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CASE REPORT

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Pneumocystis jiroveci pneumonia in a patient with rheumatoid arthritis as a complication of treatment with infliximab, anti-tumor necrosis factor α neutralizing antibody

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Abstract We report that a-63-year-old woman developed *Pneumocystis jiroveci* pneumonia (PCP) as a complication from treatment with infliximab for rheumatoid arthritis. Although there was neither symptoms of dyspnea nor typical observations on a chest X-ray examination, low levels of oxygen saturation and findings of high-resolution chest computed tomographic scanning suggested a possibility of interstitial pneumonia. A polymerase chain reaction-based detection of *Pneumocystis jiroveci* in induced sputum allowed an early diagnosis of PCP and subsequent effective treatment.

Key words Infliximab · *Pneumocystis jiroveci* · Polymerase chain reaction · Rheumatoid arthritis (RA) · Tumor necrosis factor

Introduction

Tumor necrosis factor α (TNFα) is a pleiotropic cytokine mainly produced by macrophages and lymphocytes. It has been recognized that an overproduction of TNFα probably causes serious chronic synovitis and joint destruction in rheumatoid arthritis (RA). The introduction of TNF-blocking agents is reported to dramatically improve the outcome of RA.[1–4] Infliximab is a chimeric mouse/human monoclonal antibody against human TNFα and etanercept is a recombinant human TNF receptor p75 dimer attached to the Fc domain of human IgG_1. Both are most commonly used worldwide as the TNF-blocking agent, and their uses are approved in Japan for RA therapy. A combination of methotrexate (MTX) and infliximab or etanercept is more effective compared with each treatment alone in RA patients.[2–4]

Tumor necrosis factor α is known to induce expressions of adhesion molecules and cytokines, both important mediators of inflammation. Further, TNFα plays a pivotal role in cellular immunity, suggesting that the inhibition of TNFα activities may increase a risk of intracellular microorganism-induced infectious diseases.[5–8] First, an increased risk of tuberculosis, especially extrapulmonary and/or disseminated types, has been reported in infliximab-treated patients.[9] Further, serious infections caused by *Listeria monocytogenes*, *Coccidioides immitis*, *Mycobacterium avium*, and *Pneumocystis jiroveci* (formerly called *Pneumocystis carinii*) have been noticed in patients under TNF blockade therapy.[5–8] Thus TNFα is very likely to be essential for an elimination of these microorganisms, and the use of TNF-blocking agents is associated with the increased risk of opportunistic infection. A rapid detection of these microorganisms is therefore needed to minimize serious infections in the course of TNF blockade therapy.

More recently, the detection of *P. jiroveci* using a polymerase chain reaction (PCR) technique has been established with specimens from induced sputum. This method may improve the clinical diagnosis of *P. jiroveci* pneumonia (PCP).[10] Since a delay in the diagnosis of PCP increases the risk of death, sensitive and specific detection of *P. jiroveci* is essential (U.S. Public Health Service: Consensus Statement on the Use of Corticosteroids as Adjunctive Therapy for *Pneumocystis* Pneumonia in the Acquired Immunodeficiency Syndrome, http://www.cdc.gov). Here we report the early diagnosis of PCP in an RA patient receiving infliximab therapy, using the PCR-based detection of *P. jiroveci* DNA.

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Case report

A 63-year-old woman who had seropositive, anti-cyclic citrullinated peptide antibody-positive and HLA-DR4-positive RA for 8 years was referred to our hospital for a routine medical check in the course of infliximab treatment. She had an ongoing active disease in spite of 8 mg/week of MTX treatment. Four weeks prior to referral, she received a first infusion of infliximab (3 mg/kg) and she had a further infusion of the same dosage 2 weeks before admission. Before the second infusion of infliximab, the therapy for RA was successful, namely, the disease activity score for 28 joints was decreased from 6.8 to 4.6. The values of serum C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were below 0.3 mg/dl and 30 mm/h, respectively.

