【 CASE REPORT 】

Inflammatory Cerebellar PML with a CD4/CD8 Ratio of 2.9 Showed a Favorable Prognosis in a Patient with Rheumatoid Arthritis

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Abstract:
The patient was a 74-year-old woman with rheumatoid arthritis who developed ataxia. MRI revealed T2-hyperintense lesions predominantly in the left middle cerebellar peduncle. Punctate or linear Gd enhancement was also observed on T1-weighted images. A brain biopsy was conducted and the pathology revealed a mild demyelinated lesion. Polymerase chain reaction (PCR) of biopsied brain tissues revealed the presence of JC virus (JCV) DNA, but JCV-infected oligodendroglia-like cells were not apparent on immunohistochemistry. Sensitive *in-situ* hybridization, however, detected three JCV-positive cells and the infiltration of CD4^{+} and CD 8^{+} T cells and plasma cells was also observed. Immunosuppressants were tapered off and mirtazapine and mefloquine administered, resulting in a favorable outcome.

Key words: progressive multifocal leukoencephalopathy (PML), rheumatoid arthritis (RA), JC virus, inflammation, methotrexate (MTX), cerebellum

Introduction

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease that occurs due to reactivation of persistent JC virus (JCV) infection. PML has been known as a fatal complication in acquired immunodeficiency syndrome (AIDS) and generally results in a poor prognosis. To date, however, PML has attracted attention due to its development in multiple sclerosis (MS) patients who are undergoing disease modifying therapies (DMTs), such as natalizumab (NTZ) and fingolimod (FTY) (1, 2). In addition, PML may occur in patients with autoimmune disorders, including systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) under treatment with immunosuppressants such as methotrexate (MTX) and prednisolone (PSL) (3). In drug-associated PML, however, a favorable prognosis may be expected with an early diagnosis, when the JCV titer is limited. An early diagnosis is typically challenging to make and prognostic factors have not been well understood.

Inflammatory reactions are rare in AIDS-associated PML, whereas NTZ-associated PML (NTZ-PML) frequently shows gadolinium (Gd)-enhancement on T1-weighted magnetic resonance imaging (T1W-MRI) (4). The disease may be diagnosed as PML with immune reconstitution inflammatory syndrome (PML-IRIS) and the prognosis might vary among cases. Although temporary clinical deterioration can be observed, a relatively large number of cases show favorable outcomes (designated as "inflammatory PML"). However, some cases of PML-IRIS are fatal (fatal PML-IRIS). Thus, the definition of IRIS, "a paradoxical clinical deterioration, typically occurring in the face of immunologic recovery", is

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a clinical concept for which the pathology and immunology have not fully been elucidated. In the post-mortem brain, fatal PML-IRIS is characterized by excessive CD8+ T-cell infiltration without CD4+ T-cells, even with the clearance of JCV antigen (5-7). In contrast, in biopsied cases, pathological examinations revealed T-cell infiltration with a well-balanced CD4/CD8 ratio in the presence of plasma cells (8). Quantitative polymerase chain reaction (qPCR) detects a relatively low, but distinct, JCV titer. Thus, the CD4/CD8 ratio in the presence of plasma cells may be one of the prognostic factors and define the nature of "inflammatory PML". However, this hypothesis has not been fully elucidated (9).

We herein present a case of PML with inflammatory reactions, which developed in a patient with RA under MTX treatment. Mild T-cell infiltration with a CD4/CD8 ratio of 2.9 was found in biopsied brain tissues, and the prognosis was favorable.

Case Report

The first visit and brain MRI

A 74-year-old woman who had suffered from progressive gait disturbance for five months and progressive nausea for one month was admitted to our hospital. She had tested positive for anti-cyclic citrullinated peptide (anti-CCP) antibody and had been treated with low-dose PSL (approximately 1-5 mg/day for 26 years). MTX (12 mg/week) was also initiated at 65 years of age and her disease activity had been well controlled for 9 years. At admission, a neurological examination revealed ataxic dysarthria, left pyramidal signs, slight left hemiparesis, and marked ataxia in the left upper and the lower extremities. The patient was almost unable to walk without support, due to ataxia. The scale for the assessment and rating of ataxia (SARA) score was 22 points. Her total blood count exhibited no abnormality and a normal CD4/CD8 ratio of 1.6. An examination of her cerebrospinal fluid (CSF) indicated a cell count of 1 cell/μL and a slightly elevated protein level (45 mg/dL), as well as an IgG index of 2.27.

