Cerebral Toxoplasmosis Diagnosed by Nested-polymerase Chain Reaction in a Patient with Rheumatoid Arthritis

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Abstract:
A 65-year-old woman with rheumatoid arthritis (RA) visited our hospital because of right facial sensory hypoesthesia. Cerebral toxoplasmosis was suspected on brain magnetic resonance imaging. We discontinued methotrexate for RA and started a sulfamethoxazole/trimethoprim (ST) mixture. Although ST treatment was interrupted because of adverse reactions, her prognosis was favorable. The Toxoplasma 18S rDNA gene was detected by nested-polymerase chain reaction (PCR) from blood and cerebrospinal fluid. Detecting the Toxoplasma 18S rDNA gene by nested-PCR is useful for the diagnosis and safer than a brain biopsy. In addition, the discontinuation of immunosuppressants may be recommended in patients compromised by those immunosuppressants.

Key words: cerebral toxoplasmosis, rheumatoid arthritis, nested-PCR, methotrexate

Introduction
Toxoplasmosis is one of the most common opportunistic infections caused by the protozoan Toxoplasma gondii, presenting with neurological and ocular manifestations. Toxoplasmosis is a self-limited disease in an immunocompetent host (1). However, in immunocompromised individuals, cerebral toxoplasmosis is a well-recognized life-threatening complication (1). A definite diagnosis of cerebral toxoplasmosis is usually difficult and can be established by direct detection of the parasite on a brain biopsy, although the procedure is invasive and sometimes impossible depending on the site of the disease.

We herein report a rheumatoid arthritis (RA) patient with cerebral toxoplasmosis who had been medicated with an immunosuppressant for six years; she was diagnosed by a noninvasive method, the nested-polymerase chain reaction (PCR). The patient showed a favorable prognosis after the discontinuation of immunosuppressive therapy.

Case Report
A 65-year-old woman with well-controlled RA visited the emergency department of our hospital because of sensory hypoesthesia on the right side of her face that had persisted for the previous two days. As emergent brain magnetic resonance imaging (MRI) excluded acute cerebral infarction and revealed a high-intensity signal on T2-weighted and Fluid attenuated Inversion Recovery (FLAIR) images at the medulla oblongata to inferior cerebellar peduncle (Fig. 1a), we suspected an infectious disease or malignant tumor.

Serology for human immunodeficiency virus (HIV) was negative. The levels of soluble interleukin-2 receptor (711

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U/mL; normal: 124–466 U/mL), lactate dehydrogenase (LDH) (298 IU/L; normal: 100-225 IU/L), and C-reactive protein (CRP) (1.571 mg/dL; normal: 0-0.3 mg/dL) were slightly high. Other laboratory findings were mostly within their normal ranges. However, elevated levels of serum anti-Toxoplasma antibodies (IgM: 1.3 IU/mL; normal: <0.8 IU/mL, IgG: 18 IU/mL; normal: <6 IU/mL) and rheumatoid factor (1,014 U/mL; normal range: <15 U/mL) were noted (Fig. 2). The Toxoplasma IgG avidity index was 0.68, indicating chronic infection (chronic infection: >0.35) (2). Cell numbers, cytology, proteins, and glucose in the cerebrospinal fluid (CSF) were within their normal ranges. The CSF was negative for anti-Toxoplasma antibodies. Whole-body contrast-enhanced computed tomography (CT) and gallium scintigraphy showed no abnormalities.

She had taken care to avoid eating raw or undercooked meat. She had also avoided contact with kittens and gardening. Considering that she had been medicated with methotrexate (immunosuppressant; 8 mg/week) for 6 years for RA, we suspected that the reactivation of toxoplasmosis had occurred due to the immunocompromised condition. Therefore, on day 22, we discontinued methotrexate and treated her with a sulfamethoxazole/trimethoprim (ST) mixture (trimethoprim: 360 mg/day) simultaneously, as pyrimethamine and sulfadiazine, the most common chemotherapy used against symptomatic toxoplasmosis, are unapproved in Japan at present. However, the ST treatment was administered for only eight days because she experienced adverse reactions, such as multiple rashes. Despite this short-term anti-Toxoplasma treatment, the sensory hypoesthesia and ring-enhanced lesion gradually improved (Fig. 1c, 2). Serum anti-Toxoplasma antibodies were still high (IgM: 1.6 IU/mL, IgG: 22 IU/mL) on day 64 (Fig. 2).

