Journal of NeuroVirology, 13: 268–273, 2007

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280701291796

informa
healthcare

Short Communication

Longitudinal study of two cases of progressive multifocal leukoencephalopathy with a clinical benign evolution

Serena Delbue,[1] Enrico Marchioni,[2] Giovanni Sotgiu,[3] Marina Saresella,[4] Eleonora Tavazzi,[2] Elena Colombo,[4] Franca Rosa Guerini,[4] Renato Maserati,[5] Elena Sinforiani,[2] Maria Rita Schifino,[2] and Pasquale Ferrante[1]

[1]*Department of Biomedical Science and Technology, University of Milan, Italy;* [2]*Neurology Department, Mondino Hospital, IRCCS, Pavia, Italy;* [3]*Hygiene and Preventive Medicine Institute, University of Sassari Medical School, Italy;* [4]*Laboratory of Molecular Medicine and Biotechnology, Don Carlo Gnocchi Foundation, IRCCS, Milan, Italy;* [5]*Clinic of Infectious Diseases, Policlinico San Matteo, Pavia, Italy*

Progressive multifocal leukoencephalopathy (PML) usually is a rapid and fatal demyelinating disease of the central nervous system (CNS), caused by JC virus (JCV). After the introduction of Highly active antiretroviral therapy (HAART), its prognosis has been modified in some cases but remains a relevant cause of morbidity in human immunodeficiency virus–seropositive (HIV+) patients. The authors report here two cases of PML, followed over time, sharing a benign course and a JCV antigen–specific T-cell response, but with different cerebrospinal fluid (CSF), magnetic resonance imaging (MRI), and clinical features. In both cases, JCV DNA detection in brain biopsies samples and specific antigenic response preceded its isolation in the CSF by several months. In one patient, during the first stage of the disease, the presence of CSF and MRI inflammatory findings, associated with the lack of JCV detection in the CSF, made the diagnosis more challenging. Given that to date a reformation of the laboratory parameters for PML diagnosis is strongly needed, this report highlights the following considerations: (a) indications for performing brain biopsy in HIV-related leukoencephalopathies of uncertain origin, and (b) the role of JCV immunologically specific T-cell response as an additional marker for PML diagnosis and indicator for good prognosis of the disease. *Journal of NeuroVirology* (2007) **13,** 268–273.

Keywords: brain biopsy; cerebrospinal fluid; JC virus; JCV antigen–specific T cells; leukoencephalopathy

Introduction

JC virus (JCV) is the etiological agent of progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the central nervous system (CNS) affecting up to 5% of patients with acquired immunodeficiency syndrome (AIDS) (Berger *et al*, 1999). The classic form of PML has often a fatal outcome within 6 months from the onset, but cases of atypical PML, characterized by clearance of JCV from cerebrospinal fluid (CSF) and prolonged survival time, have been reported after the introduction of highly active antiretroviral therapy (HAART) (Mayo *et al*, 1998; Clifford *et al*, 1999; Hoffmann *et al*, 2003). Paradoxically, HAART-related immune restoration can lead to an inflammatory and lethal form of PML and, conversely, also to a PML variant with a relatively good prognosis (Safdar *et al*, 2002; Koralnik, 2004; Di Giambenedetto *et al*, 2004).

Because the onset of demyelination is unpredictable and the early diagnosis of PML is important, descriptions of new cases of HIV-associated leukoencephalopathy are needed in order to draw a profile of their pathogenesis, and to find new diagnostic and prognostic disease markers and targets for a still-lacking therapy.

Address correspondence to Pasquale Ferrante, MD, Department of Biomedical Science and Technology, University of Milan, Milan, Italy. E-mail: pasquale.ferrante@unimi.it

This work was supported by NIH grant no. MH072528 and by grant from Italian Minister of Health (ISS 2004) to PF.

Received 11 January 2007; revised 6 February 2007; accepted 18 February 2007.

In this article we describe two cases of histologically and laboratory-proven PML, followed over time, characterized by an unusually benign clinical evolution, different neuroimaging patterns, and significant JCV-specific immunological responses.

