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JC virus load in cerebrospinal fluid and transcriptional control region rearrangements may predict the clinical course of progressive multifocal leukoencephalopathy

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Abstract

Progressive multifocal leukoencephalopathy (PML) is a severe disease of the central nervous system (CNS), caused by infection with the Polyomavirus JC virus (JCV). Because there are no known treatments or prognostic factors, we performed a long-term study focusing mainly on cerebrospinal fluid (CSF) samples from PML patients to describe the virological features akin to the different forms of the disease. Twenty-eight PML patients were enrolled: 10 HIV-1+ patients with classical PML (CPML), 9 HIV-1+ patients with slowly progressing or stable neurological symptoms (benign PML), 3 HIV-1+ asymptomatic patients and 6 HIV-1-negative patients. CSF, urine and blood samples were collected at the enrollment (baseline) and every six months afterwards when possible. The JCV DNA and HIV-1 RNA loads were determined, and the JCV strains were characterized.

At baseline, the mean CSF JCV load was $\log 6.0 \pm 1.2$ copies/ml for CPML patients, $\log 4.0 \pm 1.0$ copies/ml for benign PML patients, $\log 4.2 \pm 0.5$ copies/ml for asymptomatic PML patients and $\log 5.8 \pm 1.3$ copies/ml for HIV-1-negative PML patients (CPML versus benign: $p < 0.01$; CPML versus asymptomatic: $p < 0.05$; HIV-1 negative versus benign: $p < 0.01$). Organization of the JCV transcriptional control region (TCR) showed unusual archetype structures in 2 long-term survival patients; the NF1 sequence was found most commonly, whereas the Sp1 binding site was the most common for both CPML patients and HIV-1 negative patients.

Our results suggest that the JCV load in the CSF and the organization of the TCR should be considered as indicators of PML clinical outcome.

Keywords

JC Virus; Cerebrospinal Fluid; Transcriptional Cellular Factors; Molecular organization

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INTRODUCTION

Progressive multifocal leukoencephalopathy (PML) is a fatal and progressive demyelinating disease of the central nervous system (CNS) that results from lytic infection of oligodendrocytes by the human polyomavirus JC virus (JCV) (Brooks and Walker, 1984).

PML occurs in severely immunocompromised patients, such as in the context of HIV-1 infection, hematological malignancy, exposure to chemotherapy or immunomodulatory antibodies, transplantation and various causes of lymphocyte depletion (Berger, 2010; Dworkin, 2002).

Before the introduction of highly active antiretroviral therapy (HAART), the prevalence of PML among HIV-1+ patients varied from 0.3% to 8%, and typically less than one-tenth of these patients survived for more than one year after the diagnosis (Clifford et al., 1999). To date, clinical stabilization of PML is seen in 50% of patients, and a survival rate as high as 63% has been reported. However, survivors are often left with severe neurological sequelae. PML remains the third most common neurological infectious disease in HIV/AIDS, following Toxoplasma encephalitis and HIV-1 encephalopathy (Gray et al., 2003; Lima et al., 2010). In addition, several reports have indicated that the risk for HIV-1-negative individuals to develop PML is increased as a result of treatment with natalizumab, rituximab and other biological immunomodulatory drugs (Berger, 2010). To date, there is no known treatment for the disease, and there are no well-defined prognostic factors. However, a high CD4+ T cell count (Berenguer et al., 2003), a low plasma HIV-1 RNA load, the presence of JCV-specific cytotoxic T cells and a low JCV load in cerebrospinal fluid (CSF) seem to be related to a good prognosis for PML patients (Berenguer et al., 2003; Bossolasco et al., 2005; Clifford et al., 1999; Martin et al., 1985; Taoufik et al., 1998).

