception (Case 4, 20 pg/ml), the values of serum ß-D-glucan were below 10 pg/ml. Serum levels of lactate dehydrogenase (LDH) were within the normal range in all the carriers of *P. jiroveci*. Forty-two healthy volunteers (21 men and 21 women, mean age 50.8 ± 5.9 yrs) were also examined for the presence of *P. jiroveci* DNA in induced sputum, and no positive PCR results were obtained.

To prevent the development of PCP during therapy for RA, we decided to eliminate this organism in 5 patients (Table 2) using oral administration of trimethoprim-sulfamethoxazole (TMP-SMX) for 4 cases (Cases 2–5) and intravenous drip of pentamidine isethionate for 1 case (Case 1). Case 1 was not given TMP-SMX as primary prophylaxis against PCP because this patient showed an elevated level of serum creatinine. The prophylactic therapy was started within 2 weeks after the patients tested positive for *P. jiroveci* by PCR. MTX, prednisolone, and other immunosuppressive agents were all discontinued during prophylaxis against PCP. Two weeks later, 3 cases (Cases 1, 2, and 4) tested negative for *P. jiroveci* by PCR analysis of induced sputum. One month after the start of prophylactic treatment, the other 2 cases (Cases 3 and 5) obtained negative PCR results. At this point we stopped the prophylactic antibiotic treatment and resumed the same therapy for RA that had been used before. These cases have not developed PCP to date; they have continued to test negative for *P. jiroveci* by PCR for 15–27 months (Table 2). Case 6 refused prophylactic use of antibiotics and did not receive regular checkups, but she has not developed PCP in the 13 months thereafter.

The remaining 3 among the 9 asymptomatic carriers developed PCP before starting prophylactic intervention (Cases 7–9, Table 2). All 3 developed PCP within 1 month after testing positive for *P. jiroveci* by PCR. These patients' respiratory symptoms were not severe. At the onset, their oxygen saturation was 96%–98% at rest, but it dropped to 91%–94% after mild exercise. HRCT scans showed extensive interstitial infiltrates in all cases. A considerable amount of ß-D-glucan (> 100 pg/ml) was detected in all patient sera, while serum LDH concentrations remained within the normal range. Case 9 exhibited a low lymphocyte count (728/μl), but that in Case 8 was within the normal range (1317/μl). These 3 patients tested positive for *P. jiroveci* by PCR of both induced sputum and BAL fluids. We suspended immunosuppressive therapy for RA and started treatment against PCP with TMP-SMX, together with intravenous methylprednisolone drip (40 mg/day for 5 days) and oral prednisolone (started at 30 mg/day and tapered to 7.5 mg/day). After short periods of PCP treatment (1–2 weeks), all 3 obtained negative PCR results, and the abnormalities seen on their HRCT scans improved. Frequent followup examinations have revealed that HRCT improvement in response to TMP-SMX is associated with negative PCR results. Cases 7 and 8 are now being treated with MTX and prednisolone for RA at the same doses that had been used before. In Case 9, MTX therapy was suspended halfway through the followup because of development of acute hepatitis B, and this patient is now being treated with 5 mg/day of prednisolone alone. None of these cases has developed PCP to date, and negative PCR results have been maintained for 6–18 months (Table 2).

DISCUSSION

Prophylaxis against PCP is effective, as evidenced by a dramatic reduction in morbidity and mortality from this pneumonia in individuals infected with human immunodeficiency virus (HIV)[14,15]. However, guidelines for the administration of prophylaxis to non-HIV-infected immunocompromised patients, especially patients with connective tissue diseases (CTD) who are receiving immunosuppressive agents, remain less clear[1,16,17]. PCP prophylaxis often induces severe adverse effects such as myelosuppression. In addition, a combination of MTX and TMP-SMX may not be viable since it may induce pancytopenia[18]. It is therefore necessary to identify the patients whose risk of developing PCP is great enough to warrant prophylaxis in spite of the adverse effects.

Recently, the Japanese Ministry of Health, Labor and

Table 2. PCP prophylaxis and outcomes in *P. jiroveci* carriers during immunosuppressive therapy for RA.