Results

Patient 1

A 35-year-old, female drug abuser was found to be human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV) positive in 1991. In September 2003 the patient was referred to the Infectious Diseases Institute of the University of Pavia, Italy, because of sensory-motor left hemiparesis. She was naive to antiretroviral drugs and had 210 cps/ml of HIV RNA in plasma and a CD4+ cells count of 192/mm^3. Human leukocyte antigen (HLA) molecular characterization, performed in order to synthesize specific HLA-restricted peptides, showed HLA-A*0201 genotype on both chromosomes, indicating a homozygous pattern for this allele.

The clinical neurological examination was quantified by means of the Scripps Neurological Rating Scale (SNRS). At the disease onset the clinical score was 80. Neuropsychological assessment revealed impairment of attentive functions, abstract reasoning, and manual dexterity. CSF analysis showed lymphomonocytic pleocytosis (with up to 26 cells/microscope field) and albumin increase (89 mg/dl), and a CSF/serum albumin ratio of 1.9% corresponding to a mild blood-brain barrier breakdown with several CSF oligoclonal bands.

Brain magnetic resonance imaging (MRI) showed a diffuse but asymmetric leukoencephalopathy more evident in the right hemisphere, where the lesions involved the subcortical and periventricular white matter of the frontoparietal region. The subcortical frontoparietal white matter was mainly involved in the left hemisphere. Small focal gadolinium-enhanced lesions, hyperintense on T2 images and hypointense on T1 images, were detected at the cortical-subcortical junction in frontal lobes and in the basal ganglia. This latter MRI pattern resembled the results usually observed in cerebral vasculitis (Figure 1). The patient started HAART with zidovudine (300 mg twice a day [bid]), lamivudine (150 mg bid), and efavirenz (600 mg four times per day [qd]). During the following 16 months, clinical, MRI, CSF and laboratory assessments were performed and have been summarized in Table 1. After 4 weeks of anti-HIV therapy, worsening in clinical and laboratory parameters was observed: right mild cerebellar ataxia (SNRS = 70), increased plasmatic HIV viremia (1207 cps/ml), but stable CD4+ T-cell count. PML was not confirmed until February 2004, when a stereotactic biopsy of the right periventricular white matter lesions was performed and JCV DNA detected by means of polymerase chain reaction (PCR). The histopathological pattern of three biopsy specimens was characterized by a remarkable inflammatory reaction with significant presence of both B (CD20+) and T (CD3+) lymphocytes. MRI examination showed a progression of the leukoencephalopathy.

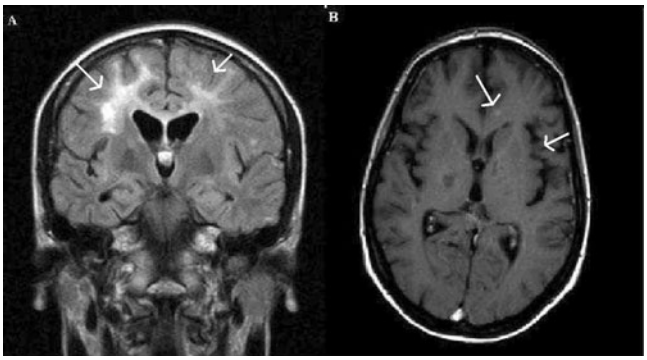

Figure 1 Patient #1, panel A) Coronal FLAIR (fluid attenuated inversion recovery) sequences showed diffuse signal hyperintensity of the subcortical and deep lobar white matter, more pronounced in the right emysphere (arrows). Panel B) Small foci of contrast enhancement in the white matter (arrows) were showed on T1 weighted image post contrast injection at T1.

In June 2004, MRI examination showed stable leukoencephalopathy, but no more evidence of contrast-enhancing lesions. CSF pleocytosis and the increase of albumin content disappeared.

In September 2004 (T7), despite clinical improvement (autonomous walk, SNRS = 90), JCV DNA was also found in the CSF (5208 copies/ml; it was characterized as genotype 2B, MAD4 strain). At T8 (November 2004) a mild clinical improvement with an unmodified brain MRI pattern was observed; plasmatic HIV RNA was undetectable. These aspects, likely due to a good response to HAART, did not correlate with a significant CD4+ cell count increase (232/mm^3). PCR performed for the detection of human herpes viruses in all the CSF samples were negative and CSF HIV RNA was undetectable. Neuropsychological follow-up showed a slight improvement of attentive functions despite ideomotor slowing.