Nestled PCR for amplification of the T. gondii 18S rDNA gene was performed as described previously (3). The primers used were as follows: for the first-round PCR, forward (5’-CCATGCATGTCTAAGTATAAGG-3’) and reverse (5’-GTTACCCGTCACTGCCAC-3’); for the second-round PCR, forward (5’-CTAAGTATAAGCTTTTATACGGC-3’) and reverse (5’-TGCCACCGTAGTTACCAATAC-3’). The thermal profile consisted of 40 amplification cycles of a 1-minute denaturation at 94°C, 1-minute primer annealing at 42°C, and 1-minute primer extension at 72°C and it was performed in a PCR Thermocycler (Takara Bio, Kusatsu, Japan). In the second-round PCR reaction, the amplification mixtures consisted of 1/50 of the first-round product. The Toxoplasma 18S rDNA gene was detected in the DNA extracted from frozen-stored blood and CSF sampled just prior to ST treatment (Fig. 3). In the CSF, only one out of nine samples was positive, indicating that the copy number of T. gondii DNA

Figure 1. MRI. (a) FLAIR imaging at her first visit showing an abnormal signal at the medulla oblongata to the inferior cerebellar peduncle. (b, c) Gadolinium-enhanced T1-weighted imaging 11 (b) and 35 (c) days after symptom onset. FLAIR: fluid attenuated inversion recovery
in the CSF was very low. We were therefore finally able to obtain a definitive diagnosis of cerebral toxoplasmosis.

At two months after discharge, methotrexate was restarted because of RA deterioration. However, as the liver function subsequently worsened, this drug was stopped at 20 months after discharge. The patient now has almost no neurological symptoms. The clinical course and laboratory data are shown in Fig. 2.

**Discussion**

Data on the prevalence and incidence of toxoplasmosis in immunosuppressed, non-transplant, and HIV-negative patients are lacking, and decisions on the optimum treatment and utility of prophylaxis have yet to reach a consensus (4). Table summarizes seven cases of toxoplasmosis with RA involving patients who had received several immunosuppressants to control RA reported in the English and Japanese literature, including the present case (5-9). In general, treatment against symptomatic toxoplasmosis involves a combination of pyrimethamine and sulfadiazine plus folinic acid for at least four to six weeks after the resolution of signs and symptoms (1). Only two authors discontinued immunosuppressants and started treatments such as pyrimethamine and sulfadiazine plus folinic acid for six and eight weeks, respectively, for toxoplasmosis (6, 8). However, there have been no reports in which only the discontinuation of immunosuppressants led to improvement of the toxoplasmosis.

In the present case, the patient’s symptoms improved soon after the discontinuation of methotrexate and initiation of

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**Figure 2.** Clinical course. IgM (closed triangle), anti-*T. gondii* immunoglobulin M antibody in the serum; IgG (closed circle), anti-*T. gondii* immunoglobulin G antibody in the serum. ST: sulfamethoxazole/trimethoprim, RA: clinical activity of rheumatoid arthritis.

**Figure 3.** Nested-PCR products of *Toxoplasma* 18S rDNA amplified from blood (lane 1) and aliquots of CSF (lanes 2-10) were electrophoresed along with a negative control (N), positive control (P), and size marker (M). The amplified band was detected in the blood and 1 aliquot (lane 3) of 9 CSF samples. Arrows: 291 bp. PCR: polymerase chain reaction, CSF: cerebrospinal fluid.
### Table. Summary of Reported Cases of Toxoplasmosis Associated with Rheumatoid Arthritis.