JCV, the etiological agent of PML, is a human polyomavirus characterized by a circular DNA genome that is divided into three regions: the large tumor antigen (LT) and small tumor antigen (sT) coding regions, the viral proteins coding region (VP) and the hypervariable untranslated transcriptional control region (TCR) (Frisque et al., 1984). Based on genotyping analysis of the VP1 gene sequence, the most frequent JCV genotypes described in Europe are genotypes 1 and 4, although different distribution patterns have been observed in specific areas (Agostini et al., 1996). The archetypal form (IIS) of the TCR is detected in urine, while rearrangements (IS, IR, IIR) within this region can generate pathogenic variants with altered tissue tropism and increased infectivity (Ault and Stoner, 1993; Raj and Khalili, 1995). Different reports have suggested that the presence of JCV genotype 2 and the rearranged neurotropic pattern of the TCR could be risk factors for PML development (Agostini et al., 1997; Agostini et al., 1998; Ferrante et al., 2001).

While the classical disease course of PML is tumultuous and a fatal outcome commonly occurs within a few months, several recently reported forms of the disease have been shown to have a better prognosis and a longer survival rate for infected individuals. To our knowledge, scant clinical or biological data exists regarding the follow-up of long-term survival of PML patients in the era of HAART. The significance of studying these patients is notable, as these cases could help to delineate the complex interactions between the virus and the immune system and identify the host and viral factors related to better prognosis.

Thus, we performed this extensive study to describe the JCV features of HIV-1+ and HIV-1-negative patients affected with PML. Herein, the follow-up analysis of such patients is reported, and a particular focus was made on the analysis of CSF samples from patients with prolonged survival.

MATERIAL AND METHODS

Patients

From 2007 to 2010, twenty-two HIV-1+ patients with a confirmed molecular diagnosis of PML were identified from those patients who underwent lumbar puncture at the Infectious Diseases Department of San Matteo Hospital, Pavia and at the Neurological Department of the Mondino Institute, Pavia. CSF, peripheral blood and urine samples were collected at the time of lumbar puncture, coinciding with the time of PML diagnosis. After the baseline collection, all patients were given a neurological examination and lumbar puncture and clinical specimens were collected every 6 months to monitor the clinical course of the disease. Unscheduled visits and lumbar punctures were performed in cases with the sudden appearance of new signs/symptoms or the severe worsening of pre-existing conditions. The patients' immune-virological characteristics (CD4 cells count and HIV plasma viral load) were acquired from clinical records. Based on the symptomatology at the onset and during the clinical course of the disease, we decided to define three different groups of HIV-1+ PML: 10 PML patients with multisymptomatic onset typical for PML and a classical disease course, who died within one year from the time of diagnosis (CPML); 9 PML patients who had a typical multisymptomatic onset, but benign disease course, including those with slowly progressing or stable neurological symptoms and those with an improving status (benign PML); and 3 asymptomatic PML patients, showing lesions resembling PML at MRI and JCV in the CSF.

In addition, 6 HIV-1 negative patients who underwent lumbar punctures and had a confirmed molecular diagnosis of PML were included in the study to analyze and compare the CSF JCV load and its molecular characterization at the time of enrollment. These HIV-1-negative patients were affected with different forms of hematological malignancy (4 cases of chronic lymphocytic leukemia [CLL] and 2 cases of non-Hodgkin's lymphoma) and were not subjected to follow-up examinations. Of these HIV-1-negative patients, two with CLL and one with non-Hodgkin's lymphoma died within a few months of disease onset, two others are still alive but have severe neurological conditions and the remaining patient was unable to be contacted for follow-up.

The study protocol was approved by the local ethical review boards (IRB numbers 00001835 and 00002319), and all patients provided written informed content.

Virological assays

Real-Time PCR (Q-PCR) to amplify viral genomic sequences—The JCV loads were quantified after the viral DNA was extracted from 0.15 ml CSF or urine and from 0.4 ml peripheral blood, which was done using the Nucleospin RNA virus kit (Macherey Nagels, Germany) and the Qiamp blood mini kit (QIAGEN, USA), respectively, according to the manufacturers' instructions. The HIV-1 loads were quantified after the viral RNA was extracted from 0.14 ml CSF using the Qiamp viral RNA mini kit (QIAGEN, USA), according to the manufacturer's instructions. The Q-PCR protocol for the detection of JCV DNA, particularly for targeting the LT region, has been described elsewhere (Delbue et al., 2005). The test sensitivity was 2 copies/reaction, as certified by the external quality control programs for the quantitative assays of Polyomaviruses, provided by the Quality Control for Molecular Diagnostics (QCMD) organisation. To detect the presence of the HIV-1 genome, a Q-PCR targeting the gag gene was performed using the following primers and probe: forward primer GAC ACT AAG CAG CCA TGC AA; reverse primer CTA TCC CAT TCT GCA GCT TCC T and probe FAM- TGT TAA AAG AGA CCA TCA AT-MinorGrooveBinder. The Q-PCR protocol for HIV-1 detection consisted of the following steps: a reverse-transcription step at 48°C for 30 min, denaturation at 95°C for 10 min, and a

