

# Progressive multifocal leukoencephalopathy in common variable immunodeficiency: mitigated course under mirtazapine and mefloquine

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**Abstract** Demonstration of survival and outcome of progressive multifocal leukoencephalopathy (PML) in a 56-year-old patient with common variable immunodeficiency, consisting of severe hypogammaglobulinemia and CD4+ T lymphocytopenia, during continuous treatment with mirtazapine (30 mg/day) and mefloquine (250 mg/week) over 23 months. Regular clinical examinations including Rankin scale and Barthel index, nine-hole peg and box and block tests, Berg balance, 10-m walking tests, and Montreal Cognitive Assessment (MoCA) were done. Laboratory diagnostics included complete blood count and JC virus (JCV) concentration in cerebrospinal fluid (CSF). The noncoding control region (NCCR) of JCV, important for neurotropism and neurovirulence, was sequenced. Repetitive MRI investigated the course of brain lesions. JCV was detected in increasing concentrations (peak 2568 copies/ml CSF), and its NCCR was genetically rearranged. Under treatment, the rearrangement changed toward the archetype sequence, and later JCV DNA

became undetectable. Total brain lesion volume decreased (8.54 to 3.97 cm<sup>3</sup>) and atrophy increased. Barthel (60 to 100 to 80 points) and Rankin (4 to 2 to 3) scores, gait stability, and box and block (7, 35, 25 pieces) and nine-hole peg (300, 50, 300 s) test performances first improved but subsequently worsened. Cognition and walking speed remained stable. Despite initial rapid deterioration, the patient survived under continuous treatment with mirtazapine and mefloquine even though he belongs to a PML subgroup that is usually fatal within a few months. This course was paralleled by JCV clones with presumably lower replication capability before JCV became undetectable. Neurological deficits were due to PML lesions and progressive brain atrophy.

**Keywords** CD4+ T lymphocytopenia · Idiopathic primary hypogammaglobulinemia · Mefloquine · Mirtazapine · Progressive multifocal leukoencephalopathy · Noncoding control region

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## Introduction

Progressive multifocal leukoencephalopathy (PML) is caused by JC polyomavirus (JCPyV or JC virus (JCV)) infection of the central nervous system. PML risk factors are HIV infection, immunosuppressive therapy after organ and stem cell transplantation, and immunomodulatory agents used in the treatment of autoimmune disorders (Steiner and Berger 2012; Weber 2008; Gheuens et al. 2011; Bellizzi et al. 2013).

JCV can have a strong neurotropism, not only lytically infecting oligodendrocytes and, to a lesser extent, astrocytes, but can also spread to gray matter (Bellizzi et al. 2013; Elphick et al. 2004; Wüthrich and Koralnik 2012). JCV enters cells via N-linked glycoprotein with  $\alpha$ -(2,6)-linked sialic acid and the serotonergic 5-HT-2A receptor, two components of a putative JCV receptor. JCV is internalized with the 5-HT-2A receptor by clathrin-mediated endocytosis (Bellizzi et al. 2013; Elphick et al. 2004). The archetype JCV is not associated with PML. Increased virulence and neurotropism of JCV are associated with genetic rearrangement mainly in its hypervariable noncoding control region (NCCR) (Bellizzi et al. 2013). The NCCR of the archetype JCV contains a bidirectional promoter, the viral origin of replication, enhancers, and six regions termed box A to F which contain binding sites for host transcription factors and the viral large T antigen. The NCCR is involved in virus replication, and its genetic rearrangement has been associated with poor PML outcome (Bellizzi et al. 2013; Hirsch et al. 2013). Impairment of the host immune system with CD4+ and CD8+ T lymphocytopenia, NCCR rearrangement, activation/upregulation of transcription factors binding to recombinant NCCR, and migration of JCV—freely or via B lymphocytes—are conditions that, in combination, will lead to PML (Bellizzi et al. 2013).