MRI showed multiple T2/fluid-attenuated inversion recovery (FLAIR)-hyperintense lesions in the infratentorial regions, including bilateral cerebellar peduncles, cerebellar white matter, and pontine basilar regions (Fig. 1A and B). The smaller T1-hypointense lesions were present within the T2/FLAIR-hyperintense lesions (Fig. 1C). Faint dot-shaped or linear Gd enhancement was observed on post-contrast T1 weighted images (T1WI) (Fig. 1D). Although diffusion-weighted imaging (DWI) revealed slight hyperintense areas in the left side of the middle cerebellar peduncle (Fig. 1E), the lesion corresponded to isointensity on the apparent diffusion coefficient (Fig. 1F), which was considered to be a T2 shine-through pattern. Differential diagnoses included MTX-associated lymphoproliferative disorder and vasculitis. The interpretation of the Gd enhancement was variable and the

clinical status was deteriorating with an elevated SARA score of 25. Thus, MTX and PSL were tapered off and a stereotactic needle brain biopsy was carried out. One burr-hole craniotomy was performed, and tissues were obtained from the border of the T1 hypointense and Gd contrasted areas (Fig. 1D). Retrospectively, cerebellar PML was also considered likely; however, PCR was not performed to detect JCV DNA before the biopsy.

Pathological findings of mild demyelinating lesions, suggesting PML

Intraoperatively, the left cerebellar hemisphere showed slight discoloration with edema and mild vascular proliferation. Four pieces of brain tissue, approximately 2 mm in size, were obtained. The brain tissue specimens were notably small but included affected areas (Fig. 2A). With Hematoxylin and Eosin (H & E) staining, numerous macrophages, lymphocytes, and plasma cells were seen, but no oligodendroglia-like cells with typical JCV inclusions in the markedly enlarged nuclei were found. Instead, glia-like cells with small but mildly enlarged nuclei were observed. The nuclei were slightly larger than those of lymphocytes (Fig. 2B). Klüver-Barrera (KB) staining revealed that the myelin sheath was not preserved and that the macrophages harbored myelin particles in their cytoplasm (Fig. 2C). Bodian staining revealed that the neuronal axons were well-preserved. Taken together, these observations indicated the presence of demyelinating lesions (Fig. 2D).

DNA was extracted from biopsied brain tissues and quantitative real-time PCR revealed the presence of JCV DNA at 131,575 copies/μL (human β-actin 377 copies/μL), which was estimated to correspond to 698 copies/cell. Multiplex real-time PCR targeting JCV T antigen and the non-coding control regions (NCCR) was also performed (10). The DNA sample showed that 99.97% of the JCV population had PML-type rearrangements within the NCCR, while archetype or archetype-like sequences were remarkably limited (0.03%) (Fig. 2E). Immunohistochemistry with anti-JCV antibodies (VP1, VP2/VP3C), however, did not reveal any apparent JCV-infected cells. The more sensitive *in situ* hybridization (ISH) method demonstrated the presence of three JCV-positive oligodendroglia-like cells with relatively small nuclei (Fig. 2F-H). Postoperatively, quantitative real-time PCR detected JCV DNA (2,124 copies/mL) in the CSF.