| Patient number | Age (yrs), sex | Immuno suppressive drugs for RA | Clinical findings | anti- *T. gondii* IgM and IgG, IgG avidity | MRI and/or CT findings | Methods to diagnose toxoplasmosis | Toxoplasmosis lesions | Therapy for toxoplasmosis | Adverse reactions to anti-toxoplasmic treatment | Prognosis | HIV | Ref. number |
|----------------|----------------|---------------------------------|-------------------|------------------------------------------|------------------------|------------------------------|------------------------|---------------------|-----------------------------------------------|------------|------|------------|
| 1              | 36, F          | prednisone, methotrexate, leflunomide, infliximab | headache, shurred speech, weakness in the left face and the left arm, a grand mal seizure | anti- *T. gondii* IgM: negative (serum) anti- *T. gondii* IgG: positive (serum) | Two lesions within the right hemisphere on FLAIR Two peripherally enhanced lesions in the right hemisphere | Brain biopsy | Brain | Pyrimethamine, folinic acid, and dapsone (because of allergy against sulfa) | - | Improved | Negative | 5 |
| 2              | 43, M          | methotrexate, prednisone, leflunomide, infliximab | sudden onset of decreased vision of the left eye | anti- *T. gondii* IgM: strongly positive (serum and aqueous humor) | No abnormalities | PCR of aqueous humor | Eye | Pyrimethamine, sulfadiazine, and folinic acid for 6 weeks, Discontinuation of anti-TNF-α | Not improved because of a scar due to toxoplasmic choriorretinitis | Negative | 6 (case 1) |
| 3              | 40, F          | prednisone, methotrexate, etanercept | vision loss in the lower quadrant of the left eye | anti- *T. gondii* IgM: negative (serum) anti- *T. gondii* IgG: positive (serum) | No abnormalities | Fluorescein angiography | Eye | Pyrimethamine, sulfadiazine, and folinic acid | Moderately improved | Negative | 6 (case 2) |
| 4              | 60, F          | prednisolone, methotrexate | no symptom | anti- *T. gondii* IgM: negative (serum, CSF) anti- *T. gondii* IgG: positive (serum, CSF) IgG avidity index: high | Lesions in the right thalamus, both pallida, and left cerebellar dentate nucleus High intensity on T1WI Low intensity but the border is high, and the inside is heterogeneous on T2WI and FLAIR | MRI Multiplex-PCR of CSF | Brain | Sulfamethoxazole/trimethoprim (ST) mixture for 10 weeks but no improvement. So, the ST mixture was discontinued, and pyrimethamine + sulfadiazine were started. But these were not continued because of side effects. Then, the ST mixture was restarted. | No change | Mildly improved on MRI | Negative | 7 |
| 5              | 67, M          | adalimumab | headache, bilateral peripheral facial palsy, mild left hemiparesis right sided weakness, shurred speech, right facial droop | anti- *T. gondii* IgM: negative (serum) anti- *T. gondii* IgG: positive (serum) | A round-shaped rim-enhanced lesion centered in the right thalamus | MRI, Brain biopsy, PCR of brain tissue | Brain | pyrimethamine, sulfadiazine, and folinic acid for 8 weeks, Discontinuation of adalimumab | - | Improved | No description | 8 |
| 6              | 76, F          | methotrexate, leflunomide | sensory hypohesia on the right side of the face | anti- *T. gondii* IgM: positive (serum, CSF) anti- *T. gondii* IgG: positive (serum, CSF) IgG avidity index: high | An irregular lesion centered in the left putamen with thin peripheral enhancement and a number of smaller punctate cortical enhanced lesions involving the brain parenchyma, mostly in cortical locations | CT, MRI, brain biopsy | Brain | Because of allergy to sulfa, pyrimethamine (75 mg), clindamycin (600 mg), and leucovorin (25 mg) | - | Neurologically stable | Negative | 9 |
| 7              | 65, F          | methotrexate | sensory hypohesia on the right side of the face | anti- *T. gondii* IgM: positive (serum, CSF) anti- *T. gondii* IgG: positive (serum, CSF) IgG avidity index: high | Ring-enhanced signal at medulla oblongata to inferior cerebellar peduncle by contrast-enhanced T1-weighted MRI | MRI Nested PCR of CSF | Brain | sulfamethoxazole/trimethoprim (ST) mixture (trimethoprim: 360 mg/day) for 8 days, Discontinuation of methotrexate | Multiple rashes | Improved | Negative | This case |

F: female, M: male, RA: rheumatoid arthritis, CSF: cerebrospinal fluid, PCR: polymerase chain reaction, HIV: human immunodeficiency virus
short-term treatment with an ST mixture (for only eight days). Thus, the discontinuation of the immunosuppressant was suggested to be more useful than the ST treatment, as that led to the recovery of her natural immunity and improvement of her condition. This improvement may have been caused in part by only one immunosuppressant being used and the mild nature of her symptoms.

