Progressive Multifocal Leukoencephalopathy in a Patient with Multifocal Neurological Manifestations Caused by Solitary Brainstem Involvement

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Abstract:
A Japanese man in his 60s on medication for chronic lymphocytic leukemia presented with progressive, multifocal neurological manifestations. Magnetic resonance imaging showed a small, solitary region of brainstem involvement. Sensitive real-time polymerase chain reaction testing detected a small amount of JC virus (JCV) DNA (170 copies/mL) with pathogenic mutation in cerebrospinal fluid. We diagnosed the patient with progressive multifocal leukoencephalopathy (PML). The small PML lesion may have caused multifocal neurological symptoms because of its focal brainstem involvement. This case contributes to knowledge regarding the diagnosis and treatment of brainstem PML in the context of hematologic malignancies and other underlying diseases.

Key words: brainstem, JC virus, lesion volume, progressive multifocal leukoencephalopathy, real-time polymerase chain reaction, solitary lesion

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Introduction
Progressive multifocal leukoencephalopathy (PML) is a rare central nervous system demyelinating disease caused by JC virus (JCV). JCV establishes subclinical persistent and latent infections in the general population, and 60-80% of adult individuals are seropositive for this virus (1). In patients with compromised immune system competence, particularly cellular immunity, JCV can reactivate and proliferate in oligodendrocytes of the brain, leading to PML. Pathogenic JCVs in lytic infection have mutations in the non-coding control region (NCCR) of the viral genome and are called prototypes (2).

Pathological and clinical criteria have been proposed for the diagnosis of PML. The gold standard for diagnosing PML is a brain biopsy, which is based on histopathology, immunohistochemistry, and polymerase chain reaction (PCR) findings for JCV. In the absence of a brain biopsy, the clinical manifestations, magnetic resonance imaging (MRI) findings, and PCR findings for JCV in the cerebrospinal fluid provide evidence to support a diagnosis of PML (3). On MRI, PML is characterized by multifocal brain lesions that are usually hyperintense on T2-weighted images including fluid-attenuated inversion recovery (FLAIR) sequences, and hypointense on T1-weighted images (3).

PML lesions are found mainly in the subcortical and juxtacortical white matter of supratentorial or infratentorial structures (1). Although few cases are recorded, it has been reported that PML can occur in the brainstem as the primary lesion (4, 5). However, more data are needed to clarify the pathogenesis of PML of the brainstem and to enable its diagnosis.

We herein report a Japanese patient who developed a small and solitary brainstem lesion extending to left tectum mesencephali that was detected on brain MRI during treatment for chronic lymphocytic leukemia (CLL). An ultra-
sensitive real-time PCR test detected a small amount of prototype JCV in a sample specimen of the patient’s cerebrospinal fluid (CSF), leading to a diagnosis of PML.

**Case Report**

A Japanese man in his 60s presented to the neurology clinic with a 28-day history of visual disturbance (with Day 0 being the day of onset). His medical history and peripheral absolute lymphocyte counts are summarized in Fig. 1. He had been diagnosed with CLL 5 years earlier and treated with fludarabine, rituximab, and bendamustine. There was an increase in the peripheral blood lymphocyte count after discontinuation of rituximab medication. Most recently, the patient had been receiving ibrutinib, and at the time of the onset of the neurological manifestation, the lymphocyte count had decreased to 30,000/μL from 70,000/μL three months earlier. Suspecting that ibrutinib was contributing to his symptoms, the patient discontinued it three weeks after the onset.

On Day 27, the patient visited the ophthalmology clinic and underwent MRI of the brain, which revealed a solitary lesion in the brainstem that was hypointense on T1-weighted imaging and hyperintense on T2-weighted fluid-attenuated inversion recovery (FLAIR) imaging (Fig. 2a-c). The lesion extended to the left tectum mesencephali and showed no contrast enhancement. The volume of the lesion was 1.57 mL, as measured on FLAIR sequences (1-mm slice thickness) using a 3-dimensional image analysis system (SYNAPSE VINCENT, version 5; Fujifilm Medical Corporation, Tokyo, Japan). MRI also showed mucosal thickening of the right maxillary, ethmoid, and sphenoid sinuses, as well as thickening of the epipharynx wall. An old infarction seen in the left occipital lobe was presumed to be unrelated to the present condition.