Inflammatory reactions of CD4+, CD8+ T-cells and plasma cells

Host inflammatory reactions were examined using immunohistochemistry. In addition to Olig-2 or glial fibrillary acidic protein (GFAP)-positive glial cells, a relative abundance of inflammatory cells was observed. CD3+ T-cells were diffusely distributed in the brain parenchyma and the number of infiltrating CD4+ T-cells was greater than the number of CD8+ T-cells. The CD4+/CD8+ ratio was 2.9 (Fig. 3A-C). A small number of CD20+ B cells was found (only located around blood vessels), while a notable number
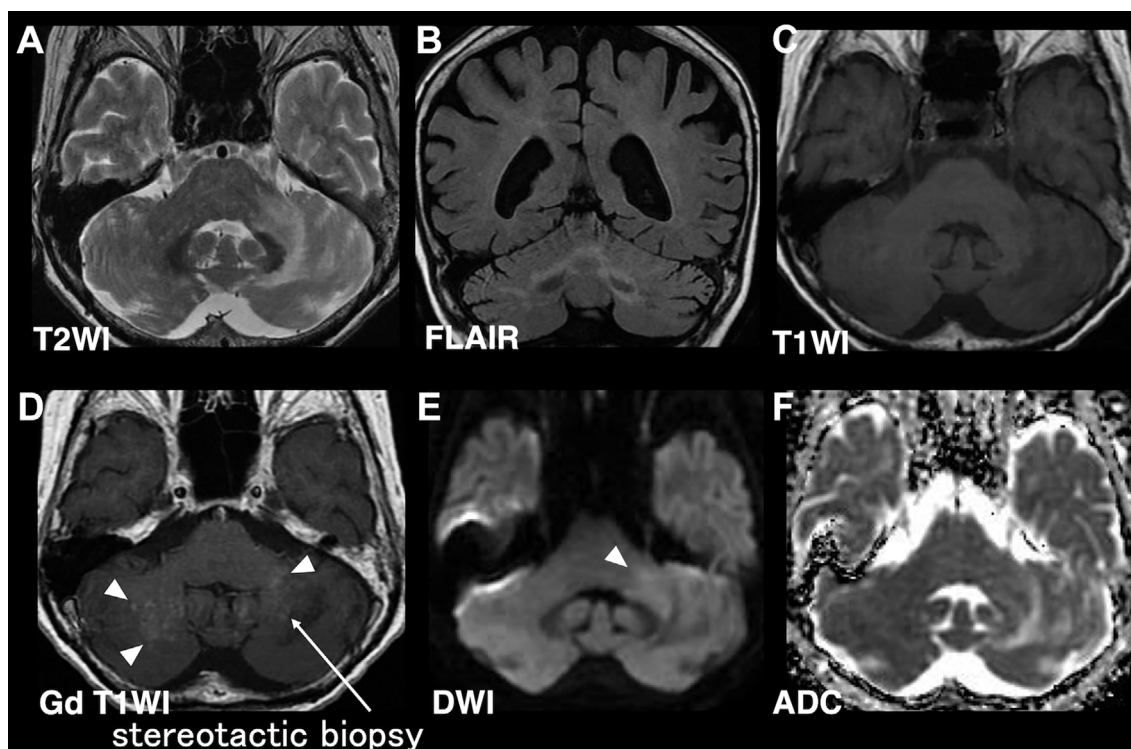
Figure 1. MRI findings at the time of admission. A: T2-weighted images (T2WI) showed high-intensity areas in the cerebellar peduncles and cerebellar white matter. The signals were detected bilaterally but were dominant on the left side. B: FLAIR images also showed high intensity areas in the cerebellar white matter and vermis, which corresponded well to T2WI. C: T1-weighted images (T1WI) showed hypointense signals within the hyperintensive areas detected by T2WI and FLAIR. D: Post-contrast T1WI showed faint dot-shaped or linear enhancement (arrowheads) partially surrounding the T1WI hypointense area. Stereotactic needle brain biopsy was performed, aiming at the border of the T1 hypointense area and Gd-enhanced area in the cerebellar hemisphere (arrow). E: Diffusion-weighted imaging (DWI) showed a slight hyperintense area (arrowhead) in the left side of middle cerebellar peduncle, which was at the edge of T2WI-hyperintense area. DWI also showed a hypointense area, which was consistent with the T1WI-hypointense area. F: While an increased signal on the apparent diffusion coefficient (ADC) corresponded to the DWI-hypointense area, the DWI-hyperintense lesion was corresponded to the isointense area on ADC.

of CD79a⁺ and CD138⁺ cells appeared diffusely in the brain parenchyma (Fig. 3D-F).

As a limited number of JCV-positive cells were found in the tiny biopsied tissues, the pathological diagnosis was not definitive for PML. However, due to the associated MRI findings and PCR positivity, the pathology was regarded as being compatible with that of PML. Thus, treatment with mefloquine and mirtazapine was initiated. After the biopsy, the CD4⁺/CD8⁺ ratio of the peripheral blood mononuclear cells was 2.6.

