INTERNAL MEDICINE

□ CASE REPORT □

Brain Biopsy Is More Reliable than the DNA test for JC Virus in Cerebrospinal Fluid for the Diagnosis of Progressive Multifocal Leukoencephalopathy

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Abstract

The current standard diagnostic approach for progressive multifocal leukoencephalopathy (PML) is to perform a DNA test to identify the presence of the JC virus in cerebrospinal fluid (CSF). A 32-year-old woman with a 5-year history of systemic lupus erythematosus developed right hemiplegia and motor aphasia. MRI revealed a large white matter lesion in the left frontal lobe. JC virus DNA was undetectable in the CSF, but a brain biopsy showed typical histopathology and a high DNA load of the JC virus. The patient was treated with mefloquine and mirtazapine, and is currently alive at 24 months after onset. An early brain biopsy may therefore be important for making a timely diagnosis of PML.

Key words: progressive multifocal leukoencephalopathy, JC virus, DNA test, brain biopsy, demyelination, slow virus infection

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Introduction

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system (CNS) caused by a lytic infection of oligodendrocytes due to the presence of the JC polyomavirus (1). This disease occurs almost exclusively in immunocompromised patients, and is associated with a high mortality rate. The diagnosis of PML is made based on a positive polymerase chain reaction (PCR) assay for JC viral DNA in patients with clinical manifestations and neuroimaging findings (2). Recently, potentially useful medications have been proposed for PML (3), and therefore the early diagnosis of this disease is essential. We herein report a case of PML with a negative DNA test result in the cerebrospinal fluid (CSF), but it was confirmed by a brain biopsy, in which the patient demonstrated a marked improvement in both the clinical and neuroradiological findings after treatment.

Case Report

A 32-year-old, right-handed woman was referred to us because of right hemiplegia and motor aphasia. She had been diagnosed with systemic lupus erythematosus (SLE) 5 years previously and had been taking prednisolone (initial dose 30 mg/day reduced to 27.5 mg/day). Recently, she received six cycles of cyclophosphamide pulse therapy (intravenous administration of 500 mg every 2 weeks) for lupus nephropathy, after which she began to take additional tacrolimus at 3 mg/day. One month later, she noticed weakness in her right arm and difficulty in walking. She visited another hospital where neurological deficits and abnormal findings on brain magnetic resonance imaging (MRI) were found.

On examination at our institute, her general physical findings were unremarkable except for a slight moon face. Neurologically, her consciousness was clear, but she had very limited pronunciation due to motor aphasia. In addition to

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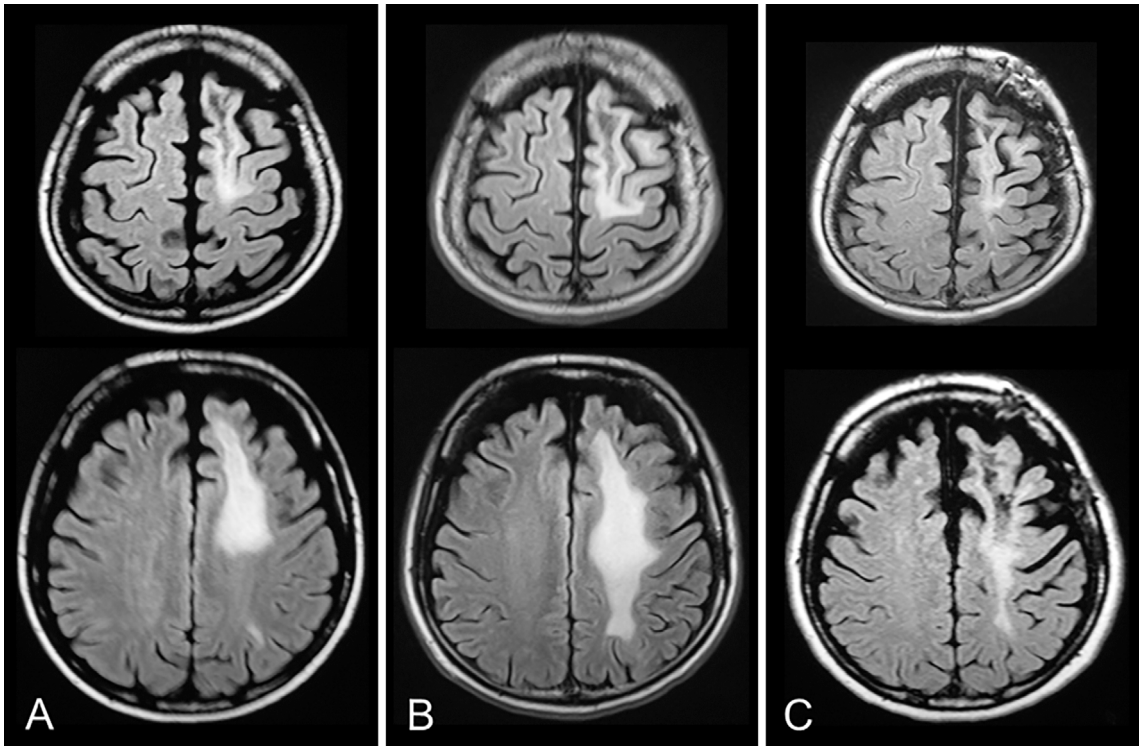