As yet, no specific treatment exists against JCV. Of the several compounds tested, we would like to focus on mirtazapine and mefloquine. Mirtazapine can pass the blood–brain barrier (BBB) and inhibits central noradrenergic, serotonergic, and histaminergic receptors. Its binding to the 5-HT-2A receptor is believed to block JCV entry into cells (Elphick et al. 2004). Mefloquine equally passes the BBB and is believed to accumulate in the brain in significantly higher concentrations than in plasma due to its long plasma half-life, lipophilicity, and inhibition of an ATP-binding cassette (ABC) transporter called multidrug resistance protein 1 (MDR-1) that is an efflux pump at the BBB. Mefloquine's assumed anti-JCV activity is inhibition of DNA replication by binding to the T antigen, a JCV helicase (Brickelmaier et al. 2009). PML treatment with mirtazapine or mefloquine was mainly described in patients with HIV, organ transplant, dermatomyositis, polycythemia vera, sarcoidosis, systemic lupus erythematosus, and idiopathic CD4+ T lymphocytopenia (Loyaga-Rendon et al. 2013; Delgado-Alvarado et al. 2013; Hohlfield et al. 2012; Verma et al. 2007; Vulliemoz et al. 2006;

Beppu et al. 2012; Naito et al. 2012; Clifford et al. 2013; Cettomai and McArthur 2009). Combined treatment with mefloquine and mirtazapine has been reported in PML cases in the context of HIV infection, multiple sclerosis (MS) (Gheuens et al. 2011; Iannetta et al. 2013; Moenster and Jett 2012; Schröder et al. 2010), and in one seemingly immunocompetent PML patient (Christakis et al. 2013). One case series compared the effect of mirtazapine to mirtazapine and mefloquine in 34 HIV-negative patients with PML (Gheuens et al. 2011), including nine patients who had hematological disorders including Waldenstrom macroglobulinemia, common variable immunodeficiency (CVID), and idiopathic CD4+ or CD8+ T lymphocytopenia. Combined treatment did not improve 1-year survival of the 34 patients compared to mirtazapine alone. Neither survival rate nor presence or lack of therapeutic effect was presented for the subgroup with hematological disorders and, in particular, for the patients with CVID or idiopathic CD4+ or CD8+ T lymphocytopenia.

CVID encompasses a diagnostic group of approximately 150 different primary immunodeficiencies, which have a common set of features including hypogammaglobulinemia, but which have different underlying causes (Salzer et al. 2012). Late-onset combined immune deficiency (LOCID) is a CVID subgroup, defined by the additional occurrence of opportunistic infections and/or a CD4+ T cell count  $< 200 \times 10^6$  cells/L (Malphettes et al. 2009).

The overall 1-year survival rate of HIV-negative PML patients is around 50 % (Marzocchetti et al. 2009), with dramatic variations depending on the underlying immunocompromising disorder. The 1-year survival rate of PML patients with CVID or LOCID is not well known. HIV-negative PML patients with CVID or idiopathic CD4+ or CD8+ T lymphocytopenia were reported to survive at least 1 year under mirtazapine alone or in combination with mefloquine, but their numbers were not specified (Gheuens et al. 2011). Further PML cases with constitutive immunodeficiency were reported: three were CVID patients and three most likely fulfilled (according to us) the LOCID criteria (Supplementary Table 1) or had Good's syndrome (Squintani et al. 2010). None was treated with mirtazapine and/or mefloquine, but with different compounds, or did not receive treatment. Survival was less than 10 months or not reported.

We present an adult patient with CVID and CD4+ T lymphocytopenia (i.e., LOCID) who contracted PML and was treated with mirtazapine and mefloquine since October 2012. His regular follow-up included clinical examination and measurements of motor performance, cognition, brain lesion volume, and JCV DNA quantification in cerebrospinal fluid (CSF). The NCCR of JCV was DNA-sequenced, as it is typically rearranged in PML with deletion and/or duplication of archetype sequence elements. This patient is still alive without active PML signs 23 months after diagnosis, has residual neurological deficits, and has a reasonable quality of life.

## Patient and methods

Our study complies with the Declaration of Helsinki and was approved by the local ethics committee of Bern. Written informed consent was obtained. The 56-year-old patient was followed up at ~3 monthly intervals between September 2012 and August 2014, when he was last seen, with general clinical and neurological examinations, assessments of Rankin scale and Barthel index (disability, independence in daily activities), Berg balance test (for static and dynamic balance abilities), 10-m walking test, Montreal Cognitive Assessment (MoCA, cognition), and nine-hole peg and box and block tests (manual fine and coarse motor skills performed with right hand under physiotherapist supervision). Laboratory diagnostics included differential blood count; serum protein electrophoresis; and quantification of IgG, IgM, and IgA concentrations, absolute B lymphocyte as well as CD4+ and CD8+ T lymphocyte counts. From DNA isolated out of CSF, (i) the JCV copy number was determined by quantitative polymerase chain reaction (PCR) and (ii) the NCCR of JCV was selectively amplified by PCR. PCR products were separated on agarose gel, excised, and sequenced as previously described (Hirsch et al. 2013). Repetitive cerebral MRIs were performed and segmented manually slice by slice on high-resolution fluid-attenuated inversion recovery (FLAIR) sequences (pulse parameters: TR/TE of 8500/89 ms, TI 2500 ms; matrix size 512 × 512) in order to determine the cerebral lesion volumes, using a slice thickness of 1 mm<sup>3</sup> and the open source Software 3-D Slicer Version 4.2.2.3 ([www.slicer.org](http://www.slicer.org)).