### The clinical course of PML with a favorable prognosis

The patient's clinical status improved and her symptoms resolved at two months after the start of treatment. She was able to walk a few meters by herself and her SARA score was 17 points. She was then discharged from our hospital. Repeated PCR testing of the patient's CSF revealed that the copy number of JCV DNA gradually decreased until it

eventually became undetectable at five months after the discharge. Although faint Gd enhancement remained on T1WI MRI, the affected lesions did not expand. The patient's IgG index remained high over the five months after discharge; however, the patient's symptoms did not worsen (Fig. 4).

Discussion

During the AIDS era, the 1980s and 1990s, PML associated with AIDS (AIDS-PML) was usually diagnosed in an advanced stage when the CSF showed a high JCV DNA titer. When MRI showed T2/FLAIR-hyperintense lesions, typically in the fronto-parietal lobe (including in the precentral gyrus), the detection of a high titer of JCV DNA in the CSF was sufficient for a definitive diagnosis, and brain biopsy was rarely performed. Thus, in the AIDS era, pathological examinations were usually performed on the postmortem brain. In advanced demyelinated lesions, typical JCV-infected cells with markedly enlarged nuclei were read-
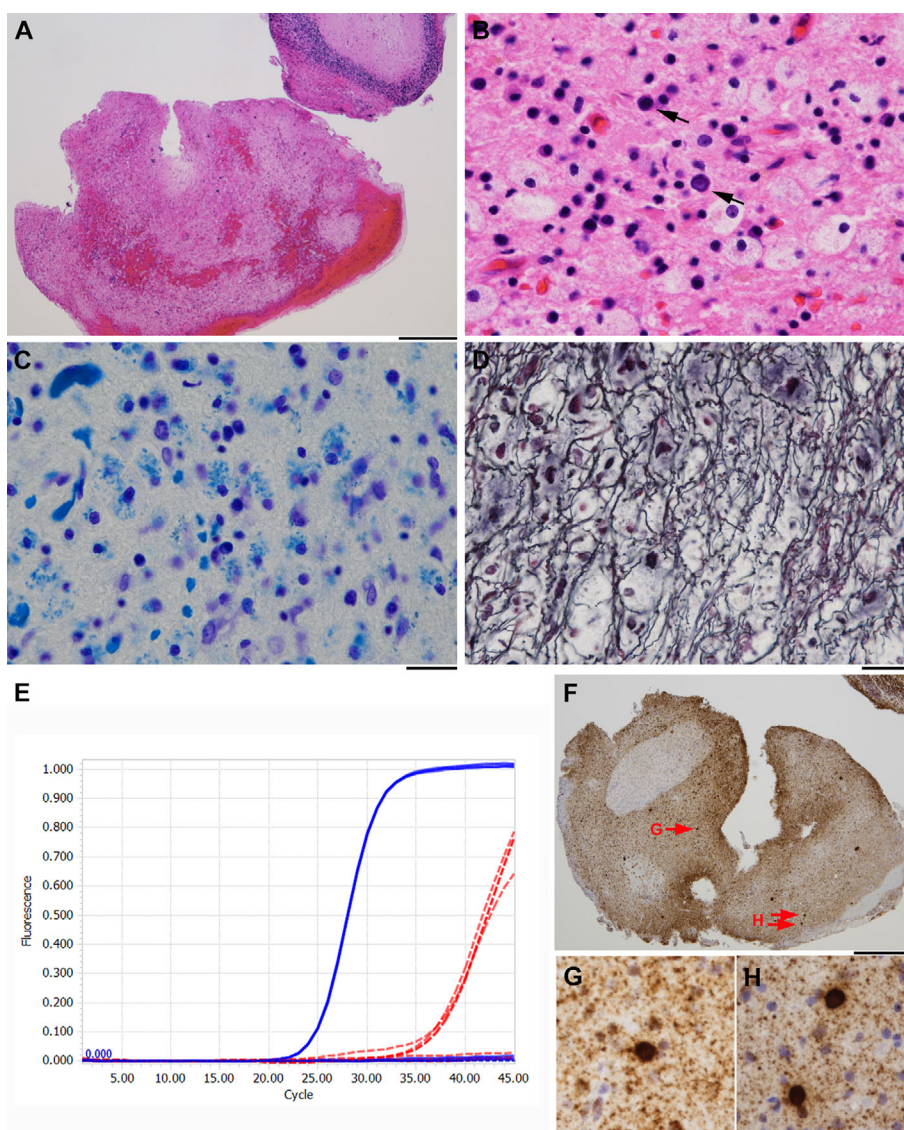
Figure 2. Neuropathology and detection of JCV DNA. A: Tiny brain tissues obtained by stereotactic biopsy [Hematoxylin and Eosin (H&E) staining]. B: Pathology revealed infiltration of macrophages and lymphoid cells. Glia-like cells with mildly enlarged nuclei were also present (arrows) (H&E staining). C: Numerous macrophages harboring myelin particles (KB staining). D: Neuronal axons remained (Bodian staining). E: Multiplex real-time PCR revealed the presence of JCV genomic DNA. Multiplex primers detected mainly rearranged PML-type genome (blue lines, 99.97%) and archetypal genome was markedly low in copy number (red line, 0.03%). F: Sensitive *in-situ* hybridization (ISH) revealed the presence of three JCV-positive cells in tiny biopsied specimens. G and H: A high-power view of the JCV-positive cells in F. Scale bars. A: 500 μm; B-D: 25 μm; F: 500 μm; G, H: 25 μm.