Figure 1. Serial MRI findings on FLAIR images. A: On admission, a large left frontal white matter lesion was seen. B: On brain biopsy, the left white matter lesion was enlarged, involving the parietal lobe. C: At 18 months after diagnosis, the size of the white matter lesion was found to have markedly decreased in association with slight atrophy of the left frontal lobe.

right hemiplegia, deep tendon reflex was exaggerated in the right upper and lower limbs, accompanied with ankle clonus. Pathological reflexes, including Babinski's sign, were not seen and there was neither any sensory disturbance nor autonomic dysfunction. Routine laboratory examinations showed normal renal and liver functions. Notable laboratory findings were as follows: white blood cell count, was 11,910/μL; lymphocyte count, 1,900/μL, including 362 $CD4^+$ cells/μL. Serum IgG level was 615 mg/dL (normal: 870-1,700 mg/dL) and serum CH_{50} level was 58.8 mg/dL (normal: 30-53 mg/dL). Anti-ds-DNA antibody was 29.1 IU/dL (normal: 0-12 IU/dL) and antibodies to human immunodeficiency virus (HIV) were undetectable. CSF showed no increase in the cell count with normal levels of protein and glucose (protein: 39 mg/dL; glucose 53 mg/dL). On brain MRI, a large white matter lesion was seen in the left frontal lobe, with small dot-like lesions in both occipital lobes (Fig. 1A). These lesions were located mainly in the white matter, and suggested tacrolimus-related encephalopathy, lupus encephalopathy, or malignant lymphoma. The use of tacrolimus was thus stopped and the intravenous administration of dexamethasone (8 mg/day) was performed for 5 days, but the patient showed no significant improvement. She was then suggested to have PML, but the findings of the DNA test for JC virus in CSF, as described previously (4), did not support this diagnosis. Despite reducing the daily dose of prednisolone from 27.5 to 22.5 mg/day, her neurological condition deteriorated rapidly with the expansion of the white matter lesions (Fig. 1B), leading to a bed-ridden state. She underwent brain biopsy on hospital day 30.

The biopsied small piece of the left frontal lobe showed an abnormal histopathology, including the presence of oligodendrocytes with enlarged nuclei and atypical astrocytes (Fig. 2A), and immunohistochemistry revealed positive reactivity for JC virus-related antigens on these abnormal glial cells (Fig. 2B). Real-time PCR was performed for the analysis of the DNA extracted from the fresh-frozen brain tissue (4) and JC virus DNA was detected (568 copies/cell). In addition, paraffin-embedded tissue of the brain lesion contained a high viral load (1,690 copies/cell), although the viral DNA was below the limit of detection in her CSF obtained at operation. Finally, a diagnosis of PML was made and the oral administration of mefloquine was started (275 mg daily for 3 days followed by the same dose once weekly). Two weeks later, mirtazapine (15 mg daily) was added and the daily dose of prednisolone was further reduced to 19 mg/day. After these therapies, her motor disability gradually improved and 2 months later she could walk without aid. She was discharged from our hospital, and at 18 months after the diagnosis MRI showed a marked decrease in the size of the left frontal lesion (Fig. 1C). At 24 months after onset, she requires minimal aid in daily life, although her mental activity has declined possibly due to damage to the left frontal lobe. The white blood cell count was 14,260/μL and that of lymphocytes was 1,412/μL, in-