MRI of this case revealed an irregular lesion at the medulla oblongata to the inferior cerebellar peduncle, and contrast-enhanced T1-weighted MRI of the brain showed a ring-enhanced signal at the lesion. These findings should be differentiated from metastatic brain tumor, malignant lymphoma, abscess, neurocysticercosis, and other granulomatous diseases such as tuberculosis and syphilis, as images of these lesions are very similar. As biochemical tests (e.g., antibody titers) are sometimes unreliable in immunocompromised patients, such as patients with AIDS, those undergoing organ transplantation, and those taking immunosuppressive agents like the present case (4), the diagnosis of cerebral toxoplasmosis is difficult. In the present case, laboratory data, including imaging findings, did not lead to the definitive diagnosis of any of the above-mentioned diseases except malignant lymphoma. As the levels of soluble interleukin-2 receptor, LDH, and CRP were only mildly elevated and whole-body contrast-enhanced CT and gallium scintigraphy showed no abnormalities, we did not perform bone marrow aspiration or positron emission tomography (PET) to assess the possibility of malignant lymphoma. In addition, since serum anti-Toxoplasma IgM and IgG antibodies and nested-PCR targeting \( T. gondii \) 18S rDNA were positive, we concluded that she had toxoplasmosis. However, we should be alert for potential malignant lymphoma, as we were unable to rule it out completely in this case, and it is well-known that malignant lymphoma associated with methotrexate will improve directly after the discontinuation of methotrexate (10).

The diagnostic strategy for toxoplasmosis has already been discussed: the isolation of the parasite in tissue, serology, and PCR (1, 4). Each diagnostic method has both advantages and disadvantages. Table shows that five out of seven cases involved brain diseases, and a brain biopsy was carried out in three of these five cases to achieve a definitive diagnosis. A brain biopsy is sometimes impossible in cases in which lesions are located in deep areas. PCR-based assays to detect \( T. gondii \) DNA have been developed, although they cannot always differentiate between active and latent infections (1). Nested-PCR, multiplex nested-PCR, and real-time PCR of \( B1 \) and/or other genes of \( T. gondii \) in CSF have been reported to be useful to diagnose toxoplasmosis in the early stage because of their high sensitivity (7, 11-15). As \( T. gondii \) has 110 copies of 18S rDNA, nested-PCR targeting 18S rDNA was reported to be the most useful for diagnosing toxoplasmosis using body fluids (3). The sensitivity and specificity of 18S rDNA nested-PCR with clinical samples were reported to be 50% and 100%, respectively (3). Thus, PCR, especially nested PCR, is a useful and safe method of diagnosing toxoplasmosis.

Table shows that four out of seven cases were negative for anti-\( T. gondii \) IgM antibody. Anti-\( T. gondii \) IgM antibody is not very useful for a definitive diagnosis because this antibody is generally considered to be good evidence of a recent infection, and the titer tends to decrease after a few months, although the persistence of IgM antibody to \( T. gondii \) in sera has been reported in some patients (16). The presence of rheumatoid factor in serum can cause false-positive results for IgM (17). Thus, in this case, the high level of anti-Toxoplasma IgM antibody (1.3 IU/mL) on admission might have been a false-positive, as her level of rheumatoid factor was high (1,014 U/mL). Her Toxoplasma IgG avidity index also showed a chronic infection.

In conclusion, the findings in the present case suggest that nested-PCR is a valuable test for the definitive diagnosis of toxoplasmosis in the early stage and that the discontinuation of immunosuppressant use may improve cerebral toxoplasmosis in some cases. Serious complications, such as toxoplasmic infection, during methotrexate therapy for RA should always be kept in mind.

The authors state that they have no Conflict of Interest (COI).

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