

Case Report

An autopsy case of progressive multifocal leukoencephalopathy after rituximab therapy for malignant lymphoma

Reiji Muto,^{1,2}  Yasuo Sugita,¹  Seiya Momosaki,³ Yuriko Ito,⁴ Yoshiyuki Wakugawa⁴ and Koichi Ohshima¹

¹Department of Pathology, Kurume University School of Medicine, Kurume, ²Department of Pathology, National Hospital Organization, Kumamoto Medical Center, Kumamoto, Departments of ³Pathology and ⁴Cerebrovascular Disease and Clinical Research Institute, National Hospital Organization, Kyushu Medical Center, Fukuoka, Japan

Progressive multifocal leukoencephalopathy (PML) is a rare fatal demyelinating disease of the central nervous system caused by reactivation of the JC virus (JCV), which is named after the initials of the patient from whom the virus was first isolated. JCV is highly prevalent worldwide, infects humans in early childhood, and the infection persists throughout the course of life in latent form. The present paper deals with the second autopsy case report of rituximab-associated PML in Japan. A 63-year-old woman who had undergone chemotherapy for non-Hodgkin lymphoma developed progressive dysarthria and cerebellar ataxia. Head magnetic resonance imaging (MRI) revealed small, scattered, hyperintense areas in the midbrain, pons and thalamus, and the patient was first diagnosed as having cerebral infarction. Follow-up MRI showed tendency toward cerebellar atrophy and multiple system atrophy cerebellar type was suggested, which we concluded must have coincidentally occurred. It was challenging to perform biopsy due to the location of the foci and the patient's condition. Twelve months later she died of aspiration pneumonia caused by the bulbar lesion. At autopsy, the histological examination suggested the presence of demyelinating foci with numerous foamy macrophages. In the foci, oligodendrocytes with enlarged ground-glass like nuclei were found in a scattered manner and astrocytes with bizarre nuclei were also detected. These findings verified the case as PML. The first diagnosis of cerebral infarction was later withdrawn,

although appropriate disorders were not recalled even after testing with various antibodies. The rate of PML development tends to increase after treatment with molecular-targeted therapies, which directly or indirectly attenuate the cellular-mediated immune system. Various novel molecular-targeted and immunosuppressive drugs have been released on the market; the cases of PML have consequently increased. Accordingly, pathologists should keep this disease in mind in the differential diagnosis when neural symptoms newly emerge in patients who are treated with these drugs.

Key words: demyelinating diseases, JC virus, leukoencephalopathy, progressive multifocal, rituximab.

INTRODUCTION

Progressive multifocal leukoencephalopathy (PML) is a rare demyelinating disorder of the central nervous system (CNS) caused by opportunistic reactivation of the JC virus (JCV), which is named after the initials of the patient from whom the virus was first isolated.^{1,2} JCV is prevalent worldwide but its seropositivity differs among countries, with approximately 70% of adults reportedly being seropositive in Japan.^{3,4} PML has a high mortality rate. The mortality rate of human immunodeficiency virus (HIV)-negative patients with PML after rituximab therapy is reportedly as high as 90%.⁵ Recent studies have confirmed that molecular-targeted and/or immunosuppressant drugs are potential causative agents of this disease.^{6–8} Given the widening indication of these drugs and the increasing frequency with which these medications are being used, the frequency of PML has increased in recent years.⁸

Here, we present a rare autopsy case of rituximab-associated PML.

Correspondence: Reiji Muto, MD, Department of Pathology, School of Medicine, Kurume University, Asahi-machi 67, Kurume-city, Fukuoka 830-0011, Japan. Email: sugarless610@hotmail.com

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CASE PRESENTATION

Clinical summary

A 63-year-old woman was diagnosed with malignant lymphoma and had started therapy with bendamustine and rituximab approximately 12 months before death. Eight months before death, the last dose of rituximab was administered. Thereafter, the disease had remained in complete remission.

Approximately 6 months before death, the patient developed dysarthria and cerebellar ataxia. The cerebrospinal fluid (CSF) was clear with cells $3/\text{mm}^3$ (100% small mononuclear), and contained 35 mg/dL protein and 57 mg/dL glucose. No organisms were found in the CSF including cryptococcus. Head magnetic resonance imaging (MRI) revealed small, scattered, hyperintense areas in the midbrain, pons, medulla oblongata and thalamus, and the patient was first diagnosed with cerebral infarction, which was later discounted (Fig. 1A–C). Follow-up imaging showed tendency toward cerebellar atrophy and hot cross bun sign in the pontine base area (Fig. 1D). The clinicians wondered whether the cerebellar atrophy and the mass lesion in the brain were signs of a single disease or of separate diseases. They concluded that two separate diseases merged because the patient's condition could not be

explained by a single disease. They considered the possibility of a drug-induced condition or a para-neoplastic syndrome, but there were no findings or data suggesting such potentialities. They concluded that a degenerative disease such as multiple system atrophy cerebellar type (MSA-C), which was suggested by imaging, coincidentally occurred. However, even if this was a case of MSA-C, they judged that the progress of atrophy was fast and that possibly another disease was involved.