ily detected and inflammatory reactions were typically poor. In contrast, the frequency at which brain biopsy is performed is increasing, especially in Japan since the approval of NTZ for MS patients in 2014. Brain biopsy may be performed to aid the diagnosis of PML in its relatively early stages, not only for patients with MS undergoing DMT, but also for those with SLE and RA undergoing steroid or MTX treatment (3, 11).

The early pathological diagnosis of PML according to the biopsied brain tissue specimens would be challenging because the typical diagnostic hallmark of JCV-infected oli-godendroglia may not be contained in small specimens. Previously, the diagnostic criteria for PML were proposed on the basis of a histopathological triad: 1) demyelination; 2) astroglia with bizarre nuclei; and 3) oligodendroglia with enlarged nuclei (12). However, how these three criteria should be assessed in tiny fragments of biopsied brain tissue has not been fully elucidated. Glial nuclei, which represent early infection with JCV are generally small (13, 14) and frequently unable to be detected by insensitive immunohistochemistry using anti-JCV antibodies. Alternatively, JCV-infected cells may be detectable with the more sensitive ISH
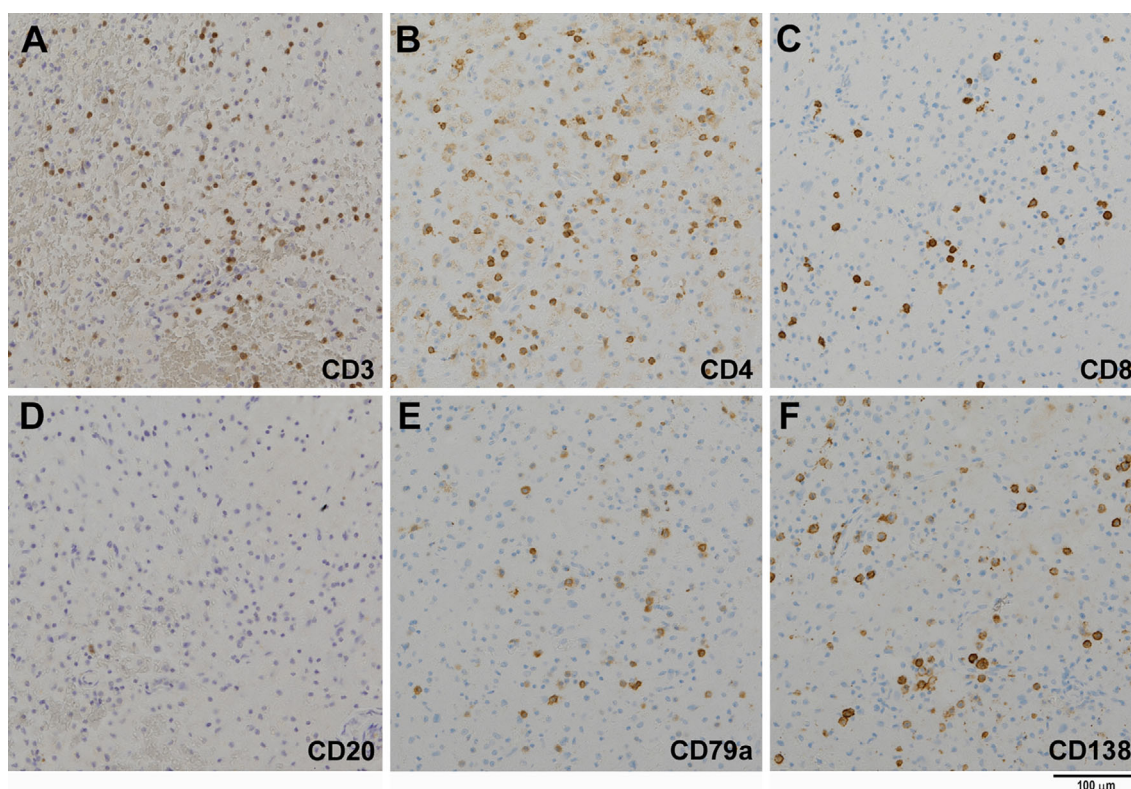
Figure 3. Immunohistochemistry for the detection of inflammatory cells. Immunohistochemistry revealed the infiltration of both CD4+ and CD8+ T-cells and plasma cells. The CD4/CD8 ratio of the brain tissue sections was 2.9. The infiltrating B-cells were mostly CD138+ plasma cells and CD79a+ B-cells differentiating to plasma cells. The number of CD20+ cells was relatively small. A: CD3; B: CD4; C: CD8; D: CD20; E: CD79a; F: CD138. Scale bar: 100 μm.