When she visited our clinic for a routine medical check of infliximab therapy, the CRP value in serum was 2.01 mg/dl, regardless of a decrease in the number of swollen and tender joints. She revealed no clinical symptoms such as dyspnea, fever, or dry cough, except for slight general fatigue. A chest X-ray film showed an obscure margin of cardiac shadow at the left lower lobe, and there was no greater change compared with that taken before the infliximab therapy (Fig. 1). Despite lacking obvious clinical symptoms and chest X-ray findings, the patient had a low level of oxygen saturation (91%), and her arterial blood gas measurement on room air yielded pH 7.466, PaO_2 56.8 mmHg, and $PaCO_2$ 37.7 mmHg. Moreover high-resolution chest computed tomographic scans (HRCTs) demonstrated distant mosaic patterns (Fig. 2A,B). Serum level of lactate dehydrogenase (LDH) was elevated (336 IU/l). Other hematological and biochemical data were normal.

Since in Japan *P. jiroveci* is supposed to be one of the major causal agents of interstitial pneumonia during infliximab therapy (postmarketing surveillance [PMS] report in Japanese by Japan College of Rheumatology [JCR], http://www.ryumachi-jp.com), we discontinued MTX treatment and started the oral administration of trimethoprim–sulfamethoxazole (TMP-SMX, 3200 mg of TMP and 640 mg of SMX). To make an early diagnosis of PCP, we performed PCR for *P. jiroveci*. Template DNAs were extracted from induced sputum and bronchoalveolar lavage (BAL) samples, using an Amplicor Respiration Specimen Preparation kit (Roche Molecular Systems, Bellville, NJ, USA), and 25 µl of samples were subjected to PCR as templates. Oligonucleotide primers (5′-GAT GGC TGT TTC CAA GCC CA-3′ and 5′-GTG TAC GTT GCA AAG TAG TC-3′) were used at 100 pmol in 50 µl of amplification reaction mixtures, with denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 90 s, for 40 cycles. Amplified products were subjected to electrophoresis on 2% agarose gels. On the day following admission, considerable amounts of DNA products with lengths of 376 bp were amplified from DNA isolated from both induced sputum and BAL samples (Fig. 3). These data are indicative of the existence of *P. jiroveci* in this patient. Concerning interstitial pneumonia markers, the serum levels of surfactant protein-A (SP-A) and surfactant protein-D (SP-D), but not KL-6, were elevated. High-resolution computed tomography findings were consistent with those seen in PCP.[11] In consideration of all these data, we reached the final diagnosis of PCP, and TMP-SMX treatments were continued for this patient. A large amount of β-D-glucane (123.6 pg/ml), a composition of fungus cell walls, was detected in patient serum. This result also supported our diagnosis.

The clinical course is shown in Fig. 4. The treatment with TMP-SMX was successful without the use of steroid pulse therapy and the distant mosaic pattern shown in HRCT on admission was improved (Fig. 2C,D). On day 13, the levels of SP-A, SP-D, and LDH decreased to within the normal range, and improvement of oxygenation and normalization of serum CRP levels were seen. Serum β-D-glucane levels on days 13 and 27 were 240.4 and 109.4 pg/ml, respectively. On day 3 of the TMP-SMX treatment, the oral administration of these agents was changed to drip infusion because of their adverse effects, such as severe nausea and vomiting.