At the time of presentation, the patient had lucid consciousness, and a neurological examination revealed double vision, limitation of right eye abduction, and right-sided hemiparesis with paresthesia of his face and extremities. His pupillary light reflex was normal. On Day 35, he was admitted with suspected fungal sinusitis, tumor, or demyelinating or inflammatory disease. Laboratory tests were negative for serum anti-neutrophil cytoplasmic antibodies specific to myeloperoxidase or proteinase, anti-human immunodeficiency virus type-1/2 (HIV-1/2) antibodies, and beta-D-glucan. Serum cytomegalovirus (CMV) DNA was detected by standard real-time PCR with a detection limit of 200 copies/mL. Cervical, thoracic, and lumbar spine MRI performed on Days 37-38 showed no pathological signal change. A CSF examination revealed that glucose (60 mg/dL), protein (24.2 mg/dL), cell count (1/μL), IgG index (0.5), myelin basic protein (31.2 pg/mL), and opening pressure (130 mm H2O) values were all within normal ranges, and the CSF was negative for oligoclonal bands. An excisional biopsy of the right maxillary sinus, ethmoid sinus, sphenoid sinus, and epipharynx revealed leukemic cell infil-
Figure 2. MRI findings of a small brainstem lesion involving the left tectum mesencephali. The lesion is hypointense on T1-weighted imaging (a) and hyperintense on T2-weighted FLAIR imaging (b, c) performed 27 days after the onset. FLAIR images acquired at 49 and 84 days after the onset (d and e, respectively) in similar coronal sections as (c) show caudal extension of the lesion. The line in (f) indicates the position of these images on a sagittal section. The timeline (g) summarizes the clinical course of the patient, who was admitted 35 days after the onset. An ultra-sensitive real-time PCR assay targeting JCV was conducted for the CSF sampled 36, 105, and 137 days after the onset. The single, double, and triple dots in the line labeled “brainstem lesion” correspond to the timing of acquisition of MRI images (c), (d), and (e), respectively.

On Day 49, MRI of the brain performed on Day 49 showed expansion of the brainstem lesion to involve the right ventral midbrain and caudal extension of the lesion in the left brainstem.

On Day 52, he was started on mirtazapine 15 mg for insomnia suspected to be caused by the brainstem lesion. His neurological symptoms, including dizziness and worsening right-sided paresthesia of his face and extremities, were considered to be related to cerebral edema, and he was started on prednisolone 20 mg on Day 59. 18-Fluorodeoxyglucose positron emission tomography (FDG-PET) of the brain performed on Day 62 showed no intense FDG uptake in the left brainstem.
cipation of the brainstem lesion.

PML was suspected based on his medical history, immunocompromised status, and use of immunomodulators, along with the clinical findings. He was started on mefloquine 275 mg on Day 63. CSF sampled on Day 45 was subjected to real-time PCR targeting JCV, but JCV DNA was not detected, possibly because of the detection limit of 200 copies/mL of CSF in standard real-time PCR in a commercial laboratory. Thus, an ultra-sensitive real-time PCR assay with a detection limit of 20 copies/mL of CSF (6) was conducted at the National Institute of Infectious Diseases, Japan, in CSF sampled on Day 36, showing positivity for JCV (170 copies/mL). Duplex real-time PCR targeting the T gene and NCCR (7) demonstrated that JCV detected in the CSF was a pathogenic prototype. A brain biopsy was not performed. Based on these findings, a diagnosis of PML was made. His mirtazapine dose was increased to 45 mg to prevent the spread of JCV infection to glial cells (8), and the prednisolone dose was gradually reduced. MRI of the brain obtained on Day 84 revealed extension of the brainstem lesion to the left side of the ventral medulla (Fig. 2d-f).

As beneficial neurological effects of systemic administration have been reported in non-AIDS-related PML (9), cytarabine 110 mg was administered intravenously on Days 106-110. Fig. 2g shows the patient’s clinical course. The neurological symptoms continued to deteriorate throughout the course, along with enlargement of the lesions on MRI. The paresis with paresthesia of the face and extremities was limited to the right side throughout his course. Consistent with the clinical findings, the copy number of JCV in the CSF increased sequentially. The patient became somnolent and died of aspiration pneumonia after developing dysphagia on Day 137.

Discussion

The diagnostic approach by a differential diagnosis in the present case was challenging. Along with the absence of an intense FDG uptake, the brainstem lesion showed mainly vertical extension, suggesting progression via nerve fibers rather than a tumor. The patient had been receiving ibrutinib at the onset, for which efficacy against CLL CNS involvement and Richter transformation has been reported (10, 11). Given that the CSF examination showed a normal cell count, protein, and IgG index, and the lesion showed no contrast enhancement on MRI, there was low suspicion of inflammatory disease. In addition to the CSF and MRI findings, the negative results of increased myelin basic protein and oligoclonal bands in CSF suggested multiple sclerosis as a less likely diagnosis. Although we did not examine anti-aquaporin-4 IgG in the CSF, neuromyelitis optica spectrum disorder was not a strong consideration due to the lack of evidence of optic neuropathy or transverse myelitis throughout his course. As anti-HIV-1/2 antibodies and CMV DNA were negative, we did not consider these infectious diseases. However, rather than ruling out differential diagnoses, the final diagnosis was made because adequate evidence for a diagnosis of PML was obtained.