Immunological analysis performed on peripheral blood mononuclear cells (PBMCs) revealed tumor necrosis factor alpha (TNF-α)–producing CD8+ T cells and interleukin-2 (IL-2)–producing CD4+ T cells at T5 (0.63% and 0.16%, respectively), T7 (0.37% and 0.45%), and T8 (0.20% and 0.10%); interferon gamma (IFN-γ)–producing CD4+ T cells at T6 (0.04%) and T9 (0.13%); whereas proliferation indices were positive at T2 (2.3), T4 (2), and at T9 (2.5).

From September 2004 to April 2006 the clinical, MRI, and CSF variations were unremarkable. During this time, the patient showed a mild sensory-motor hemiparesis with an autonomous walk.

Patient 2

On February 2004, a 46-year-old female, diagnosed HIV-1 positive in 1989, was admitted to the Neurological Department of the University of Pavia, Italy, because of the development of a subacute cerebellar syndrome, characterized by ataxic gait, dysarthria,

Table 1 Clinical, virological, and immunological data at baseline and during follow-up time of HIV+ patient 1 affected by PML

Time	CD4+ (mm^3)	Plasma HIV RNA (copies/ml)	Anti-HIV drugs	JCV DNA (copies/ml)	Clinical features and SNRS score	MRI	IL2 by CD4+ (%)	IFN-γ by CD4+ (%)	IFN-α by CD8+ (%)	Proliferation index
T1 (SEP 2003)	192	210	AZT, 3TC,EFV	0	Left hemiparesis 80	Vasculitis/BBB damage	0.3	0	0	1.5
T2 (OCT 2003)	192	1207	AZT, 3TC,EFV	0	Cerebellar ataxia 70	n.d.	0	0	0	2.3
T3 (FEB 2004)	138	<50	AZT, 3TC,EFV	POS in brain biopsy	Stable	demyelination	0	0	0	n.d.
T4 (MAR 2004)	168	<50	AZT, 3TC,EFV	0	Stable	n.d.	0.01	0	0	2
T5 (APR 2004)	168	<50	AZT, 3TC,EFV	n.d.	Stable	n.d.	0.16	0	0.63	n.d.
T6 (JUN 2004)	168	<50	AZT, 3TC,EFV	n.d.	Stable	Stable	0	0.04	0	n.d.
T7 (SEP 2004)	186	<50	AZT, 3TC,EFV	POS in CSF (5208)	Autonomous walk 90	n.d.	0.49	0	0.37	n.d.
T8 (NOV 2004)	232	<50	AZT, 3TC,EFV	n.d.	Improvement	Stable	0.10	0	0.20	0.9
T9 (JAN 2005)	238	<50	AZT, 3TC,EFV	n.d.	Stable	n.d.	0	0.13	0	2.5

MRI: magnetic resonance imaging; AZT: zidovudine; 3TC: lamivudine; EFV: efavirenz; POS: positive; n.d.: not determined.

nystagmus, and bilateral segmental dysmetria (SNRS = 65). During the 2 previous months, she had also presented with recurrent focal seizures of the right frontal lobe. She had been on HAART (stavudine 40 mg bid), lamivudine (150 mg bid), nelfinavir (1250 mg bid), since 1999. Neuropsychological evaluation showed impairment of both short-term and long-term verbal memory, linguistic functions, and logical abilities.