Patient	Primary Prophylaxis (dosages, duration)	Clinical Course	Treatment for PCP (dosages, duration)	Outcomes
1	PI (150 mg/day, 10 days)	No PCP/neg PCR for 27 mo	—	—
2	TMP-SMX* (4 tablets/day, 2 wks)	No PCP/neg PCR for 15 mo	—	—
3	TMP-SMX (1 tablet/day, 1 mo)	No PCP/neg PCR for 19 mo	—	—
4	TMP-SMX (3 tablets, 2 wks)	No PCP/neg PCR for 7 mo**	—	—
5	TMP-SMX (1 tablet/day, 1 mo)	No PCP/neg PCR for 15 mo	—	—
6	None	No PCP for 13 mo	—	—
7	None	Developed PCP 2 weeks later	TMP-SMX† (9 tablets/day for 4 days then 6 tablets/day for 1 wk)	Responded No PCP/neg PCR for 6 mo
8	None	Developed PCP 1 mo later	TMP-SMX†† (6 tablets/day, 2 wks)	Responded No PCP/neg PCR for 6 mo
9	None	Developed PCP 1 mo later	TMP-SMX (12 tablets/day, 1 wk)	Responded No PCP/neg PCR for 18 mo

* Case 2 had agranulocytosis 2 weeks later as an adverse effect of TMP-SMX. ** Case 4 choked on food and died. † Case 7 needed a reduced dosage 4 days after starting treatment because of nausea and vomiting. †† Case 8 developed rash 2 weeks after starting treatment. PI: pentamidine isethionate; TMP-SMX: trimethoprim-sulfamethoxazole. One tablet of TMP-SMX contains 80 mg TMP and 400 mg SMX.

Welfare Study Group recommended the use of TMP-SMX or pentamidine isethionate as a prophylactic against PCP for (1) patients with CTD over the age of 50 years receiving corticosteroids equivalent to ≥ 1.2 mg/kg/day prednisolone; (2) those receiving corticosteroids equivalent to ≥ 0.8 mg/kg/day prednisolone and immunosuppressive agents; or (3) those whose peripheral lymphocyte counts are < 500/μl during immunosuppressive therapy[19]. Reports from Western countries similarly recommend that PCP prophylaxis be given (when not contraindicated) to patients receiving prolonged systemic corticosteroid therapy at a level equivalent to ≥ 16–20 mg prednisolone daily who also have an underlying immunosuppressive condition[4,20]. Meanwhile, Harigai, *et al* reported that even low doses of prednisolone contribute substantially to the increased risk of PCP in patients with RA during infliximab therapy[21]. Further, it has been noted that the use of low-dose MTX is also a predisposing factor for PCP development in patients with CTD, even when dosages of prednisolone are low or when patients are not treated with any corticosteroids[2,5,8,22].

PCP is usually considered an opportunistic infection, that is, one that occurs under immunocompromised conditions such as HIV infection; accordingly, preventive measures are recommended for HIV-infected individuals with peripheral CD4+ lymphocyte counts < 200/μl[23]. Patients with CTD and PCP are observed to have lymphocytopenia just as HIV-infected individuals do[9,24]. At the same time, PCP often occurs in non-HIV patients with peripheral lymphocyte counts > 500/μl or with circulating CD4+ lymphocyte counts > 200/μl[6,7,22,25,26]. Thus, peripheral lymphocyte counts > 500/μl and/or CD4+ lymphocyte counts > 200/μl may not always guarantee prevention of this pneumonia in non-HIV patients receiving immunosuppressive drugs for underlying diseases. In our study, all the *P. jiroveci* carriers with RA maintained lymphocyte counts > 500/μl while receiving low-dose MTX with or without low-dose prednisolone, but even so, 3 developed PCP before primary prophylaxis against PCP could be instituted. These findings support the idea that asymptomatic carriers of *P. jiroveci* should be recognized as having a high risk of developing PCP during immunosuppressive therapy for RA even if they maintain lymphocyte counts > 500/μl and even if they do not receive high-dose prednisolone.