subsequent 40 cycles of 95°C for 15 s and 60°C for 1 min. The limit of detection was 1 copy/reaction.

For both the JCV and HIV-1 assays, each sample was analyzed in triplicate, and each run contained a negative control composed of the reaction mixture without the DNA template. Standard curves for the quantification of the viral genome were constructed using serial dilutions of a plasmid containing all or a portion of the viral genome (range: 10 to 10⁶ plasmid copies), as described by the supplier (Advanced Biotechnology). The viral copy concentrations were log-transformed and expressed as log [copies/CSF ml] for the CSF and urine samples and as log [copies/ug of isolated DNA] for the peripheral blood samples.

As the Q-PCR technique is highly sensitive, strict precautionary measures were taken to avoid cross-contamination (Kwok and Higuchi, 1989), including the use of separate rooms to extract the nucleic acids, prepare the amplification mixtures and run the Q-PCR. Multiple negative controls (containing water instead of DNA templates) were included with each Q-PCR batch.

Analysis of JCV genotypes and rearrangements from CSF—The molecular analysis of the JCV strains was performed only on CSF samples. JCV genotyping was accomplished by amplifying a 215-bp fragment of the JCV VP1 gene using a single set of primers: JLP15 and JLP16 (Pagani et al., 2003). The analysis of the rearranged TCR regions was performed using a previously described protocol (Delbue et al., 2005) and the primers JRE1 and LP2 (outer) and RFOR and RREV (inner), which amplify a 353-bp fragment belonging to the JCV TCR. Each PCR fragment was sent to an external facility for the direct sequencing of both viral strands (Primm srl, Milan). Sequence homology searches were performed using a nucleotide BLAST search, database search set “others”, from NCBI site (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), according to Agostini *et al.*, 1996 for JCV genotyping and Ault *et al.*, 1993 and Jensen and Major, 2001 for JCV TCR rearrangements.

Statistical analysis

Differences between continuous variables were analyzed using the Student's t-test (one-way analysis of variance, ANOVA), and correlations between continuous variables were tested by Pearson's analysis. All analyses were performed using the GraphPad InStat software.

RESULTS

Patient characteristics at baseline

Twenty-two HIV-1+ patients and 6 HIV-1 negative patients, including 21 males and 7 females with confirmed PML and a mean age of 42 years (range: 20 – 70 years), were enrolled in the longitudinal study. Of the HIV-1+ patients, 50% were intravenous drug users. All but one of the HIV-1+ patients were on HAART at the time of PML diagnosis. The mean baseline CD4+ cell count was 70.9 ± 29.8/ul for the CPML patients, 220.2 ± 78.4/ul for the benign PML patients and 424 ± 157.9/ul for the asymptomatic PML patients (CPML vs. benign and vs. asymptomatic PML: p<0.05). The mean baseline HIV-1 load was log 4.3 ± 4.1 copies/ml, log 3.3 ± 3.2 copies/ml and log 4.7 ± 4.6 copies/ml for the CPML, PML and asymptomatic PML patients, respectively. These data are summarized in table 1.

