

Progressive Multifocal Leukoencephalopathy Diagnosed by Brain Biopsy, not by the DNA Test for JC Virus

Abstract

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system caused by a lytic infection of oligodendrocytes due to the presence of JC polyomavirus (JCV). The disease occurs mostly in immunocompromised patients and is associated with a high mortality rate. The diagnosis of PML is based on a polymerase chain reaction (PCR) assay for JC viral DNA in cerebrospinal fluid (CSF). However, case reports of the diagnosis of PML established with brain biopsy despite negative JCV CSF PCR analysis when clinical and neuroimaging features are suggestive of PML have been published. A 44-year-old male with a 6-year history of acquired immunodeficiency syndrome developed mental confusion and memory impairment despite 3 months of highly active antiretroviral therapy. Magnetic resonance imaging revealed multiple subcortical white matter lesions in bilateral hemispheres and subcortical nuclei including the thalamus and basal ganglia. JCV DNA was not detected in CSF study, but a brain biopsy showed a high JCV DNA titer. The diagnosis of PML was established with brain biopsy. An early brain biopsy may be important in the diagnosis of PML in patients with clinical manifestations and neuroimaging findings if JCV DNA is undetectable in the CSF PCR.

Keywords: *Acquired immunodeficiency syndrome, brain biopsy, demyelination, DNA test, human immunodeficiency virus, JC virus, progressive multifocal leukoencephalopathy*

Introduction

Progressive multifocal leukoencephalopathy (PML) is an infectious demyelinating disease of the brain, caused by the human JC polyomavirus (JCV).^[1] The JCV is a small ubiquitous DNA polyomavirus with a 5.13 Kb circular enclosed double-stranded DNA. It is a neurotropic virus that infects only humans.^[1] JCV was first isolated in 1971 from the brain of a patient with Hodgkin's disease who died of PML and was named based on the patient's initials.^[1,2] In the first two decades after its initial description, PML remained a rather rare disease, occurring mainly in patients with hematopathies, solid organ malignancies, and inflammatory disorders, as well as in organ transplant recipients. This changed drastically in the 1980s, in the advent of the human immunodeficiency virus (HIV) epidemic. PML was soon recognized as a major opportunistic infection of acquired immunodeficiency syndrome (AIDS), occurring in up to 5% of patients, and did not decrease significantly despite advances in antiretroviral treatment.^[1] Interests in

PML increased further by withdrawal of natalizumab (Tysabri®) from the marketplace. The drug, which was promising in the treatment of multiple sclerosis (MS) and Crohn's disease, was withdrawn after the report of two cases of PML in MS patients and a patient with Crohn's disease.^[1] Several classes of immunomodulatory medications for autoimmune diseases have been reportedly associated with PML, including natalizumab for MS and Crohn's disease,^[3] rituximab for lupus,^[4] and efalizumab for psoriasis.^[5]

The diagnosis of PML is suggested by clinical and neuroimaging features and is further established by demonstrating the presence of JCV DNA in the cerebrospinal fluid (CSF) using polymerase chain reaction (PCR).^[6,7] Although CSF PCR for JCV is highly specific (92%–99%) and sensitive (74%–93%), false negatives do occur.^[8–11] If ascertaining the diagnosis of PML remains elusive, a brain biopsy should be undertaken.^[10] This remains the gold standard for the diagnosis of PML, specifically when clinical and neuroradiological features are suggestive

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of PML despite negative JCV CSF PCR analysis.^[10,11] We describe a case of PML with false-negative CSF JCV PCR, which reinforces the importance of obtaining histologic confirmation when clinicoradiological findings are suggestive of PML, even with initially normal CSF studies.

Case Report

A 44-year-old male patient presented with dysphasia, confusion, and memory impairment that developed following a generalized seizure. He had been living in Jakarta, Indonesia, for the last 6 years. He was diagnosed as AIDS during a routine health examination in 2011. However, he willingly did not seek any medical treatment. Two months prior to presentation, an unremitting diarrhea developed and he returned to his hometown for medical evaluation. A diagnosis of cytomegalovirus (CMV) colitis was made through colonoscopic biopsy. The titer of reverse transcriptase-PCR (RT-PCR) against HIV at the time of admission was 126,629 copies/ml and absolute count of CD4 was 10/UL. Ganciclovir (antiviral agent for CMV) and highly active antiretroviral therapy (HAART, comprising lopinavir, ritonavir, tenofovir, and emtricitabine) were started. After recovery from CMV colitis, his condition was stable. Despite the improvement of his diarrhea, a generalized seizure followed by dysphasia and memory impairment developed 2 months later. He denied any homosexual behavior and his medical history was unremarkable, including transfusion or drug addiction.