## Results

### PML diagnosis in September 2012

The patient first noticed persistent diplopia, worse on gaze to the right shortly after a severe sinusitis in June 2012. Over the next 2 months, gait disturbance, coordination problems of the right arm and leg, and severe fatigue appeared.

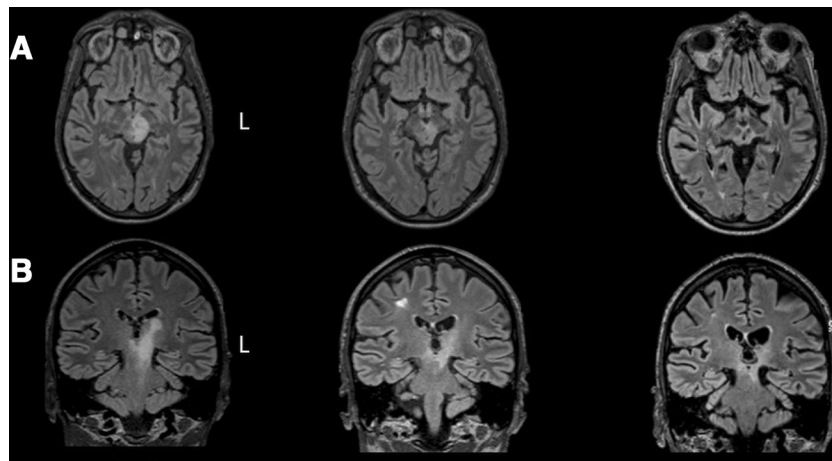
At first admission in September 2012, neurological examination showed a complex pretectal syndrome (abduction deficit of the right eye, hypotropia and esotropia of the right eye, and a vertical gaze palsy), mild right-sided hemiparesis (including facial palsy), and right-sided hemi-ataxia including gait ataxia. Cranial MRI (T2-weighted and FLAIR images) showed hyperintense confluent lesions in the left-sided medial thalamus, hypothalamus, mesencephalon, and tegmentum pontis (Fig. 1). Further lesions were found in the colliculus superior and inferior, medulla oblongata, right gyrus frontalis medius, and in the right cerebellar hemisphere with the latter two lesions enhancing with gadolinium (not shown). CSF contained six mononuclear cells per microliter, protein

0.61 g/l, glucose 2.85 mmol/l, lactate 1.6 mmol/l, and no oligoclonal bands. The cytological analysis was negative for neoplastic cells. Cultures of CSF were negative for general bacterial growth, *Mycobacterium tuberculosis*, and *Cryptococcus neoformans*. Serology was negative for various neurotropic bacteria and viruses including HIV. PCR with DNA from CSF was negative for *Mycoplasma pneumoniae*, *Tropheryma whipplei*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, herpes simplex 1 and 2, varicella zoster, cytomegalovirus, Epstein-Barr virus, enteroviruses, and human herpesvirus 6 but showed 1167 copies/ml and, a week later, 2568 copies/ml of JCV DNA (Table 1, Fig. 2). Together with the neurological deficits, the MRI lesion pattern, and the positive JCV DNA PCR, the diagnosis of PML was established. The diagnosis of CVID was based on familial severe hypogammaglobulinemia (his 2 sisters were also affected) and a history of immune thrombocytopenia (ITP), autoimmune hemolytic anemia (AIHA), and pseudo T cell lymphoma. The patient first presented with hepatosplenomegaly at the age of 3 years and was diagnosed with AIHA 2 years later (Roth et al. 1975). He suffered from lifelong recurrent sinopulmonary infections. For their efficient prevention, he was regularly and successfully treated with intravenous immunoglobulins (IvIgG). Blood analysis at the time of PML diagnosis showed hypogammaglobulinemia (IgG 3.19 g/l, under replacement therapy with IvIgG 30 g every 4 weeks; IgM <0.05 g/l; IgA <0.06 g/l), as well as a severe depletion of CD4+ T (97/μl) and B lymphocytes (0/μl). Our patient belongs to the only 10 % of CVID patients with very low B cell counts. In contrast, the CD8+ T cell count was normal (Table 1). His immunodeficiency matches the definition of LOCID. PCR amplification and sequencing of the NCCR of JCV DNA from September 3, 2012, revealed a genetic rearrangement. Four sequences were obtained with a common signature deletion *del*(C<sub>54–55</sub>D<sub>1–64</sub>) of most of the D box, starting in the end of the C box (variants V1C120903, V2C120903, V1C120907, and V2C120907), together with partial duplications of the sequence boxes A, B, and C whereas two variants with continuous duplications (V1C121205 and V2C121205) were identified in the last CSF sample with lower JCV loads (Fig. 2).