Quantitative virological results at baseline

At the time of enrollment, the mean JCV load in the CSF was log 6.0 ± 1.2 copies/ml for the CPML patients, log 4.0 ± 1.0 copies/ml for the benign PML patients, log 4.2 ± 0.5 copies/ml for the asymptomatic PML patients and log 5.8 ± 1.3 copies/ml for the HIV-1 negative PML patients (CPML vs. benign PML: p<0.01; CPML vs. asymptomatic PML: p<0.05; HIV-1

negative vs. benign PML: $p < 0.01$). The mean HIV-1 RNA load in the CSF was $\log 2.7 \pm 1.3$ copies/ml for the CPML patients, $\log 2.2 \pm 1.0$ copies/ml for the benign PML patients and $\log 2.7 \pm 1.7$ copies/ml for the asymptomatic PML patients. In general, there was a positive correlation at baseline between the JCV DNA levels and the HIV-1 RNA levels in the CSF ($R^2 = 0.65$ for CPML; $R^2 = 0.21$ for benign PML and $R^2 = 0.99$ for asymptomatic PML). JCV DNA was detected from the peripheral blood in 4 of 7 samples from CPML patients ($\log 3.15 \pm 1.1$ copies/ug), in 1 of 7 samples from the benign PML patients and in none of the samples from the asymptomatic PML patients. JCV DNA was also found in 4 out of 7 urine samples from CPML patients ($\log 6.6 \pm 1.2$ copies/ml), in none of the samples from the benign PML patients and in 1 sample from the asymptomatic PML patients (table 1).

Quantitative virological results during follow-up

When possible, patient follow-up was performed every six months and included the collection of clinical specimens for virological analysis. Two CPML patients both had one follow-up examination six months after enrollment, whereas the other CPML patients died within less than six months of their PML diagnosis. Follow-up study was possible for 6 patients from the benign PML group (mean time of follow-up: 28 months [range: 6–60 months]) and for each of the 3 patients in the asymptomatic PML group (mean time of follow-up: 16 months [range: 6–48 months]). In three patients, the JCV DNA viral load had increased compared to the baseline level, while the JCV viral load decreased in each of the other patients, and four patients demonstrated clearance of the JCV genome from the CSF.

HIV-1 RNA was only detectable in the CSF samples from 3 PML patients during the follow-up period, but all patients experienced clearance of the virus during the follow up. The longitudinal virological CSF findings are summarized in table 2. Viral loads in the peripheral blood and urine were also monitored during the follow-up period for benign and asymptomatic PML patients. In the peripheral blood, JCV DNA was repeatedly detectable in one patient (from 3 follow-ups), was detectable in one out of ten consecutive samples from another patient and was not detectable in the other patients. In the urine, JCV DNA was found during two of the 11 follow-up examinations in one patient and was repeatedly detected in one of the asymptomatic PML patients (from 5 follow-ups).

Molecular characterization of JCV strains isolated from CSF

All of the CSF samples were analyzed to characterize the JCV genotype. Of these, 20 samples were identified as containing genotype 1 (13 → 1a and 7 → 1b), 7 samples contained genotype 2 (4 → 2b and 3 → 2c) and 1 sample contained genotype 4, but there was no significant difference in the distribution of these genotypes by PML patient group (Agostini et al., 1996).

JCV TCR organization was analyzed for 25 CSF samples at baseline, and the molecular organization was found to be rearranged in 23 of these samples (4 IR rearrangements and 19 IIR rearrangements), whereas two samples from asymptomatic patients demonstrated an archetypal form (IIS), as shown in figure 1 (panels A–C). Each of the CPML patients and all but one of the HIV-1 negative PML patients showed IIR rearrangements; one patient from the benign PML group, one from the asymptomatic PML group and one from the HIV-1-negative PML group had a Mad1 (PML-like) rearrangement, while one benign PML patient had a Mad4 rearrangement (Frisque et al., 1984; Martin et al., 1985).

The A, C, and F blocks were always present as single copy or were completely or partially duplicated, whereas a complete lack of block B was observed in four patients (2 benign PML, one asymptomatic PML and one HIV-1-negative), an absence of block D was observed in 9 cases (3 CPML, 4 benign PML, one asymptomatic and one HIV-1-negative

PML) and an absence of block E was observed in one case of benign PML. Region E was found to be present in duplicate or triplicate most frequently, but this region is not known to contain any binding motifs.