Magnetic resonance imaging (MRI) of the brain revealed multiple confluent subcortical lesions with high signal intensity on T2-weighted image, involving bilateral frontal, right temporal and parietal lobes, and left thalamic and subcapsular regions [Figure 1a and b]. Irregular marginal

enhancement on gadolinium was evident [Figure 1c]. Laboratory findings were as follows: hemoglobin of 15.1 g/dL, white blood cell (WBC) count of $4.62 \times 10^9/L$, platelet count of $293 \times 10^9/L$, erythrocyte sedimentation ratio of 27 mm/h, and C-reactive protein level of 0.17 mg/dL. CSF examination showed WBC count of 2 cells/UL, red blood cell count of 1 cell/UL, protein level of 33.5 mg/dL, glucose of 51 mg/dL, and chloride of 124 mEq/L. HIV titer was 80,148 copies/mL and CD4 count was 35/UL. The results of the CSF PCR examination for toxoplasmosis, tuberculosis, and polyoma viruses including BK and JCV were negative.

To confirm the pathology of the brain lesion, a brain biopsy was requested. Under three-dimensional MRI guidance, small craniotomy of the left frontal was performed. Portions of the middle frontal gyrus and subcortical white matter showing enhancement on MRI were taken for pathologic examination [Figure 1d]. The histologic examination revealed multifocal demyelinated areas with reactive astrocytosis and perivascular lymphocytic cuffing. Several large infected oligodendrocytes with inclusion-bearing dark nuclei or clear nucleoplasm having marginated chromatin were present in the periphery of demyelinated area [Figure 2a]. The demyelinated areas revealed small round lesions with myelin breakdown in the luxol-fast blue staining for myelin [Figure 2b]. Numerous macrophages had infiltrated and phagocytosis containing fragmented myelin was apparent. The hyperchromatic, inclusion-bearing oligodendrocytes displayed high ki-67 proliferative index [Figure 2c] and intense p53 nuclear immunostaining. Electron microscopy revealed infected oligodendrocytes filled by viral particles evident as spheres measuring up to 40 nm in diameter [Figure 2d]. JCV

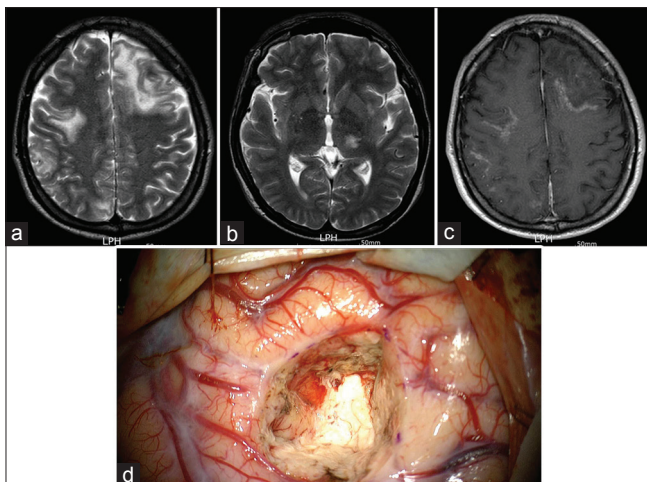


Figure 1: Magnetic resonance imaging and intraoperative findings in progressive multifocal leukoencephalopathy. (a) A T2-weighted axial image showing multiple convoluted irregular cortical and subcortical lesions in bilateral frontal and parietal lobes. (b) A T2-weighted axial image showing small irregular high signal in the left thalamus. (c) An enhanced T1-weighted axial image showing irregular marginal subcortical enhancement in multiple frontal lesions. (d) An intraoperative photograph taken after removal of a portion of middle frontal gyrus for brain biopsy. The subcortical white matter and surrounding gyri seem normal in gross inspection