methods (2, 3). For example, a previous case report stated that despite the lack of definitive JCV-positive cells using immunohistochemistry, ISH detected more than 20 JCV-positive cells, which led to the diagnosis of PML (3). In the present case, however, even with the sensitive ISH method, only three JCV-positive cells were revealed. Additionally, because the copy number of JCV-DNA was markedly low, its detection was accompanied by relatively high background signals. Thus, the histopathological diagnosis was not definitive; however, the low copy number of JCV DNA likely contributed to the favorable prognosis.

Different levels of inflammatory reactions may be observed in biopsied PML brain tissues and some cases have shown a favorable prognosis, designated as "inflammatory PML" or "PML with controlled inflammation" (8, 9). The prognostic factors are not well understood and there is no established indicator to distinguish inflammatory PML (favorable prognosis) from fatal PML-IRIS (poor prognosis). However, in fatal PML-IRIS, autopsied cases frequently show excessive cytotoxic/CD8+ T-cell infiltration in the post-mortem brain (5, 6). In contrast, in "inflammatory PML" or "PML with controlled inflammation", inflammatory cells of both T- and B-cell lineages appear cooperatively.

Plasma cells (CD138+) and B-cells with plasma cell differentiation (CD79a+) were moderately infiltrated in this case. In contrast, a limited number of CD20+ B-cells were found. A similar tendency for infiltration of the B-cell lineage has been observed in other immune disease-based cases of PML with favorable prognoses (15). Since plasma cell infiltration in the brain is rarely observed in RA, it would be a part of the anti-viral response in combination with CD4+ and CD8+ T-cells (6). In addition, the IgG index in the peripheral blood was persistently high, which is not generally observed in RA patients and likely reflects the production of antibodies in the brain. Recently, a clinicopathological study reported that a satisfactory prognosis in PML is related to CD 138+ cell infiltration with the expression of PD-1 on the surface of T-lymphocytes. Similarly, in the present case, it is possible that CD138+ plasma cells and plasmablasts controlled excessive inflammation via inhibitory cytokine production (15). Although the functions of plasma cells and anti-JCV antibodies have not been revealed in PML, the infiltration of plasma cells might be also a prognostic factor.