The patient's condition rapidly deteriorated with progression of right-sided hemiataxia, gait ataxia, pretectal oculomotor syndrome, and onset of dysarthria. In October 2012, we started treatment with mirtazapine 30 mg daily and mefloquine (250 mg daily for 3 days, then 250 mg weekly) and as of then continued therapy.

### Follow-up examinations over the course of 23 months

Between September 2012 and August 2014, the Barthel (60 to 100 to 80) and Rankin (4 to 2 to 3) scores first improved and then fell to a level still better compared to treatment start. The



**Fig. 1** Brain MRI, depicting axial (**a**) and coronal (**b**) fluid attenuated inversion recovery (FLAIR) sections on different time points: *left column* on September 2012, *middle column* on June 2013, and *right column* on September 2013. Note the typical PML affection of midline structures and subcortical white matter (U fibers) as, e.g., seen in **b** in the *left* and *middle column*, respectively. Also, note shrinking of total lesion volume,

as well as progressive brain atrophy from *middle* to *right column*. *L* left. Cerebral MRIs from January, May, and August 2014 are not depicted because the increase in brain atrophy and decrease in lesion volume were quantified and given numerically in the “Results” section and Table 1, respectively

nine-hole peg test (300, 50, 300 s) and the box and block test (7, 35, 25 pieces during follow-up) showed first an improvement of manual skills and then a deterioration due right-sided ataxia in this right hander. On the Berg balance test, which was not accomplishable at the time of diagnosis, the patient scored independent with a tendency to worsen. His walking speed over 10 m remained unaltered (1.0–1.25 m/s) (Table 1; reference ranges for all tests in respective figure legend). B cell counts were always 0/ $\mu$ l. The hypogammaglobulinemia (varying IgG concentrations due to monthly IvIgG replacement) and the CD4+/CD8+ T cell counts remained essentially unchanged (Table 1). In CSF from September 7, 2012, 2568 JCV copies/ml were found with NCCR sequences that still contained the common signature deletion *del*(C<sub>54–55</sub>D<sub>1–64</sub>) but different partial duplications of the boxes C, D, and E than on September 3 (Fig. 2). While under mirtazapine and mefloquine for about 2 months, JCV concentration decreased to 363 copies/ml on 5 December 2012. Now, the signature deletion of box D was lost and a less complex NCCR rearrangement was found interrupting the continuity of the D box at D<sub>35</sub> by inserting the sequences B<sub>20–23</sub>, C<sub>1–55</sub>, and D<sub>1–35</sub> (Table 1, Fig. 2). JCV DNA was not detectable by PCR as of March 2013. The patient’s cognitive performance (MoCA) showed a slight deficit which remained stable (Table 1). Over the course of 23 months, total brain lesion volume decreased from 8.54 to 3.97 cm<sup>3</sup>, as did the midline-lesion volume (hypothalamic, mesencephalic, pontine) from 7.31 to 0.50 cm<sup>3</sup>. The midline-lesion volume was assumed to be responsible for the oculomotor deficit, dysarthria, and the motor symptoms (Table 1). While the midline-lesion volume decreased upon treatment initiation, the total lesion volume first increased due to appearance of new subcortical lesions and then decreased

(Table 1, Fig. 1). Brain atrophy visibly increased between PML onset in September 2012 until September 2013 (Fig. 1). Between September 2013 and August 2014, brain atrophy further advanced and was quantified by a decrease in volume of gray matter (735 to 640 cm<sup>3</sup>, 13 %) and white matter (436 to 411 cm<sup>3</sup>, 6 %).