The frequency of occurrence of the binding motifs for cellular transcriptional control factors was also determined and is reported in table 3. The most frequent transcription binding sites found were NF1 and Sp1, and these were followed in frequency by pseudo NF1 and Gf1 (Marzocchetti et al., 2009). Region F repeatedly retained all of the transcription factor binding sites, although there was a lack of pseudoNF1 and p53 sites in the samples from three patients. Within region C, the deletion of the AP1 and Tar regions was found in four patients (3 CPML and one benign PML). In addition, the Sp-1 binding site and the pentamut sequence were more frequently identified in CPML and HIV-1-negative PML patients than in asymptomatic PML patients ($p < 0.05$).

The Oct-6/tst-1A and pentamut sites were identified only once, and region A was found to contain a binding site for Oct-6/tst-1A that was present in all of the sequences.

No newly created binding sites by deletions and changes have been detected.

The same results were observed for the analysis of the virus isolated from the CSF during follow-up (data not shown).

DISCUSSION

Here we presented a multidisciplinary clinical and molecular longitudinal assessment of PML patients in order to understand the pathogenesis of this disease.

Twenty-two HIV-1+ PML patients were divided into three different groups, based on the presence/severity of symptoms at the onset and during the progression of the disease at follow-up. These included the classical group (10 patients), benign group (9 patients), and asymptomatic group (3 patients). Eight patients affected with CPML died within a few months, and the two remaining patients had only one follow-up examination after six months, whereas the benign and asymptomatic PML patients were monitored during a follow-up period of 6 to 60 months.

The analysis of the immunological and virological features at baseline demonstrated that CPML patients had the lowest CD4+ cell counts followed by the benign and then the asymptomatic cases, whereas the mean JCV viral load in the CSF was significantly higher in the CPML group than in the other HIV-1+ PML groups. The HIV-1-negative PML patients were characterized by a very high rate of JC virus replication in the CNS. Our findings confirm previously published data in the literature, which indicated a poor prognosis for HIV-1-negative PML patients and an 80% mortality rate within 6 months (www.ninds.nih.gov/disorders/pml/pml.htm). In our study, three out of six patients died within one year of disease onset, and two remain alive but are severely disabled. The clinical and virological similarities between the HIV-1-negative PML and CPML cases seem to suggest that factors related to both the JCV infection (high CSF viral load) and the host immune system (low CD4+ cell count) can influence the prognosis of the disease more significantly than the presence of HIV-1 (Berenguer et al., 2003; Berger et al., 1998; Bossolasco et al., 2005; Engsig et al., 2009; Khanna et al., 2009; Tretiakova et al., 1999). However, the HIV-1 TAT-responsive element (TAR) was duplicated in 13 primarily HIV-1+ PML patients, thus confirming the synergistic effect of HIV-1 and JCV (Tretiakova et al., 1999), although the JCV load in the CSF did not significantly correlate with the HIV-1 load.

During follow-up, the JCV load in the CSF remained stable in two CPML patients, was decreased in four of the six benign PML patients and was decreased in all of the asymptomatic PML patients. In particular, virological remission, as defined by the clearance of JCV DNA from the CSF (Giudici et al., 2000; Marzocchetti et al., 2009; Taoufik et al., 1998), was evident in four cases. Our results support previous findings that viral clearance from the CNS occurs after 5 to 25 months of HAART as well as that reduced JCV replication in the brain is related to prolonged survival rate (Bossolasco et al., 2005; Garcia et al., 1999). The reduced viral presence that accompanied the slow progression of the disease was also confirmed by the concomitant clearance of HIV-1 RNA from the CSF.

All of the isolated JCV strains were molecularly characterized to determine the potential risks or prognostic factors involved.

First, no mutation or substantial change occurred in terms of viral genome organization during the follow-up period (data not shown).

The JCV genotype 1 was observed most frequently among all the PML groups examined, which confirmed the geographical distribution of the viral strains, but this was also in contrast with our previous findings that have suggested a correlation between genotype 2 and the higher risk for developing PML. The low number of patients resulting from the longitudinal design of this study may represent one limitation for defining the prevalence of the viral genotypes.