Biopsy of the high intensity lesions was scheduled, but it was difficult to perform the maneuver due to difficulties in accessing the site of the foci and the patient's condition, which was carefully monitored. Subsequently, the scheduled head MRI revealed tendency toward aggravation. MSA-C was clinically suspected. However, other diseases (i.e., invasion and/or metastasis of malignant lymphoma, paraneoplastic syndrome, demyelinating disease, HIV-associated encephalopathy, and drug-induced encephalopathy) were also considered in the differential diagnosis due to the condition's rapid progression. Various antibody tests, including anti-Hu, anti-Yo, and anti-Ri antibodies, were negative, as were the intrathecal oligoclonal band and myelin basic protein; no increase was observed in interleukin-6 and the immunoglobulin G index. Since it was difficult to perform a biopsy, only the scheduled MRI

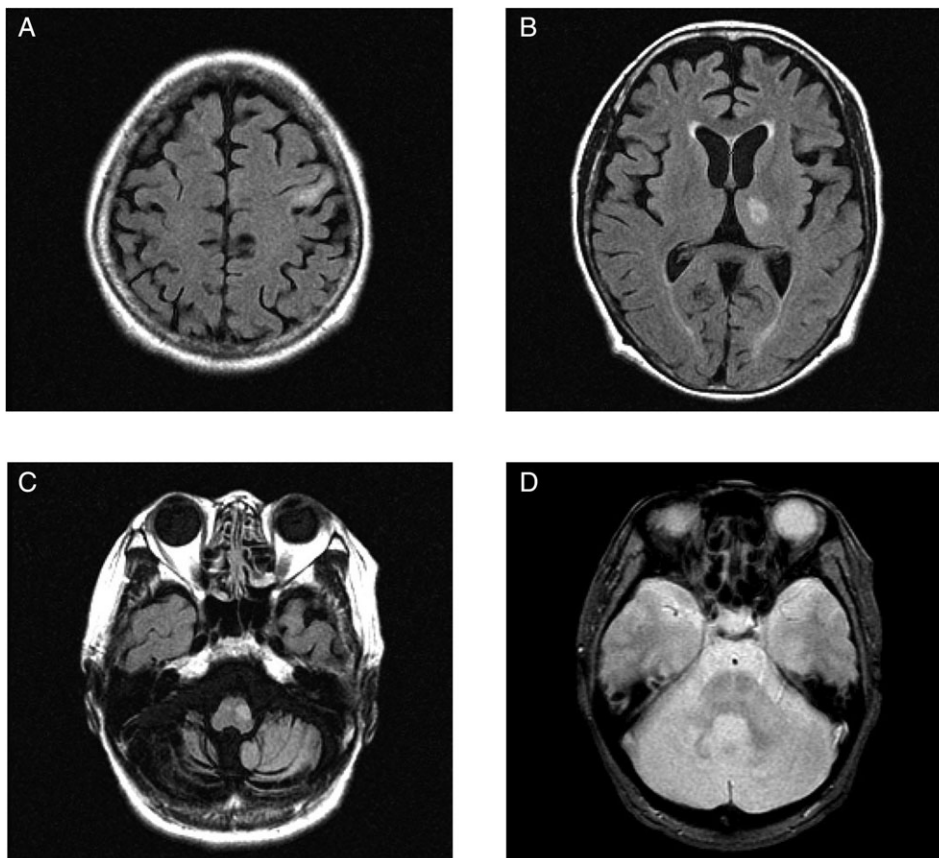


Fig. 1 MRI findings on fluid-attenuated inversion recovery (A, B), T1-weighted (C) and T2-weighted (D) images. (A, B) High intensity areas are observed in the subcortical lesion (A) and the left thalamus. (C) A plaque-like lesion can be seen in the medulla oblongata. (D) Hot cross bun sign is found in the pontine base.