All previously reported brainstem PML lesions have shown patchy hyperintensity on T2-weighted imaging, including FLAIR sequences, and hypointensity on T1-weighted imaging with no mass effect and no enhancement on contrast-enhanced imaging at the time of the diagnosis. These characteristics were observed throughout the course, and lesions continued extending in the previously reported cases as well as our own (4, 5). It is worth considering PML in the differential diagnosis of an isolated brainstem lesion showing these characteristics. In contrast to numerous reports of lesions in the pons or medulla, only two cases have been reported in the midbrain. The causative lesion for the limitation of his right eye abduction was not evident on MRI performed on Day 27. However, subsequent imaging showed the lesion extending ventrally to include the right pons. Thus, pathologic changes in the right abducens nucleus or nerve fibers may have been present from the beginning and may have caused the injury.

It is known that the T-cell cytotoxicity and effector function are reduced in patients with CLL (12). Impairment of T-cell immunity has been implicated in JCV reactivation and transformation of the virus from archetype to prototype (13) and is thus consistent with CLL being a background disease of PML. Elevated peripheral lymphocyte counts prior to the onset of neurological symptoms are thought to reflect the progression of CLL. It can be inferred that such diminished T-cell mediated immunity enables the reactivation and mutation of JCV.

It is also necessary to consider the involvement of immunomodulators, such as fludarabine, rituximab, and ibrutinib, in the development of PML, as has been reported in other cases (14-16). JCV infects the bone marrow in a latent fashion (17), and it is thought that JCV-infected hematopoietic progenitor cells differentiate into B cells and pass through the blood-brain barrier, thereby transporting JCV into the brain (18). Although the detailed mechanism remains to be elucidated, B-cell-targeted agents, including rituximab, are known to cause reactivation of JCV and the development of PML (19). In addition, most cases of PML reported in the literature were diagnosed within 6 to 12 months after the last dose of rituximab (20). In the present case, however, rituximab had been discontinued 15 months before the initial onset of neurological symptoms. Therefore, as with fludarabine, which had been discontinued more than 40 months earlier, we cannot consider rituximab as the cause of the PML in our patient. Ibrutinib, a Bruton’s tyrosine kinase (BTK) inhibitor, was used in the treatment of various B-cell hematologic malignancies (21). Although several cases of PML after ibrutinib administration have been reported in the past, the further accumulation of knowledge is needed because of the limited number of such reports (22-25). In the present case, the patient presented with neurological symptoms that first occurred four months after the start of treatment with ibrutinib. Whether or not ibrutinib induced PML,
directly remains unclear. Against the background of CLL-induced depression of T-cell immunity, JCV may have been reactivated, and ibrutinib may have acted on JCV-infected B cells, leading to extracellular release of the virus.

The most striking observation in the present case was that the primary lesion of PML developed in the brainstem, rather than in the cerebral or cerebellar white matter. To our knowledge, only 11 cases of brainstem PML have been reported this far (4, 5). In the formation of the present brainstem lesion, it was not feasible to speculate the pathway of JCV entry from the periphery to the central nervous system. In addition, the lesion was very confined at the time of the diagnosis, which is atypical compared with the more common pattern of multifocal PML lesions in the cerebrum and cerebellum.

A recent study reported a statistically significant correlation between the volume of brain lesions and the positivity rate of PCR testing for CSF JCV in natalizumab-associated PML (26). In that situation, the PML lesion volume that yielded an 80% positive rate by CSF JCV PCR was estimated to be 10 mL (26). In the present case, the volume of the solitary PML lesion in the brainstem was very small (1.57 mL), which is consistent with the failure of standard real-time PCR testing in a commercial laboratory to detect JCV DNA in CSF. Furthermore, the JCV DNA detected by ultra-sensitive PCR testing was a pathogenic prototype with a mutation in the viral genome, although the copy number was small. Over time, the amount of JCV DNA in the CSF continued to increase slowly. These observations suggest that CSF JCV testing is highly sensitive for detection and may be beneficial for diagnosing cases of focal PML lesions in the brainstem.

We also observed that the PML lesion in the brainstem extended caudally over time. It is likely that this pattern represents the propagation of JCV along the nerve fibers. Although the volume of the PML lesion was small in the present case, its location in the brainstem appears to be the cause of multifocal neurological symptoms, resulting in a serious outcome. There may also have been more extensive involvement of the lesion than could be visualized as a signal change on conventional MRI of the brain. Thus, more extensive brainstem involvement may have occurred by the time of the patient’s death. Further research is therefore needed to establish an effective treatment for brainstem PML.

**Conclusion**

We experienced a case of PML with a solitary lesion in the brainstem. The MRI findings of the lesion were atypical compared with those of PML in the cerebrum and cerebellum. As the copy number of JCV DNA in the CSF was very low, a highly sensitive testing technique was needed for the PML diagnosis. The prognosis was poor despite the small volume of the lesion. Our case will hopefully aid in the diagnosis and treatment of brainstem PML associated with hematologic malignancies and other underlying disorders in the future.

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

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