In September 2013, the patient reported ongoing improvement and no treatment side effects. Ambulation was possible without walking aid for ~1000 m. Hemiparesis, hemiataxia, and gait ataxia had improved. Dysarthria and the pretectal syndrome persisted at a stable level. His manual fine and coarse motor skills showed a tendency to worsen. In January 2014, he contracted community-acquired pneumonia and his performance could not be tested. Afterward, gait balance, right-handed manual skills, and dysarthria were worse without signs of active PML (MRI, CSF). As of then, performance remained largely stable except for manual fine motor skills (Table 1).

## Discussion

We present a patient meeting definite diagnostic certainty for PML (Berger et al. 2013) acquired on the basis of CVID, respectively, an especially severe subcategory, LOCID. The time point of JCV infection cannot be determined with certainty: would earlier sera have been available, they might have been positive for JCV antibodies due to repeated IvIgG infusions. Alternatively, even with an earlier infection, they might have been negative because of a severely defective humoral immune response caused by the severe B cell depletion. Our

**Table 1** Barthel index, Rankin scale, and nine-hole peg and box and block tests (given is the average of three performances with the right hand for each test), Berg balance, and 10-m walking tests

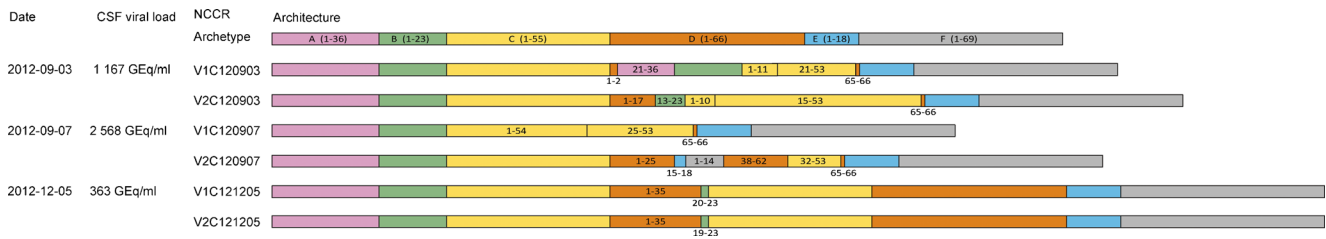
Assessments	Diagnosis		Follow-up									
	Sept 2012	Tx start	Oct 2012	Dec 2012	Jan 2013	Mar 2013	Jun 2013	Sept 2013	Jan 2014	May 2014	Aug 2014	
Barthel index			60	75	100	100	100	100	100	b	65	80
Rankin scale			4	4	3	2	2	2	2		3	3
Nine-hole peg (s)		Not possible	Not possible	73	81	50	52	90	90		240	Not possible
Box and block (pieces)		7	21	22	22	26	35	30	30		25	25
Berg balance (points)		Not possible	Not possible	Not possible	Not possible	1.00	52	50	50		40	40
Ten-minute walking test (m/s)		Not possible	Not possible	Not possible	1.25	1.00	1.00	1.11	1.11		1.0	1.0
Total lesion volume (cm <sup>3</sup> )	8.54		7.04	11.50	16.76	14.68	7.11	6.31	6.30	6.30	5.36	3.97
Midline-lesion volume (cm <sup>3</sup> )	7.31		5.96	5.27	3.28	3.12	2.56	1.25	1.21	1.21	0.87	0.50
JCV DNA copies/ml CSF	1167/2568	No LP	No LP	363	No LP	0	0	No LP	No LP	0	No LP	No LP
Leukocytes (cells/ $\mu$ l)	3300	2400	2400	1800	1400	2000	1900	1900	1900	b	1700	2200
Lymphocytes (cells/ $\mu$ l)	850	940	940	650	730	880	550	900	900		613	525
CD4+ T (cells/ $\mu$ l)		88	88	111	99	109	102	134	134		88	80
CD8+ T (cells/ $\mu$ l)		531	531	549	485	551	446	606	606		352	291
B lymphocytes (cells/ $\mu$ l)		0	0	0	0	0	0	0	0		0	0
IgG (g/l) <sup>a</sup>	3.19	15.30	15.30	8.68				7.58	7.58		8.00	9.09
IgM (g/l)	<0.05	<0.05	<0.05	0.12				<0.05	<0.05		0.09	<0.03
IgA (g/l)	<0.06	<0.06	<0.06	0.07				<0.06	<0.06		0.05	<0.05
Thrombocytes (cells/ $\mu$ l)	128,000	165,000	165,000	162,000	122,000	146,000	137,000	131,000	131,000		157,000	137,000
MoCA		Other test <sup>c</sup>	Other test <sup>c</sup>	24/30	22/30	24/30	24/30	27/30	27/30		26/30	26/30