Rheumatologists need to know when PCP prophylaxis can be safely discontinued and the immunosuppressive therapy for the underlying disease restarted. Unfortunately, the optimal duration of prophylaxis among patients with CTD has not been evaluated thoroughly. However, we showed that latent *P. jiroveci* can be eliminated from patients with RA by a very short course of prophylaxis against PCP (2–4 weeks). No development of PCP has been observed in the 15–27 months since this organism disappeared from the carriers, even after MTX therapy for RA was restarted. Four carriers did not receive prophylaxis; among these, 3 cases were diagnosed as having developed PCP within 1 month after the first detection of *P. jiroveci*. No recurrence of this

pneumonia was seen in RA patients who received TMP-SMX treatment for 1–2 weeks, even though secondary prophylaxis was withheld and immunosuppressive therapy was restarted. Similarly, Saito, *et al* observed that in almost all their patients with PCP and CTD, *P. jiroveci* disappeared within 7–10 days after commencement of TMP-SMX treatment, and no recurrence of this pneumonia was observed[9]. Godeau, *et al* also reported that, during followup of 22 months on average, no relapse of PCP was seen among survivors who had been treated with TMP-SMX for a mean of 17 days, even though they continued to receive immunosuppressive drugs for CTD without secondary prophylaxis[27]. By contrast, Suryaprasad and Stone[28] reported 3 cases of PCP that occurred in patients with autoimmune diseases after tapering or discontinuation of immunosuppressants although the patients had received primary prophylaxis against PCP during the immunosuppressive therapy. They suggested, therefore, that the need for PCP prophylaxis can extend for months beyond the time when patients receive intensive immunosuppressive therapy. The discrepancy in these observations may be due to variation in the extent of immunosuppression achieved in the different studies: all patients in the latter study[28] had profound lymphopenia at the onset of PCP (peripheral lymphocyte counts 0–158/μl), while none of our cases developed such severe lymphopenia during immunosuppressive therapy.

It has long been debated whether PCP development is due to a reactivation of latent infection or to *de novo* acquisition by person-to-person transmission[29-31]; this is an important issue when we consider short-term prophylaxis against PCP in clinical practice. In our study, 3 asymptomatic carriers of *P. jiroveci* receiving low doses of MTX and prednisolone for RA developed PCP before starting primary prophylaxis. This finding suggests that reactivation of latent infection is one of the causes of PCP in RA patients during immunosuppressive therapy. Under the reactivation theory, it seems reasonable that short-term treatment with TMP-SMX is effective in preventing new or recurrent development of PCP, as we have reported in this study and as others have reported[9,27]. In apparent contrast, new development of PCP can occur after stopping primary prophylaxis in patients with more severe immune dysfunction than our cases, as recently reported[28]. These cases may result from new acquisition of this fungus through environmental exposure. In our study, *P. jiroveci* DNA were not detected during followup in patients from whom this organism had been eliminated; however, the possibility that they may be subject to reinfection remains, as long as the immunosuppressive condition persists.

ß-D-glucan, one of the major components of fungus cell walls, has been recognized as a practical and reliable serological marker for diagnosing PCP[32,33]. We noted that serum levels of ß-D-glucan are even lower in RA patients who are asymptomatic carriers of *P. jiroveci* than they are in those developing PCP[6,9,26]. Considering that ß-D-glucan is a quantitative assay for *P. jiroveci,* the negative results for this marker may simply reflect smaller numbers of colonizing species in the lungs of asymptomatic carriers.

We performed PCR examinations on respiratory samples (BAL fluids from 8 patients and induced sputum from 74 patients), and found 9 cases (11.0%) having asymptomatic carriage of *P. jiroveci*. If all PCR tests had been done using BAL samples, the prevalence of asymptomatic colonization would have been much higher. For ethical reasons, however, it is impossible to justify performing bronchoscopy on patients with no pulmonary symptoms. Studies have revealed that PCR using induced sputum is highly sensitive[9,11], and the procedure for inducing sputum is noninvasive, which makes induced-sputum PCR look like a useful test for identification of asymptomatic carriers of *P. jiroveci*, even among healthy individuals[30]. PCR-based detection of this organism in induced sputum will lead to prompt introduction of PCP prophylaxis when rheumatologists consider immunosuppressive therapy for RA that is expected to enhance the risk of PCP.

Patients with RA with latent infection of *P. jiroveci* are at increased risk of developing PCP during immunosuppressive therapy, even if they maintain lymphocyte counts above 500/μl or do not receive high doses of prednisolone. Early detection of asymptomatic carriage of *P. jiroveci* and its eradication with short-term prophylaxis are apparently beneficial to individuals who receive immunosuppressive therapy for RA.

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