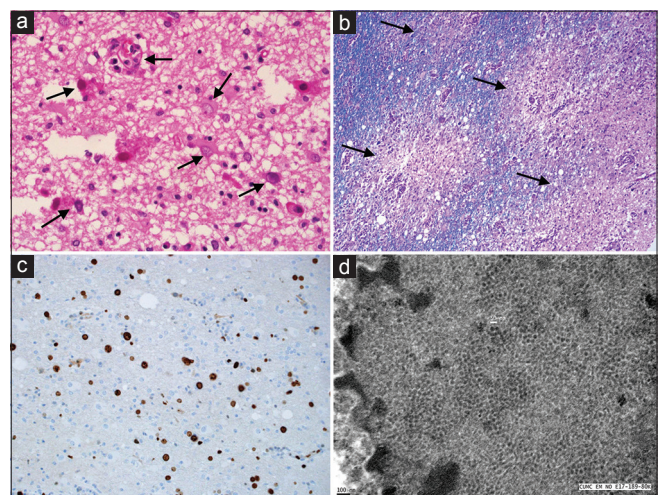


Figure 2: Histopathologic examination of progressive multifocal leukoencephalopathy. (a) Several large infected oligodendrocytes (arrows) with inclusion-bearing dark nuclei or clear nucleoplasm having marginated chromatin (H and E, $\times 400$). (b) Small round demyelinated lesions with myelin breakdown (luxol-fast blue, $\times 100$). (c) High Ki-67 proliferative oligodendrocytes (immunohistochemistry, $\times 200$). (d) Viral particles measuring up to 40 nm spheres (EM, $\times 80,000$)

DNA was detected in brain tissue by PCR. Therefore, the final diagnosis of PML was confirmed and HAART was continued. His neurologic status was stable despite continued memory impairment. A generalized seizure developed 3 weeks after brain biopsy and his mentality became stuporous. Epileptiform discharges over the left frontocentral lobes were found on electroencephalography, and anticonvulsants (levetiracetam 2000 mg/day and valproate 900 mg/day) were added. Despite anticonvulsants and HAART, his mentality did not improve. Recurrent seizures developed and additional anticonvulsants were prescribed (levetiracetam 2000 mg/day, valproate 1200 mg/day, topiramate 600 mg/day, and clobazam 20 mg/day). The patient died 3 months from the onset of neurological symptoms.

Discussion

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 JCV.^[12] JCV is a ubiquitous polyomavirus that infects 50% or more of the adult population globally.^[6] PML remains an extraordinarily rare complication of this infection in otherwise normal persons and occurs almost exclusively in immunocompromised conditions, such as HIV infection, lymphoid malignancies, and after organ and stem cell transplantations.^[11,12] While PML achieved prominence during the first two decades of the HIV endemic, effective antiretroviral treatment and restitution

Table 1: Summary of reports regarding progressive multifocal leukoencephalopathy diagnosed by brain biopsy followed by false-negative polymerase chain reaction assay in cerebrospinal fluid

Study	Age/sex	Underlying disease	Presenting symptom	MRI findings	Treatment	CSF study	Brain biopsy
Landry <i>et al.</i> , 2008 ^[9]	31/female	Job's syndrome (HIES)	Clumsiness of left hand Difficulty walking	Compatible MS White matter changes	Prednisolone IVIg Plasma exchange	Negative PCR for JCV	Polyomavirus particles on EM
Kuhle <i>et al.</i> , 2011 ^[8]	48/female	RRMS	Left-sided hypoesthesia, dysesthesia Weakness of left leg Unsteady gait	Compatible MS Nonenhancing and faintly enhancing ribbon-like lesion	Methylprednisolone Interferon β -1b Glatiramer acetate Natalizumab IVIg Plasma exchange	Negative qPCR for JCV Positive JCV-specific IgG antibody	Negative IHC VP1, large T-antigen, SV-40 Negative <i>in situ</i> hybridization for JCV Positive qPCR from paraffin-embedded tissue for JCV
Babi <i>et al.</i> , 2015 ^[10]	75/female	RA	Progressive left hemiplegia and global decline	Bilateral asymmetric nonenhancing multifocal lesions at periventricular and subcortical white matter demyelination	Methotrexate Adalimumab	Negative PCR for JCV	Positive IHC for JCV (autopsy finding)
Ikeda <i>et al.</i> , 2017 ^[11]	32/female	SLE	Right hemiparesis Motor aphasia	Left frontal white matter lesion with small dot-like lesions in both occipital	Prednisolone Cyclophosphamide Tacrolimus	Negative PCR for JCV	Positive IHC for JC virus-related antigens Positive real-time PCR for JCV DNA Paraffin-embedded tissue contained a high viral load
Current case, 2017	45/male	AIDS	Dysphasia Memory disturbance Seizure	Multifocal patch lesions involving subcortical region of both frontal, right temporoparietal, left thalamus, striatocapsular regions	Lopinavir, ritonavir Tenofovir, emtricitabine	Negative PCR for JCV	Positive PCR for JCV Numerous polyomavirus particles on EM