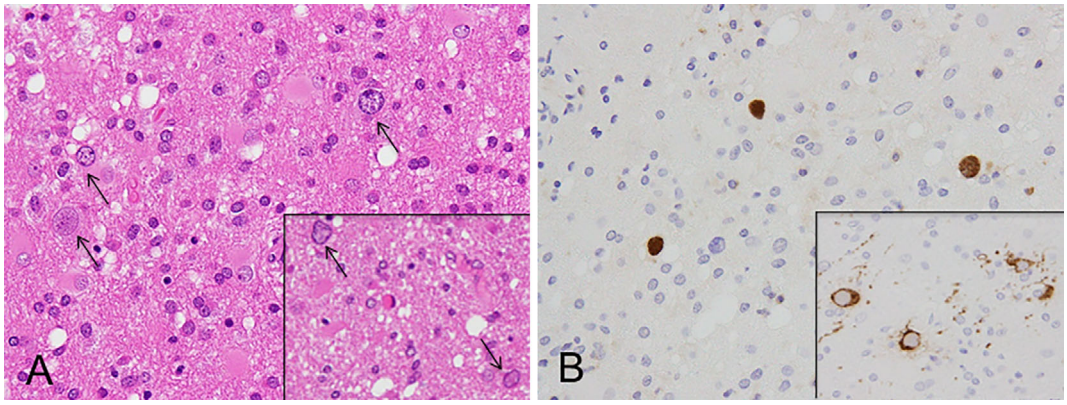

Figure 2. **Brain biopsy findings. A: Hematoxylin and Eosin staining showing the presence of abnormal oligodendrocytes with enlarged nuclei (indicated by arrows). Original magnification ×400. The insert shows astrocytes with nuclear atypia (indicated by arrows). Original magnification ×300. B: Immunohistochemistry with an anti-VP1 antibody showing a positive reaction for nuclei in some glial cells. Original magnification ×400. The insert shows a positive reaction for agnoprotein in the cytoplasm of the affected glial cells. Original magnification ×300.**

cluding 323 $CD4^+$ cells/μL.

Discussion

PML is known to develop in patients with HIV infection, lymphoid malignancies, after organ and stem cell transplantations (1), and more recently it has been noted in the context of modern immune therapies with monoclonal antibodies, such as natalizumab (5, 6), rituximab, infliximab, and efalizumab. In brain imaging by MRI, the lesions in this disorder are visualized as widespread asymmetrical areas of hypointensity on T1 sequences and hyperintensity on T2 or fluid-attenuated inversion recovery (FLAIR) sequences, and these lesions lack contrast enhancement. The natural course of the disease is progressive and it often leads to death within months if the patients remain immunocompromised. In addition, immune reconstruction inflammatory syndrome (IRIS) (7, 8) has been noted in HIV patients treated with combination antiretroviral therapy as well as in patients in whom the PML-inducing immune therapy has been terminated. PML-IRIS is characterized by the marked infiltration of lymphocytes into the preceding PML lesions (9). This condition can be seen on MRI with the presence of gadolinium enhanced lesions, and severely affected lesions with PML-IRIS occasionally producing mass effects with herniation.

The most important factor in the diagnosis of PML is the demonstration of the JC virus in CNS tissue or/and CSF by PCR (2). Although sampling of CSF is usually recommended, false-negative PCR-based analyses of JC virus in CSF, as seen in our case, have previously been reported in three patients. A 49-year-old woman that had been treated with natalizumab for 19 months developed progressive hemiparesis and expanding MRI lesion. To detect JC virus in CSF, PCR was repeated but showed negative findings, while a positive DNA test was obtained from brain biopsy tissue (10). Similar outcomes were described in a 31-year-old postpartum woman (11) and a 75-year-old rheumatoid arthritis woman maintained with methotrexate and adalimumab (12), respectively. In a series of laboratory-confirmed PML patients, the sensitivity of this DNA examination was approximately 80% (13) and these false-negative findings may have been due to low titers of JC virus DNA in CSF or technical instability at commercial laboratories. In our case, brain biopsy tissue contained high titers of JC virus DNA. When clinical and radiological findings are suggestive for PML, then a DNA test for the JC virus should first be attempted in the CSF. If the results are negative, then it is recommended to perform a brain biopsy.

Restoration of the immune function in the affected patient is a basic strategy for the effective treatment of PML: reduction or withdrawal of immunosuppressants in patients with non-AIDS PML, and the use of highly active antiretroviral therapy (HAART) in AIDS-related PML have been recommended (1). In addition, several anti-PML candidate drugs are available, including cytarabine, acyclovir, cidofovir, and mefloquine (3), and a few case reports have documented the effectiveness of mefloquine (14-16) and/or mirtazapine (17): mefloquine, a widely used antimalarial agent, has been shown to inhibit JC virus proliferation *in vitro*, and the antidepressant agent, mirtazapine, seems to block viral entry into uninvolved glial cells. A combination of mefloquine and mirtazapine is expected to be more useful than the use of either drug alone (18), although there have been no reports of clinical trials showing any significant improvement in such patients (19). Our patient was also treated with mefloquine and mirtazapine in addition to the termination of tacrolimus and a reduction in the prednisolone dose, and her neurological disability improved markedly, accompanied with a marked regression of the white matter lesions on MRI. PML-IRIS patients, the vast majority of whom are HIV-positive, are known to have a prolonged sur-

vival (20, 21) and a few can be cured from this disorder with appropriate treatment. However, as the median survival of non-AIDS patients with PML is less than 3 months (13), our PML patient without IRIS, who has survived for more than 24 months after onset, is noteworthy, and the results suggest that mefloquine and mirtazapine were effective in this case. In addition, a rare case of PML with a long-term survival (38 months after diagnosis) was recently reported, showing almost a complete resolution of the MRI lesions (22). PML is a potentially fatal disease, but a correct diagnosis at an early stage may provide a chance to successfully treat such affected individuals.

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

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