T-cells (CD4+, CD8+) are also important factors for anti-JCV reactions in PML. Regulated infiltration of CD4+, CD8+ T-cells, along with a preserved CD4+/CD8+ ratio, may contribute to a satisfactory prognosis in patients with PML (8, 15). In the present case, the CD4+/CD8+ ratio of 2.9 was slightly high in comparison to a previously reported case of PML that showed a good prognosis; in that case, the CD4+/CD8+ ratio was 1.41 (8). As MRI revealed sustained faint enhancement, we carefully assessed whether it would
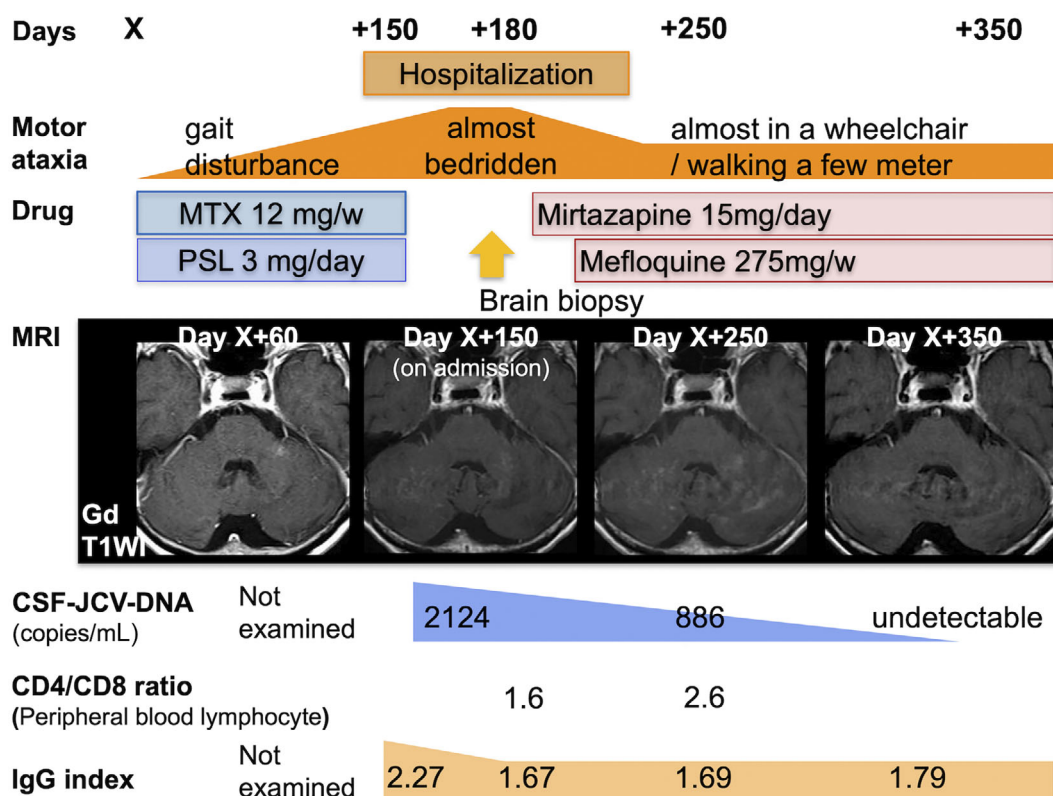
Figure 4. A summary of the clinical course of the diagnostic and therapeutic procedures with corresponding MR images. After admission, we tapered and stopped MTX and PSL. With the diagnosis of PML, mefloquine and mirtazapine were administered. Two months later, the patient's symptoms resolved, enabling her to walk for a few meters. Although, faint dot-shaped or linear enhancement was observed in the bilateral cerebellar white matter on post-contrast T1WI, no new lesions developed. Although the contrast enhancement was slightly increased at 250 days after the appearance of the symptoms, the symptoms improved and eventually we decided not to do the steroid pulse therapy. Repetitive PCR testing of the CSF revealed that the JCV DNA copy number gradually decreased and finally became undetectable at 200 days after hospitalization. During the clinical course, the IgG index was persistently high; however, the affected lesions did not expand and the patient's symptoms did not deteriorate. Additionally, the CD4/CD8 ratio of the peripheral blood increased during the clinical course.

shift to fatal PML-IRIS. Fortunately, edematous change did not occur on MRI, and the clinical symptoms did not worsen. Thus, based on the abovementioned findings, steroid pulse therapy was not performed. The CD4/CD8 ratio of 2.6 was also measured in peripheral blood. Although the number of lymphocytes in the peripheral blood might be affected by the patient's background status (e.g., RA, MTX, and PSL), the ratio turned out to be close to that of the brain pathology. However, the CD4/CD8 ratio of the CSF could not be assessed due to an insufficient number of cells.