Viroimmunological analysis evidenced undetectable plasmatic HIV-RNA and CD4+ cell counts higher than 200/mm^3 (330/mm^3). HLA molecular characterization showed an A*2402/A*6801 pattern. Brain MRI was characterized by multifocal white matter lesions, localized in the middle cerebellar pedicles and at the pontomesencephalic junction of the brainstem; they showed hypointense signal on T1 and hyperintense on T2 images with no enhancement after intravenous injection of gadolinium, as shown in Figure 2. Laboratory data at baseline and during follow-up are shown in Table 2. In April 2004, after a mild neurological worsening, the patient underwent a brain biopsy. Histopathological analysis showed moderate oligodendroglial injury and mild lymphocytic and macrophagic infiltration. Moreover, JCV DNA (characterized as genotype 4, with transcriptional control region I repeat [IR]) was detected, but polyomavirus genome remained undetectable in CSF until November 2004 (T3), when 114,598 copies/ml were found by means of real-time PCR and JCV strain IR, slightly different from the one detected in the brain, was amplified and sequenced. At the last follow-up (April 2005), CSF JCV DNA load was stable (100,000 copies/ml). Neurological examination showed a mild improvement (SNRS = 75); the patient was seizure-free and was able to walk with a cane. No brain MRI changes have been detected. The neuropsychological evaluation, performed 1 year later, showed improvement of short-term verbal memory and linguistic functions. The impairment of long-term memory and logical abilities remained unmodified.

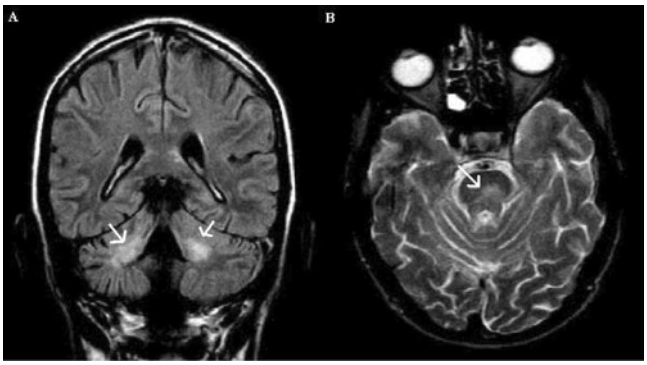

Figure 2 Patient #2, panel A) Coronal FLAIR (fluid attenuated inversion recovery) sequences showed multifocal bylateral cerebellar lesions (arrows). Panel B) Focal lesion in the left paramedian region of the pons were showed on T2 weighted axial image.

No other virus was amplified in the four collected CSF samples and HIV viral load in CSF was always undetectable excluding the possibility that biological agents other than JCV could cause demyelination.

Data from immunological assays showed IFN-γ–producing CD8+ T cells at baseline (0.01%) and at T3 (5.82%); IFN-γ–producing CD4+ T cells at T2 (0.88%), T3 (3.89%), and T4 (0.14%); and IL-2–producing CD4+ T cells at T3 (0.42%) and T4 (0.06%).

Discussion

In this paper we described two cases of "benign PML" in two HIV-infected, drug-abuser females. In the first

Table 2 Clinical, virological, and immunological data at baseline and during follow-up time of HIV+ patient 2 affected by PML

Time	CD4+ (mm^3)	Plasma HIV RNA (copies/ml)	Anti-HIV drugs	JCV DNA (copies/ml)	Clinical features and SNRS score	MRI	IL2 by CD4+ (%)	IFN-γ by CD4+ (%)	IFN-α by CD8+ (%)	IFN-γ by CD8+ (%)	Proliferation I index
T1 (FEB 2004)	330	<50	NFV,d4T, 4TC	0	Cerebellar syndrome 65	Cerebellar demyelination	0	0	0	0.1	n.d.
T2 (APR 2004)	330	<50	NFV,d4T, 4TC	POS brain biopsy	Stable	Progression	0	0.88	0	0	n.d.
T3 (NOV 2004)	750	<50	NFV,d4T, 4TC	POS CSF (114,598)	Stable	Stable	0.42	3.89	0	5.82	<2
T4 (APR 2005)	843	n.d.	NFV,d4T, 4TC	POS in CSF (100,000)	Improvement 75	Stable	0.06	0.14	0.24	0	<2

MRI: magnetic resonance imaging; NFV: nelfinavir; d4T: stavudine; 4TC: lamiduvine; POS: positive; n.d.: not determined.