With regard to the TCR organization, a large majority of IR and IIR types, as expected, were amplified from the CSF. Computer-assisted analysis showed that the 19 TCRs of the IIR type differed from all of the other TCRs in terms of sequence and length and were unique in the NCBI database. In contrast, three JCV TCR sequences identical to that of the Mad-1 strain (PML-type) and one JCV TCR sequence identical to that of the Mad-4 strain were amplified from benign, asymptomatic and HIV-1 negative PML patients.

Notably, archetype forms (IIS) were amplified from the CSF in two of the three asymptomatic PML patients. Archetype organization of the JCV TCR is usually detectable in virus that is excreted in the urine of healthy subjects and PML patients and is very rarely (and only after the introduction of HAART treatment) found in CNS specimens (Ferrante et al., 2003). In addition, the IIS form does not show infectious activity *in vitro*.

The potential role for the different molecular organizations of the JCV TCR in PML pathogenesis and in viral neurotropism has been long debated. To date, there is some evidence to indicate that the rearranged forms of the TCR play a crucial role in PML pathogenesis (Daniel et al., 1996; Jensen and Major, 2001; Pietropaolo et al., 2003; Raj and Khalili, 1995; Vaz et al., 2000). Remarkably, Gosert and colleagues recently found that different rearranged JCV TCR sequences, which were isolated from the Mad-4 strain in the CSF of PML patients and tested by *in vitro* assays, were associated with a larger and faster viral replication rate compared to the archetype form (Gosert et al., 2010). Thus, the presence of a weaker promoter, such as the JCV archetype, as well as the consequently low viral replication in the CNS of asymptomatic PML patients may at least partially explain the extremely benign course of the disease.

To define better the molecular organization of the amplified TCR, we determined the nucleotide sequence of each amplified DNA fragment. As previously reported, the C block was repeatedly retained. This block contains distinct functional elements that bind the transcriptional factors NF1, Gf1 and Ap1, which have been shown to confer glial-specific expression to the promoter and to enhance the viral replication rate. In particular, the NF1 sequence was the most frequently found in all the isolated strains, which confirmed, as

demonstrated *in vitro*, that the number of NF1 binding sites is proportional to the level of JCV transcription in glial cell lines (Kumar et al., 1996).

In contrast, block B was repeatedly retained in the CPML patients but was not identified in 4 PML patients. The B block contains the Sp1 binding site, which was the most frequently observed binding site in both the CPML patients and in HIV-1-negative patients and which seems to play a key role in PML pathogenesis. In two HIV-1-negative patients, the D block was partially deleted, but the sequence of the Sp1 binding site was retained and even duplicated. The Sp1 binding motif has been shown to be involved in the downregulation of the myelin basic protein gene promoter (Taoufik et al., 1998) and has been suggested to be essential for complete JCV replication in the absence of HIV-1 infection (Pietropaolo et al., 2003), which corroborates our findings in CPML patients and HIV-1-negative patients.

Moreover, the high frequency of the Sp1 site was also significantly and negatively correlated with the number of CD4+ cells ($p < 0.05$, data not shown).

In summary, based on this extensive follow-up study of PML patients, we have made the following conclusions: a) the JCV load in the CSF determined at the time of the diagnosis may be indicative of the prognosis of the disease; b) a low CD4+ cell count at baseline may be associated with a severe clinical course; c) long-term-survival patients are characterized by the clearance of the virus from the CNS, probably due to an improved immunological status; d) the JCV TCR molecular organization seems to be associated with the clinical course of the disease, and anomalous structures, such as the archetypal form, are present in the CNS specimens of long-term-survival patients and e) although we have reported only limited results regarding HIV-1-negative PML patients, the JCV load and the TCR molecular organization seem to confirm that PML in HIV-1-negative patients resembles the features of the classical disease pathology.

On the basis of these and previous findings, we suggest that future studies be conducted on a larger number of patients and be focused mainly on the JCV load in the CSF as well as the TCR molecular organization, which seem to be the most critical elements for the prognosis of the disease.