In conclusion, a patient who demonstrated inflammatory cerebellar PML with a CD4/CD8 ratio of 2.9 showed a favorable prognosis. A pathological analysis detected only three JCV-infected cells in tiny fragments of biopsied brain tissue; thus, the diagnosis of PML was made in combination with the MRI findings and PCR positivity. Immunohistochemistry revealed the infiltration of both T- and B-cells, indicating a host inflammatory response to JCV infection. Although the prognostic factors for PML have not been fully

determined, when brain biopsy was performed, the evaluation of inflammatory cells seemed to be important to assess the prognosis and to inform on steroid use.

References

1. Berger JR, Cree BA, Greenberg B, et al. Progressive multifocal leukoencephalopathy after fingolimod treatment. Neurology **90**: e1815-e1821, 2018.
2. Nishiyama S, Misu T, Shishido-Hara Y, et al. Fingolimod-associated PML with mild IRIS in MS: a clinicopathologic study. Neurol Neuroimmunol Neuroinflamm **5**: e415, 2018.
3. Ishii J, Shishido-Hara Y, Kawamoto M, et al. A punctate magnetic resonance imaging pattern in a patient with systemic lupus erythematosus is an early sign of progressive multifocal leukoencephalopathy: a clinicopathological study. Intern Med **57**: 2727-2734, 2018.
4. Wattjes MP, Wijburg MT, Vennegoor A, et al. MRI characteristics of early PML-IRIS after natalizumab treatment in patients with MS. J Neurol Neurosurg Psychiatry **87**: 879-884, 2016.
5. Metz I, Radue EW, Oterino A, et al. Pathology of immune reconstitution inflammatory syndrome in multiple sclerosis with natalizumab-associated progressive multifocal leukoencephalopathy. Acta Neuropathol **123**: 235-245, 2012.
6. Kleinschmidt-DeMasters BK, Miravalle A, Schowinsky J, Corboy J, Vollmer T. Update on PML and PML-IRIS occurring in multiple sclerosis patients treated with natalizumab. J Neuropathol Exp Neurol **71**: 604-617, 2012.
7. Himedan M, Camelo-Piragua S, Mills EA, Gupta A, Aburashed R, Mao-Draayer Y. Pathologic findings of chronic PML-IRIS in a patient with prolonged PML survival following natalizumab treatment. J Investig Med High Impact Case Rep **5**: 2324709617734248, 2017.
8. Sanjo N, Kina S, Shishido-Hara Y, et al. Progressive multifocal leukoencephalopathy with balanced CD4/CD8 T-Cell infiltration and good response to mefloquine treatment. Intern Med **55**: 1631-1635, 2016.
9. Shishido-Hara Y. Progressive multifocal leukoencephalopathy: dot-shaped inclusions and virus-host interactions. Neuropathology **35**: 487-496, 2015.
10. Ryschkewitsch CF, Jensen PN, Major EO. Multiplex qPCR assay for ultra sensitive detection of JCV DNA with simultaneous identification of genotypes that discriminates non-virulent from virulent variants. J Clin Virol **57**: 243-248, 2013.
11. Akagawa Y, Ueno A, Ikeda J, Ishii W, Shishido-Hara Y, Sekijima Y. [Two patients with progressive multifocal leukoencephalopathy with immune response against JC virus showing good long-term outcome by combination therapy of mefloquine, mirtazapine, and risperidone]. Rinsho Shinkeigaku **58**: 324-331, 2018 (in Japanese, Abstract in English).
12. Berger JR, Aksamit AJ, Clifford DB, et al. PML diagnostic criteria: consensus statement from the AAN Neuroinfectious Disease Section. Neurology **80**: 1430-1438, 2013.
13. Shishido-Hara Y, Yazawa T, Nagane M, et al. JC virus inclusions in progressive multifocal leukoencephalopathy: scaffolding promyelocytic leukemia nuclear bodies grow with cell cycle transition through an S-to-G2-like state in enlarging oligodendrocyte nuclei. J Neuropathol Exp Neurol **73**: 442-453, 2014.
14. Shishido-Hara Y, Ichinose S, Higuchi K, Hara Y, Yasui K. Major and minor capsid proteins of human polyomavirus JC cooperatively accumulate to nuclear domain 10 for assembly into virions. J Virol **78**: 9890-9903, 2004.
15. Sanjo N, Nose Y, Shishido-Hara Y, et al. A controlled inflammation and a regulatory immune system are associated with more favorable prognosis of progressive multifocal leukoencephalopathy. J Neurol **266**: 369-377, 2019.