In addition, brain MRI total and midline (hypothalamic and brainstem)-lesion volume, JCV DNA concentration in CSF, absolute CD4+ and CD8+ T as well as B cell counts, immunoglobulin concentrations (IgG, IgM, IgA) in serum, and Montreal Cognitive Assessment (MoCA) are given. Diagnosis, treatment start (Tx start) with mefloquine + mirtazapine, and follow-up assessments were made at given time points. Reference ranges: Barthel: 100 (points) indicate independence in grooming, toilet use, absence of incontinence, bathing, feeding, dressing, transfers, walking, and climbing stairs. Rankin: scale runs from 0 (no symptoms) to 6 (dead); 2 indicates slight disability. Nine-hole peg: normal sex/age-related cutoff for right hand  $\leq 21 \pm 5$  s (Oxford Grice et al. 2003). Box and block: normal sex/age-related cutoff for right hand  $\geq 74 \pm 1$  pieces (Mathiowetz et al. 1985). Berg balance: independence scores  $>40$  and  $\leq 56$  points. Ten-minute walking (speed): healthy male adult, decade 50–60—1.39 m/s. MoCA—26–30 points for healthy adults. Lymphocytes (cells/ $\mu$ l)—B 72–460, CD4+ T 410–1590, and CD8+ T 137–823. Immunoglobulins (g/l)—IgG 6.50–13.50, IgA, 0.75–3.12, and IgM 0.56–3.52 LP lumbar puncture, IgG/M/A immunoglobulin G/M/A

<sup>a</sup> Under four weekly IvIgG substitutions

<sup>b</sup> No clinical parameters were collected because the patient suffered from community-acquired pneumonia in Jan 2014

<sup>c</sup> A moderate cognitive deficit in a different test battery using Consortium to Establish a Registry for Alzheimer's Disease (CERAD) and material and norm values for neuropsychological diagnostic (MIND) tests



**Fig. 2** Schematic illustration of the JCV archetype noncoding control region (NCCR) and the rearranged NCCRs found in CSF at indicated time points. The NCCR of the archetype JCV is presented with six blocks *A-B-C-D-E-F* in different colors and with numbers (in brackets) indicating their size in base pairs. For the rearranged NCCRs, the size

in base pairs is indicated only for those sequence blocks in which mutations were found. The date, viral load in CSF, and the archetype and variant (see “Results” section) JCV NCCR architectures are indicated. CSF cerebrospinal fluid, GEq/ml genome equivalents (or copies) per milliliter

patient was in fact positive for anti-JCV IgG. We believe that this was rather due to the IvIgG infusions.

Our patient is still alive almost 2 years after PML onset and tolerates well the long-term treatment with mefloquine and mirtazapine. Clinical improvement, already evident 2 months after treatment initiation, was paralleled by the reduction of the midline-lesion volume, while the total lesion volume increased up to the fourth treatment month due to the appearance of new lesions. After the fifth treatment month, no new lesions appeared and the known lesions were shrinking with subsequent brain atrophy predominantly at former lesion sites. Why new neuroradiological lesions appeared during the first 4 months under mirtazapine that is supposed to block JCV entry into cells remains unclear. It has to be taken into account that amplification of viral DNA in CSF does not fully reflect viral replication in brain tissue, as shown by the lower PCR sensitivity in CSF of AIDS patients with PML on antiretroviral therapy (Marzocchetti et al. 2005). Cognition remained stable, which is important as PML is usually paralleled with cognitive decline and brain atrophy. This is partly explained by the small subcortical- and large midline-lesion volume. Had he not contracted community-acquired pneumonia in January 2014, he might have been well at an overall better performance level. We did not find any cause, i.e., no active PML signs for his deterioration after pneumonia, and hence did not treat him with interleukin-7, a promising new agent for PML patients (Alstadhaug et al. 2014). His worse performance was mainly due to increased ataxia which might be due to increased brain atrophy at the former midline-lesion site.