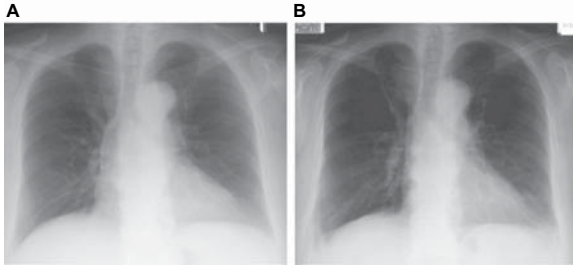

Fig.1. A Chest X-ray taken before first infusion of infliximab. **B** Chest X-ray on admission. An obscure cardiac border at left lobe is seen

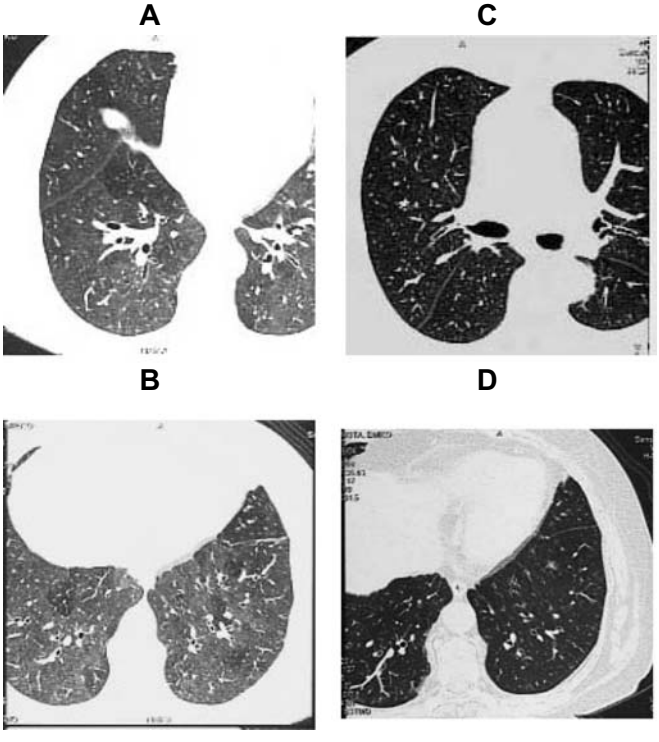

Fig. 2. A,**B** High-resolution computed tomography scans on admission show distant mosaic patterns. **C**,**D** These patterns disappeared after treatment with trimethoprim–sulfamethoxazole

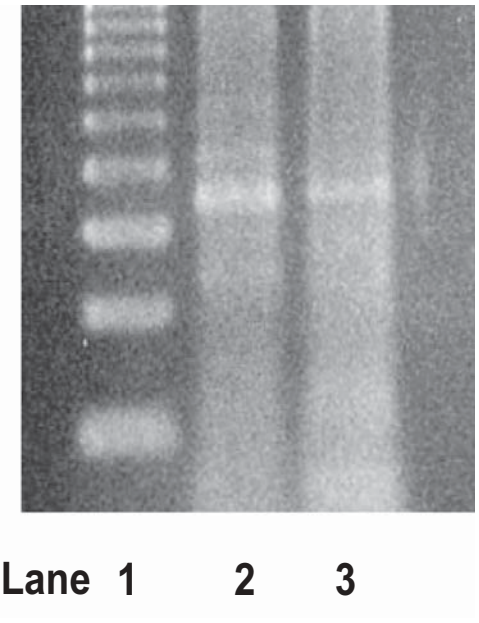

Fig. 3. Polymerase chain reaction for *Pneumocystis jiroveci*. *Lane 1*, 100 bp molecular weight markers. A total of 376 bp of DNA products were amplified from DNA prepared from induced sputum (*lane 2*) and BAL samples (*lane 3*).