patient, who was treatment naïve, PML was detected 12 years after HIV diagnosis. During the acute phase, CSF examination and brain biopsy showed a clear inflammatory pattern. Contrast-enhanced brain MRI evidenced a vasculitic-like distribution of the blood-brain barrier (BBB) breakdown, which has been previously described by other authors (Vendrely *et al*, 2005). Inflammatory PML has been associated with both better and worse prognoses (Koralnik, 2006) when compared with the classic noninflammatory variant, depending on the immunologic stage of the disease. Spontaneous inflammatory PML, which has been generally observed during a well-controlled HIV infection, is more frequently benign, whereas its variant associated with immune-reconstitution syndrome can be lethal within few months. The patient showed a surprisingly benign course with clinical and MRI improvement, besides poor immunological control with persistently low CD4 count. In the second patient, CSF, brain biopsy, and MRI data indicated a classic PML picture. No signs of inflammatory damage have been evidenced in either the acute phase and during the follow up. Nevertheless, the disease course was similar, showing clinical improvement, brain MRI stabilization, and long survival. Patient 1 is still alive, whereas patient 2 died from systemic complications after 3 years of follow up, when the neurological and brain MRI patterns were still stable.

In both PML patients, JCV DNA was detected in brain tissues, obtained by stereotactic biopsies, several months before detection in CSF samples, reflecting a previous finding (Di Giambenedetto *et al*, 2004). Before the HAART era, detection of JCV genome in CSF by PCR assays was considered the diagnostic method of choice in PML, because it is easily repeatable and minimally invasive compared with brain biopsy (Weber *et al*, 1994). However, after HAART introduction, negative JCV PCR results have become more frequent in patients with clinical manifestations indistinguishable from PML and the sensitivity of PCR has decreased from 72% to 58% (Marzoccheti A *et al*, 2005). Thus, the earlier detection of JCV DNA in brain tissues of the two patients showed that a return to the invasive brain biopsy may be necessary in HIV-related leukoencephalopathies of uncertain origin. On this basis, the presence of the virus in CSF may not be considered as a reliable diagnostic marker in the forms of PML with milder symptoms and slower progression (Bossolasco *et al*, 2005; Antinori *et al*, 2001). Consequently, as already suggested (Bossolasco *et al*, 2005; Koralnik, 2006), a reformation of the laboratory parameters for PML diagnosis is strongly needed, especially in case of doubt.

Regarding the immunological status of the patients, we observed that, when stimulated with VP1 JCV peptides, CD4+ peripheral lymphocytes had increased production of IL-2 and IFN-γ, and CD8+ peripheral lymphocytes had increased production of IFN-α and IFN-γ, already before the detection of JCV DNA in the CSF of the two patients. This specific T-cell response could be considered as a marker of viral activity in the CNS, and at the same time indicates potential virus detection by brain biopsy. In both patients, the mild clinical evolution could be associated with an improvement of immune system function, including the development of JCV antigen–specific T-cell response. As further support to this hypothesis, the first patient, who had inflammatory signs at biopsy and MRI, also displayed JCV-specific proliferation by assay. Our results are corroborated by other authors, who previously reported that reconstitution of JCV-specific T-cell reactivity was predictive for the course of PML in patients on HAART (Du Pasquier *et al*, 2004). Immunological tests, including the study of JCV antigen–specific T-cell reactivity, seem to be useful for a better definition of the early stages of the PML pathogenesis. Therefore, they could be thought as supplementary markers in case of uncertain PML diagnosis. Given the urgency for an improvement in the diagnostic and prognostic tools and given the data obtained performing the extensive immunological studies, it could be hypothesized that, when the diagnosis is uncertain, highly specific tests might be possible surrogate markers for PML diagnosis and in order to understand the evolution of the disease.

Taken together, the complete analysis of these two PML cases, with the almost unique opportunity to perform a follow up and to analyze brain biopsy

specimens, gave information that could influence the current opinion on PML diagnosis and pathogenesis.

Patients and methods

Patients

During the period September 2003 to April 2006, two HIV-1–infected patients were evaluated and followed-up at the Infectious Diseases Institute, at the Neurological Department of the University of Pavia, and at the Laboratory of Molecular Medicine and Biotechnology of Don Carlo Gnocchi Foundation, Milan, Italy. They gave informed consent for all procedures and human experimental guidelines of the Don Gnocchi Foundation and of the University of Pavia were followed in the research.