It has to be left open whether mirtazapine and mefloquine contributed to the survival of our patient. In general, survival of HIV-negative patients with PML and comparable immune status (idiopathic CD4+ T lymphocytopenia, CVID, Good’s syndrome) to our patient ranges between 2 and 3 months (longer for one patient in a vegetative state) without treatment and between 2 and 10 months under treatments including cidofovir, cytosine arabinoside, or interferon ((Squintani et al. 2010), Supplementary Table 1). Survival of PML patients with idiopathic CD4+ or CD8+ T lymphocytopenia or

CVID (immune status of individuals not specified) under mirtazapine or mirtazapine + mefloquine (without information about assignment of treatment to individuals) was at least 1 year (Gheuens et al. 2011) and even 34 months in a patient with idiopathic CD4+ T lymphocytopenia (242/μl) under mirtazapine (and mefloquine for the first few months) (Delgado-Alvarado et al. 2013). It has to be kept in mind that the B cell deficiency in CVID and the B and CD4+ T cell deficiency in LOCID patients with PML cannot be modified except for substitution with IvIgG. This is unlike PML infections in patients with, e.g., HIV, MS under natalizumab therapy, or cancer under immunosuppression, in whom the underlying condition predisposing to PML often can be modulated or reverted resulting in improved immunocompetence and abatement of PML. IvIgG therapy can contribute to partial restoration of the CD4+ T cell compartment and the reduction of CD8+ T cell activation in CVID patients (Paquin-Proulx et al. 2013). It is, however, less likely that IvIgG played an important role in the immune defense of our patient, as it did not prevent JCV infection and PML onset.

The NCCR rearrangements identified in our patient were insofar compatible with the literature, as deletions mainly affected the D box, while duplications were rather found in proximity to the origin of replication and the promoter of the early viral gene region (Hirsch et al. 2013). Although the NCCR architecture differed from so-called prototypes previously reported in PML, or in HIV patients with PML (Bellizzi et al. 2013), it becomes increasingly clear that NCCR rearrangements apparently are variable among different PML patients and frequently unique for an individual patient. It is therefore difficult to identify particular NCCR rearrangements that correlate with a poor PML outcome (Hirsch et al. 2013). Notably, different NCCR rearrangements were detected in our patient over time. It is tempting to speculate that this change toward the archetype NCCR, which is believed to be associated with lower virus replication, contributed to the patient’s survival and absent detection of JCV DNA in CSF. The inherent limitation of this and other single cases in a highly variable disease is, of course, the difficulty of generalizing the evidence of the approaches to diagnosis and therapy to larger

populations. The observation of the parallelism between changes in the NCCR and disease evolution, however, may have pathogenetic implications, and further data of this type could help in comparing potential effects of different approaches to therapy on outcome.

Multiple reasons may underlie the mixed treatment results of mirtazapine, mefloquine, or both, particularly in PML patients with constitutional immunodeficiency. First, different diseases with variable degrees of immunodeficiency predisposing to PML cannot be well compared concerning their response to treatment. Second, the delay between PML symptom onset and treatment initiation, as well as the brain lesion volume at diagnosis, is important. As suggested (Cettomai and McArthur 2009), most significant clinical improvement may be achieved with therapy start close to PML symptom onset and little lesion volume. Third, the NCCR of JCV should be systematically sequenced in future PML treatment studies to learn whether NCCR reshuffling indeed affects survival and may be influenced by treatment. Brain atrophy may constitute a future problem, as its progression in the absence of active PML is unclear and treatment options except for physiotherapy are missing.

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**Contributors** RK, MS, and HK were involved in diagnosis and treatment of the patient. RK, MS, and HK designed the study. RK, CW, and PK conducted the examinations and laboratory studies. RK, BL, MS, and HK drafted the manuscript. RW, HF, RDP, and HHH intellectually contributed to data interpretation and the manuscript. The version to be published was approved by all of the authors. HK accepts full responsibility for the data as guarantor.

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**Conflict of interest** The authors declare that they have no conflict of interest.

**Patient consent** Obtained.

## References

- Alstadhaug KB, Croughs T, Henriksen S et al (2014) Treatment of progressive multifocal leukoencephalopathy with interleukin 7. *JAMA Neurol* 71:1030–1035
- Bellizzi A, Anzivino E, Rodio DM et al (2013) New insights on human polyomavirus JC and pathogenesis of progressive multifocal leukoencephalopathy. *Clin Dev Immunol* 10:839719
- Beppu M, Kawamoto M, Nukuzuma S et al (2012) Mefloquine improved progressive multifocal leukoencephalopathy in a patient with systemic lupus erythematosus. *Intern Med* 51:1245–1247
- Berger JR, Aksamit AJ, Clifford DB et al (2013) PML diagnostic criteria: consensus statement from the AAN Neuroinfectious Disease Section. *Neurology* 80:1430–1438