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Figure 1.
Schematic diagram of the CSF TCR sequences amplified from HIV+ Classical Course PML patients (Panel A); HIV+ Benign PML patients (Panel B); HIV+ Asymptomatic PML patients (Panel B) and HIV negative PML patients (Panel C). The sequence is numbered according to Frisque et al.(1984)(18).
Legend:
PT: patient

Table 1

Demographic and virological features of HIV-1+ PML patients at baseline

	HIV-1+ PML PATIENTS			HIV-1 negative PML
	CPML 10 patients	BENIGN 9 patients	ASYMPTOMATIC 3 patients	HIV-1 – PML 6 patients
Male/Female	9/1	5/4	3/0	5/1
Mean CD4 count	70.9 ± 29.8/ul ^{a,b}	220.2 ± 78.4/ul ^a	424±157.9/ul ^b	n.a.
Mean HIV-1 viremia	log 4.3 ± 4.1 copies/ml,	log 3.3 ± 3.2 copies/ml	log 4.7 ± 4.6 copies/ml	n.a.
Mean CSF JCV load	log 6.0 ± 1.2 ^{c,d} copies/ml	log 4.0 ± 1.0 ^{c,e} copies/ml	log 4.2 ± 0.5 ^d copies/ml	log 5.8 ± 1.3 ^e copies/ml
Mean CSF HIV-1 load	3/10 log 2.7 ± 1.3 copies/ml	3/9 log 2.2 ± 1.0 copies/ml	1/3 log 2.7 ± 1.7 copies/ml	n.a.
JCV DNA in blood	4/7 log 3.15 ± 1.1 copies/ug	1/7 log 2 copies/ug	0	n.a.
JCV DNA in urine	5/7 log 6.6 ± 1.2 copies/ml	0	1/3 log 5.3 copies/ml	n.a.
JCV genotype	4→1A; 2→1B; 2→2B; 1→2C	5→1A; 1→1B; 1→2B	2→1A; 1→1B; 1→2B	1→1A; 2→1B; 2→2C; 1→4

n.a. not available

^a p<0.05^b p<0.05^d p<0.05^c p<0.01^e p<0.01

Table 2

CSF JCV and HIV-1 viral load (log [copies/ml]) during the follow-up period

	T0	T1	T2	T3	T4	T5	T6
Classical course PML							
Mean follow-up period: 6 months							
PT#1	JCV	5.3	5.9				
	HIV	neg	neg				
PT#2	JCV	6.11	5				
	HIV	neg	n.a.				
Benign PML							
Mean follow-up period: 28 months (range: 6–60)							
PT#1	JCV	4.49	3.99	3.6			
	HIV	neg	neg	neg			
PT#2	JCV	6.32	5.27	3.23	3.23		
	HIV	4.86	neg	neg	neg		
PT#3	JCV	3.23	3.23	3.23	3.48	4.34	neg
	HIV	neg	neg	neg	neg	neg	neg
PT#4	JCV	3.23	3.71	3.16	3.2	neg	neg
	HIV	2.9	neg	4.7	neg	neg	neg
PT#5	JCV	3.23	5.09	5			
	HIV	neg	neg	neg			
PT#6	JCV	4.05	5.7				
	HIV	neg	neg				
Asymptomatic PML							
Mean follow-up period: 16 months (range 6–48)							
PT#1	JCV	3.9	3.5	3.5	3.2		
	HIV	neg	neg	neg	neg		
PT#2	JCV	4.8	5.4	3.2	3.2	neg	
	HIV	4.65	5.54	neg	neg	neg	
PT#3	JCV	3.9	neg				
	HIV	neg	neg				

neg - negative

Table 3

Frequency of DNA binding sites for cellular transcriptional control factors present in the JCV TCR

DNA binding motif	Number of patients with		
	1 copy	2 copies	3 copies
Tata box	22	3	0
Oct-6/tst-1A	25	0	0
Pentanucleotide	0	4	0
SP-1	7	10	2
NF-1	2	10	13
Gf-1	11	14	0
AP-1(CRE)	10	13	0
TAR	10	13	0
Oct-6/tst-1B	5	3	0
Pseudo NF-1	2	22	0
p53	24	0	0
Pseudo AP-1	24	1	0
Pentamut	18	0	0