Peripheral blood of the first patient was collected at the first visit (T1) and during eight follow-up (T2 to T9), while CSF at T1, T2, T3, T4 and T7; stereotactic cerebral biopsy was performed at T3. With regard to the second patient, four collections of peripheral blood and CSF (T1 to T4) were executed; stereotactic brain biopsy was done at T2.

Neurological assessment

Patients were evaluated at baseline and at least every 3 months using the Scripps Neurological Rating Scale (SNRS) (Sipe *et al*, 1984).

Virus analysis

PCR analysis of CSF and brain biopsy specimens were performed in order to detect polyomaviruses (JCV and BK virus [BKV]) and human herpes viruses (herpes simplex 1 and 2, varicella-zoster virus, Epstein-barr virus, human cytomegalovirus, human herpes virus 6) DNA, using methods previously described (Ferrante *et al*, 1995, 1997; Delbue *et al*, 2005a; Secchiero *et al*, 1995). JCV viral load was determined in CSF (detection limit: 50 copies/ml) and JCV genome was characterized by means of automated nucleotide sequencing (Delbue *et al*, 2005b).

Immunological analysis

PBMC proliferation assay and cytokine (IFN-α, IFN-γ, and IL-2) production by PBMCs were evaluated by flow cytometry, before and after stimulation with VP1 JCV HLA-restricted peptides, following previously described procedures (Lyons and Parish, 1994; Clerici *et al*, 2001). The results were shown as the difference of percentage between cytokines producing stimulated T cells and nonstimulated T cells. Proliferation responses were considered positive if the stimulation index (percentage of proliferated cells in the reference population in the stimulated sample divided by the percentage of proliferated cells in the reference population in the control sample) was at least 2.

Magnetic resonance imaging

Patients were examined using 1.5 Philips Intera MR imaging system; Spin Echo (T1-weighted and T2-weighted) and Flair sequences were obtained before and after injection of a standard dose of gadolinium.

References

Antinori A, Ammassari A, Giancola ML, Cingolani A, Grisetti S, Murri R, Alba L, Ciancio B, Soldani F, Larussa D, Ippolito G, De Luca A (2001). Epidemiology and prognosis of AIDS-associated progressive multifocal leukoencephalopathy in the HAART era. *J NeuroVirol* **7:** 323–328.

Berger JR, Major EO (1999). Progressive multifocal leukoencephalopathy. *Semin Neurol* **19:** 193–200.

Bossolasco S, Calori G, Moretti F, Boschini A, Bertelli D, Mena M, Gerevini S, Bestetti A, Pedale R, Sala S, Sala S, Lazzarin A, Cinque P (2005). Prognostic significance of JC virus DNA levels in cerebrospinal fluid of patients with HIV-associated progressive multifocal leukoencephalopathy. *Clin Infect Dis* **40:** 738–744.

Clerici M, Saresella M, Trabattoni D, Speciale L, Fossati S, Ruzzante S, Cavaretta R, Filippi M, Caputo D, Ferrante P (2001). Single-cell analysis of cytokine production shows different immune profiles in multiple sclerosis patients with active or quiescent disease. *J Neuroimmunol* **121:** 88–101.

Clifford DB, Yiannoutsos C, Glicksman M, Simpson D, Singer EJ, Piliero PJ, Marra C, Francis GS, McArthur JC, Tyler KL, Tselis AC, Hyslop NE (1999). HAART improves prognosis in HIV-associated progressive multifocal leukoencephalopathy. *Neurology* **52:** 623–625.

Delbue S, Pagani E, Guerini FR, Agliardi C, Mancuso R, Borghi E, Rossi F, Boldorini R, Veggiani C, Car PG, Ferrante P (2005a). Distribution, characterization and significance of polyomavirus genomic sequences in tumors of the brain and its covering. *J Med Virol* **77:** 447–454.

Delbue S, Sotgiu G, Fumagalli D, Valli M, Borghi E, Mancuso R, Marchioni E, Maserati R, Ferrante P (2005b). A case of a progressive multifocal leukoencephalopathy patient with four different JC virus transcriptional control region rearrangements in cerebrospinal fluid, blood, serum and urine. *J NeuroVirol* **11:** 51–57.