AIDS – Acquired immunodeficiency syndrome; EM – Electron microscopy; HIES – Hyperimmunoglobulinemia E syndrome; IHC – Immunohistochemistry; IVIg – Intravenous immunoglobulin; MS – Multiple sclerosis; PCR – Polymerase chain reaction; qPCR – Quantitative PCR; RA – Rheumatoid arthritis; RRMS – Relapsing-remitting MS; SLE – Systemic lupus erythematosus; MRI – Magnetic resonance imaging, CSF – Cerebrospinal fluid

of T-cell function have led to PML being less prominent in this population.^[13] HIV infection as a predisposing factor has now been supplanted by T-cell immunodeficiency induced by a range of immune-mediated therapies, including monoclonal antibodies, such as natalizumab, rituximab, infliximab, and efalizumab, as a major cause of PML.^[13]

The onset of PML is insidious, with subtle changes in cognition and loss of memory in association with a range of progressive neurological deficits.^[6,13] These are varied and include cognitive deficits, sensory deficits, hemianopsia, aphasia, and difficulties in coordination and gait, consistent with a multifocal cerebral pathology. A high index of suspicion is required for early diagnosis, as these clinical features are not sufficiently distinctive to enable a definitive clinical diagnosis.^[13] Brain imaging reveals characteristic multiple lesions in the subcortical hemispheric white matter or the cerebellar peduncles. Contrary to the implication in the title to this article that only cerebral white matter is involved, PML lesions also occur in gray matter areas such as the basal ganglia or thalamus.^[13]

No single criterion establishes the diagnosis of PML. Rather, it requires clinical, imaging, and virologic evidences.^[6] Many authorities considered the demonstration of JCV DNA coupled with appropriate clinical and imaging features to be diagnostic of PML, obviating the need for a brain biopsy.^[6,14] However, either the clinical or imaging features are unconvincing or CSF PCR is negative or has not been performed.^[6] In the context of diagnostic uncertainty, the diagnostic criteria of PML were suggested by the American Association of Neurology in 2013. Definitive diagnosis of PML requires neuropathologic demonstration of the typical pathologic triad (demyelination, bizarre astrocytes, and enlarged oligodendroglial nuclei) coupled with the techniques to show the presence of JCV.^[6] The presence of clinical and imaging manifestations consistent with the demonstration of JCV by PCR in CSF is also considered diagnostic.^[6]

The diagnosis of PML is based on the PCR demonstration of JCV in CNS tissue and/or CSF.^[1] Although sampling of the CSF is usually recommended, false-negative PCR-based analysis of JCV in CSF, as shown in our case, has been reported several times [Table 1].^[8-11] In a series of laboratory-confirmed PML patients,^[15] the sensitivity of DNA PCR examination was approximately 80% and these false-negative findings may have been due to low titers of JCV DNA in CSF or technical instability at commercial laboratories.^[15,16] Therefore, two approaches to establishing the diagnosis of PML were suggested: securing the diagnosis with tissue or, as is more commonly practiced, establishing the diagnosis with clinical or radiographic criteria coupled with demonstrating the presence of the virus in the CSF compartment.^[6]

Conclusions

Although PML has been regarded as an incurable disease and to be associated with doomed prognosis, potentially useful medications have been proposed for PML recently.^[11] Therefore, the early diagnosis of this disease is essential. In case of negative PCR in CSF study, brain biopsy would be a useful means of establishing the diagnosis of PML.

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Conflicts of interest

There are no conflicts of interest.

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