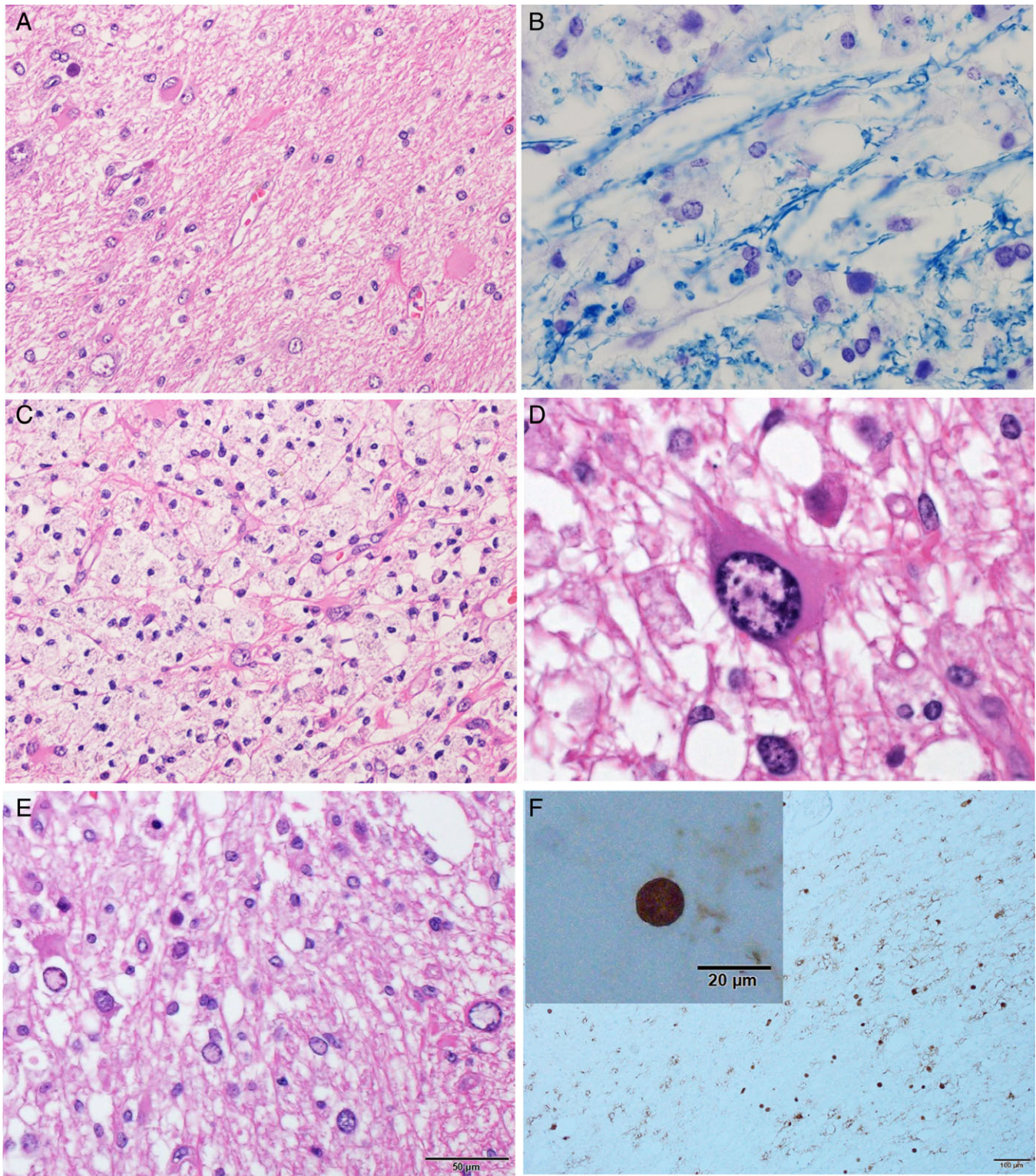


Fig. 2 Histopathological findings in the demyelinating lesions. (A) Rarefaction of neural cells and emergence of numerous gemistocytic astrocytes are seen. (B) Demyelinating foci are initially characterized by oligodendrocytes containing diffuse basophilic ground glass-like nuclei. (C) Aggregation of foamy macrophages can also be observed. (D) Astrocytes with bizarre nuclei can be seen. (E) In the demyelinating foci, oligodendrocytes with diffuse basophilic inclusion bodies are present as diagnostic hallmarks of PML. (F) Cells with dot-like inclusion bodies as well as diffuse homogenous ground glass-like nuclei (inset) show JC virus VP-1 protein immunoreactivity. HE (A, C-E), KB (B), immunohistochemistry (F). Scale bars: 200 μm (A, B), 100 μm (C, F), 50 μm (D, E).

was performed. Almost 1 year later, dysphagia, probably caused by worsening of the bulbar lesion, progressively developed. Repeated aspiration pneumonia led to disseminated intravascular coagulation, and ultimately the patient's cause of death was respiratory failure.

Pathological findings

The weight of the brain was 1290 g and age-appropriate. Although the MRI suggested cerebellar atrophy, no sign of obsolescence was found in gross pathology. The cross-sections showed small discolored lesions in the cerebellum and the subcortical areas.