Di Giambenedetto S, Vago G, Pompucci A, Scoppettuolo G, Cingolani A, Marzocchetti A, Tumbarello M, Cauda R, De Luca A (2004). Fatal inflammatory AIDS-associated PML with high CD4 counts on HAART: a new clinical entity? *Neurology* **63:** 2452–2453.

Du Pasquier RA, Schmitz JE, Jean Jacques J, Zheng Y, Gordon J, Khalili K, Letvin NL, Koralnik IJ (2004). Detection of JC virus-specific cytotoxic T lymphocytes in healthy individuals. *J Virol* **78:** 10206–10210.

Ferrante P, Caldarelli-Stefano R, Omodeo-Zorini E, Vago L, Boldorini R, Costanzi G (1995). PCR detection of JC virus DNA in brain tissue from patients with and without PML. *J Med Virol* **47:** 219–225.

Ferrante P, Omodeo-Zorini E, Zuffolato MR, Mancuso R, Caldarelli-Stefano R, Puricelli S, Mediati M, Losciale L, Caputo D (1997). Human T cell lymphotropic virus tax and Epstein Barr virus DNA in peripheral blood of MS patients during acute attack. *Acta Neurol Scand Suppl* **169:** 79–85.

Hoffmann C, Horst HA, Albrecht H, Schlote W (2003). Progressive multifocal leukoencephalopathy with unusual inflammatory response during antiretroviral treatment. *J Neurol Neurosurg Psychiatry* **74:** 1142–1144.

Koralnik IJ (2004). New insights into progressive multifocal leukoencephalopathy. *Curr Opin Neurol* **17:** 365–370.

Koralnik IJ (2006). Progressive multifocal leukoencephalopathy revisited: has the disease outgrown its name? *Ann Neurol* **60:** 162–173.

Lyons AB, Parish CR (1994). Determination of lymphocyte division by flow cytometry. *J Immunol Methods* **171:** 131–137.

Marzocchetti A, Di Giambenedetto S, Cingolani A, Ammassari A, Cauda R, De Luca A (2005). Reduced rate of diagnostic positive detection of JC virus DNA in cerebrospinal fluid in cases of suspected progressive multifocal leukoencephalopathy in the era of potent antiretroviral therapy. *J Clin Microbiol* **43:** 4175–4177.

Mayo H, Collazos J, Martinez E (1998). Progressive multifocal leukoencephalopathy following initiation of highly antiretroviral therapy. *AIDS* **12:** 1720–1722.

Safdar A, Rubocki RJ, Horvath JA, Narayan KK, Waldron RL (2002). Fatal immune restoration disease in human immunodeficiency virus type 1-infected patients with progressive multifocal leukoencephalopathy: impact of antiretroviral therapy-associated immune reconstitution. *Clin Infect Dis* **35:** 1250–1257.

Secchiero P, Carrigan DR, Asano Y, Benedetti L, Crowley RW, Komaroff AL, Gallo RC, Lusso P (1995). Detection of human herpesvirus 6 in plasma of children with primary infection and immunosuppressed patients by polymerase chain reaction. *J Infect Dis* **171:** 273–280.

Sipe JC, Knobler RL, Braheny SL, Rice GP, Panitch HS, Oldstone MB (1984). A neurologic rating scale (NRS) for use in multiple sclerosis. *Neurology* **34:** 1368–1372.

Vendrely A, Bienvenu B, Gasnault J, Thiebault JB, Salmon D, Gray F (2005). Fulminant inflammatory leukoencephalopathy associated with HAART-induced immune restoration in AIDS-related progressive multifocal leukoencephalopathy. *Acta Neuropathol* (*Berl*) **109:** 449–455.

Weber T, Turner RW, Frye S, Ruf B, Haas J, Schielke E, Pohle HD, Luke W, Luer W, Felgenhauer K (1994). Specific diagnosis of progressive multifocal leukoencephalopathy by polymerase chain reaction. *J Infect Dis* **169:** 1138–1141.