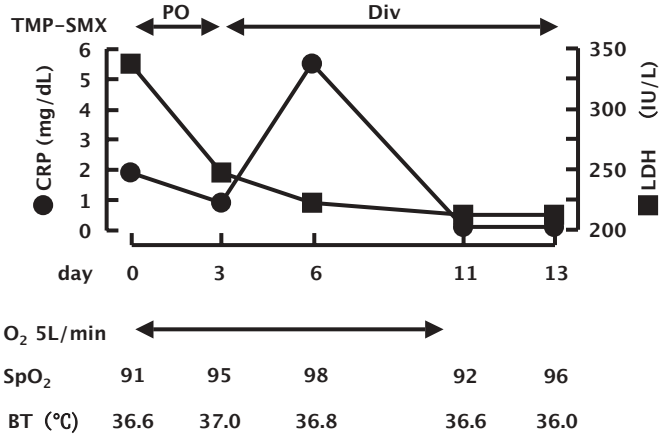

Fig. 4. Clinical course of our patient during trimethoprim–sulfamethoxazole (*TMP-SMX*) therapy. *PO*, oral administration; *Div*, drip infusion; *CRP*, C-reactive protein; *LDH*, lactate dehydrogenase; *BT*, body temperature

Discussion

Pneumocystis jiroveci pneumonia has been recognized as a rare disease occurring in immunocompromised hosts, such as malignancy and collagen disease patients. In this report, PCP was present in an RA patient as a complication of the infliximab therapy. Initially, an increased risk of tuberculosis was predicted, since Japan is an epidemic area of tuberculosis. On the other hand, data from recent PMS for infliximab in Japan indicated a high incidence of PCP. Of 4000 RA patients treated with infliximab, 15 cases were complicated with PCP (PMS report in Japanese by JCR, http://www.ryumachi-jp.com). In Western countries, PCP is a relatively uncommon infectious disease associated with infliximab therapy,[12,13] and several cases of PCP during this therapy have been reported.[14–17]

Pneumocystis jiroveci pneumonia is well known to be the most frequent complication seen in acquired immunodeficiency syndrome (AIDS) patients.[18,19] A decrease in numbers of $CD4^+$ T cells is strongly associated with the risk of developing PCP in full-blown HIV patients. Ninety percent of episodes occurred at total counts of less than 200/μl $CD4^+$ T cells, suggesting that T cells play critical roles in suppressing PCP development. However, infliximab-treated patients do not exhibit a low number of circulating $CD4^+$ T cells.[20] In our case, no severe decrease in lymphocyte count was observed. The decreased number of $CD4^+$ T cells might not be involved in the development of PCP in infliximab-treated patients.

A blocking of the TNFα function may provoke PCP. It has been proposed that TNFα and interferon-γ (IFNγ) play essential roles in the elimination of *P. jiroveci* through reactive nitrogen intermediates.[21] *Pneumocystis jiroveci* stimulates alveolar macrophages to produce TNFα, which mediates the IFNγ-dependent generation of reactive nitrogen intermediates. Further, an involvement of the TNFα-dependent expression of adhesion molecules, ICAM-1 and VCAM-1, are shown in the elimination of *P. jiroveci* in the neonatal mouse model.[22] Neutralization of TNFα through TNF blockade by infliximab may therefore possibly cause the occurrence of PCP.

Furthermore, it is possible that a loss of activated monocytes/macrophages may be the main cause of the development of PCP associated with infliximab therapy. Tumor necrosis factor α is expressed as a membrane-bound form and subsequently it is released into extracellular spaces by a TNFα-converting enzyme on cellular membranes.[23] Since immune cells produce both membrane and soluble forms of TNFα, infliximab may induce apoptosis in membrane TNFα-expressing cells, in a caspase-dependent manner. In fact, synovial specimens isolated from infliximab-treated patients revealed that macrophages, but not lymphocytes, fall into apoptosis.[24] The loss of membrane TNFα-expressing macrophages may contribute to the development of PCP.