Microscopically, multiple small foci of demyelinating lesions were observed scattered in the medulla, midbrain, thalamus and cerebellum on sections stained with hematoxylin-eosin (HE) (Fig. 2A). Klüver-Barrera (KB) staining showed near-total loss of myelin, and demyelinating foci were characterized by initially oligodendrocytes containing diffuse basophilic ground glass-like nuclei (Fig. 2B). Aggregation of macrophages was seen around the demyelinating area (Fig. 2C). Reactive hypertrophic astrocytes with bizarre nuclei were observed (Fig. 2D). In the demyelinated foci oligodendrocytes with diffuse and basophilic inclusion bodies are recognized as diagnostic hallmarks of PML (Fig. 2E). Gliosis was prominent and numerous macrophages accumulated in the lesions. Cells with dot-like inclusion bodies as well as diffuse homogeneous ground glass-like nuclei (inset) show positive immunoreactivity for the antibody against JCV VP-1 protein (VP-1, dilution 1:100; Dako Japan, Kyoto, Japan) (Fig. 2F). These findings are compatible with the presentation of PML.

DISCUSSION

Based on our literature review, nine autopsy cases have been reported from Japan in English in the past 30 years,^{9–17} and the present paper is the second autopsy case report of rituximab-associated PML in Japan (Table 1).¹⁷

The key diagnostic factors that can distinguish PML from the disorders previously mentioned, are predisposition to the disease and the histological findings.

Any form of immunodeficiency may predispose to PML.¹⁸ Various other underlying diseases and conditions, including HIV, hematological malignancies, human T-cell lymphotropic virus (HTLV)-1 infection, sarcoidosis, and collagenous diseases have been reported so far.^{19–23} In addition, immunomodulatory therapies including rituximab, which is a monoclonal antibody against the protein CD20, are increasingly being considered as potent causative agents of this disease.⁸

In addition, drugs prescribed for multiple sclerosis including natalizumab, fingolimod, and diethyl fumarate (DMF) reportedly increase the risk of PML. Natalizumab inhibits T-cell migration and extravasation.³ Fingolimod, a sphingosine-1-phosphate receptor modulator, sequesters lymphocytes in lymph nodes, and lymphocytes that can be recruited in the CNS decrease.²⁴ DMF, the precise mechanism of which is unknown, can lead to long-lasting, severe lymphocytopenia, and DMF-related PML cases have been reported.^{25,26}

In histological findings, multiple foci are found scattered in white matter within junctional lesions between the white and gray matters. In a typical case, myelin disappears, while axons are preserved. Aggregation of

Table 1 Reported autopsy cases of progressive multifocal leukoencephalopathy in the past 30 years

Authors	Age/sex	Predispositions and comorbidities	Brain weight (g)	Location of lesions
Irie <i>et al.</i> (1992) ⁹	64/F	CRF (HD)	1100	Cerebellum and brain stem
Shintaku <i>et al.</i> (2000) ¹⁰	21/M	Common variable immunodeficiency	1340	Cerebrum, basal ganglia, thalamus, brainstem, cerebellum, and spinal cord
Yogo <i>et al.</i> (2001) ¹¹	34/M	Wiskott-Aldrich syndrome	Not stated	Cerebrum, cerebellum and spinal cord
Arai <i>et al.</i> (2002) ¹²	74/F	CRF (never HD)	970	Cerebrum, cerebellum, brain stem (especially in the pons and medulla oblongata)
Matsuda <i>et al.</i> (2007) ¹³	57/M	CRF (HD), LC (hepatitis C), and HTLV-1 infection	Not stated	Cerebrum (occipital and temporal lobes)
Oshima <i>et al.</i> (2009) ¹⁴	58/M	Schizophrenia, MDS, chronic pancreatitis	1365	Corticomedullary junction of the temporal, occipital
Takeda <i>et al.</i> (2009) ¹⁵	64/M	No underlying diseases	1150	Cerebrum, mid-brain, pons, medulla oblongata, cerebellum, spinal cord (cervical to sacral segments)
Akiyama <i>et al.</i> (2010) ¹⁶	64/M	Multiple myeloma	Not stated	Cerebrum (frontal)
Sano <i>et al.</i> (2015) ¹⁷	66/M	Malignant lymphoma with rituximab therapy	1170	Cerebrum, brain stem, cerebellum

CRF, chronic renal failure; HD, hemodialysis; HTLV-1, human T-cell lymphotropic virus type 1; LC, liver cirrhosis; MDS, myelodysplastic syndrome.

macrophages is observed in the active foci. The oligodendrocytes in the peripheral lesions with enlarged nuclei, which are filled with monotonous basophilic material, are compatible with JCV DNA. Astrocytes with bizarre nuclei and vast eosinophilic cytoplasm have also been seen.

Finally, it could be concluded when pathologists encounter unidentified demyelinating lesions in patients with decreased or defective cell-mediated immunity, they should include PML in the differential diagnosis and observe the lesions carefully to confirm the cells with inclusion bodies.

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DISCLOSURE

The authors have no competing interests or funding to declare.

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