It has been long discussed whether the development of PCP is due to a reactivation of latent infection or acquisition by person-to-person spread.[18,19] In our case, PCP occurred 4 weeks after the second infliximab infusion. Data from PMS in Japan for infliximab showed that a median interval from the start of treatment to the occurrence of PCP was 74.4 days and that all episodes occurred within 22 weeks (http://www.ryumachi-jp.com), suggesting that PCP may develop through a reactivation of latent infection rather than through a new exposure to environmental microorganisms. Similarly, tuberculosis in infliximab-treated individuals is thought to be the result of a reactivation of latent infection because most cases of tuberculosis occur within 12 weeks after an introduction of infliximab therapy.[9] Cush mentioned in a review that the risk of infectious death is greatest in the first 16 weeks after drug initiation.[7] These reports support the idea that rheumatologists

should be on the lookout for occurrence of opportunistic infections, particularly during the first 6 months after initial infliximab infusion. In contrast, several studies showed the possibility that a person-to-person infection may be involved in the occurrence of PCP.[25,26] A genotype analysis of *P. jiroveci* isolates will provide a good tool for further investigation.

Infliximab therapy always carries the risk of opportunistic infections.[5–9] Isoniazide is effective as a prophylactic for tuberculosis associated with TNF-blocking therapy.[27] The use of TMP-SMX as a prophylactic is recommended for collagen disease patients receiving high-dose steroid therapy in Japan.[28] However, the preventive use of TMP-SMX against *P. jiroveci* is impossible during the MTX and infliximab treatment, since a combination of TMP-SMX and MTX is a contraindication. Furthermore, with the decrease of oxygenation and/or the alveolar–arterial oxygen difference, the risk of death is increased (U.S. Public Health Service: Consensus Statement on the Use of Corticosteroids as Adjunctive Therapy for *Pneumocystis* Pneumonia in the Acquired Immunodeficiency Syndrome, http://www.cdc.gov). Therefore, a rapid and sensitive detection of *P. jiroveci* is important for a good prognosis. Saito et al. stressed the usefulness of PCR for the early diagnosis of PCP, since this method consists of simple and easy techniques and is superior to cytology in sensitivity.[10] Durand-Joly et al. mentioned in a review that the sensitivity of PCR to diagnose PCP, using specimens from induced sputum, oropharyngeal washings, and nasopharyngeal aspirates was more than 90%, and that PCR targeting the mitochondrial ribosomal RNA (mt rRNA) was superior in its sensitivity, since mt rRNA is present in many copies in each microorganism.[29] In this study, we used the oligonucleotide primers specific for mt rRNA. Nested PCR assays and a Southern blot analysis for PCR products can also increase the sensitivity of detection. Regarding the specificity of PCR, several studies showed that respiratory samples reveal a higher number of positive PCR results, but a lower predictive value of PCP occurrence.[30–33] Most of these individuals had no development of PCP during a follow-up period of many months. They might be colonized by *P. jiroveci*. These problems will be resolved by careful assessments of patient conditions, based on clinical, radiological, and laboratory data.

PMS data for infliximab in Japan also show frequent development of noninfectious interstitial pneumonia during this therapy (PMS report in Japanese by JCR, http://www.ryumachi-jp.com). Kramer et al. reported an occurrence of MTX pneumonia after initiation of infliximab therapy for RA.[34] We need to differentiate PCP from MTX pneumonia during infliximab therapy. A typical clinical symptom of MTX pneumonia is dyspnea with fever. High levels of serum LDH and CRP are noticed in laboratory findings.[35] Further, radiological findings show ground-glass opacity.[35] These findings are similar to these observed in PCP. In this report, we confirmed the presence of *P. jiroveci* by using PCR assays. Moreover, the measurement of serum β-D-glucane value is helpful for a diagnosis of PCP because β-D-glucane is a part of the fungus cell wall. In our case, high levels of serum β-D-glucane were detected.

In conclusion, we succeeded in the early diagnosis of PCP in an infliximab-treated patient by using the PCR-based detection of *P. jiroveci*, which led to the proper and prompt management of PCP. This patient made satisfactory progress. We would like to emphasize that the use of PCR for *P. jiroveci* using a specimen from induced sputum is a great help for the diagnosis of PCP and the management of RA patients treated with TNF-blocking agents.

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