- Brickelmaier M, Lugovskoy A, Kartikeyan R et al (2009) Identification and characterization of mefloquine efficacy against JC virus in vitro. *Antimicrob Agents Chemother* 53:1840–1849
- Cettomai D, McArthur JC (2009) Mirtazapine use in human immunodeficiency virus-infected patients with progressive multifocal leukoencephalopathy. *Arch Neurol* 66:255–258
- Christakis PG, Okin D, Huttner AJ et al (2013) Progressive multifocal leukoencephalopathy in an immunocompetent patient. *J Neurol Sci* 326:107–110
- Clifford DB, Nath A, Cinque P et al (2013) A study of mefloquine treatment for progressive multifocal leukoencephalopathy: results and exploration of predictors of PML outcomes. *J Neurovirol* 19:351–358
- Delgado-Alvarado M, Sedano MJ, González-Quintanilla V et al (2013) Progressive multifocal leukoencephalopathy and idiopathic CD4 lymphocytopenia. *J Neurol Sci* 327:75–79
- Elphick GF, Querbes W, Jordan JA et al (2004) The human polyomavirus, JCV, uses serotonin receptors to infect cells. *Science* 306:1380–1383
- Gheuens S, Bord E, Kesari S et al (2011) Role of CD4+ and CD8+ T-cell responses against JC virus in the outcome of patients with progressive multifocal leukoencephalopathy (PML) and PML with immune reconstitution inflammatory syndrome. *J Virol* 85:7256–7263
- Hirsch H, Kardas P, Kranz D et al (2013) The human polyomavirus (JCPyV): virological background and clinical implications. *Acta Pathol Microbiol Immunol Scand* 121:685–727
- Hohlfeld SK, Günthard HF, Zeitz J et al (2012) Progressive multifocal leukoencephalopathy as a rare lethal complication in untreated sarcoidosis. *BMJ Case Rep*. doi: 10.1136/bcr.03.2011.4036
- Iannetta M, Bellizzi A, Lo Menzo S et al (2013) HIV-associated progressive multifocal leukoencephalopathy: longitudinal study of JC virus non-coding control region rearrangements and host immunity. *J Neurovirol* 19:274–279
- Loyaga-Rendon RY, Taylor DO, Koval CE (2013) Progressive multifocal leukoencephalopathy in a heart transplant recipient following rituximab therapy for antibody-mediated rejection. *Am J Transplant* 13:1075–1079
- Malphettes M, Gérard L, Carmagnat M et al (2009) Late-onset combined immune deficiency: a subset of common variable immunodeficiency with severe T cell defect. *Clin Infect Dis* 49:1329–1338
- Marzocchetti A, Di Giambenedetto S, Cingolani A et al (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
- Marzocchetti A, Tompkins T, Clifford DB et al (2009) Determinants of survival in progressive multifocal leukoencephalopathy. *Neurology* 73:1551–1558
- Mathiowetz V, Volland G, Kashman N et al (1985) Adult norms for the box and block test of manual dexterity. *Am J Occup Ther* 39:386–391
- Moenster RP, Jett RA (2012) Mirtazapine and mefloquine therapy for progressive multifocal leukoencephalopathy in a patient infected with human immunodeficiency virus. *Am J Health Syst Pharm* 69:496–498
- Naito K, Ueno H, Sekine M et al (2012) Akinetic mutism caused by HIV-associated progressive multifocal leukoencephalopathy was successfully treated with mefloquine: a serial multimodal MRI Study. *Intern Med* 51:205–209
- Oxford Grice K, Vogel KA, Le V et al (2003) Adult norms for a commercially available nine hole peg test for finger dexterity. *Am J Occup Ther* 57:570–573
- Paquin-Proulx D, Santos BA, Carvalho KI et al (2013) IVIg immune reconstitution treatment alleviates the state of persistent immune activation and suppressed CD4 T cell counts in CVID. *PLoS ONE* 8, e75199
- Roth P, Morell A, Hunziker HR et al (1975) *Schweiz med Wschr* 105:1584–1585

- Salzer U, Warnatz K, Peter HH (2012) Common variable immunodeficiency—an update. *Arthritis Res Ther* 14:223
- Schröder A, Lee DH, Hellwig K et al (2010) Successful management of natalizumab-associated progressive multifocal leukoencephalopathy and immune reconstitution syndrome in a patient with multiple sclerosis. *Arch Neurol* 67:1391–1394
- Squintani G, Ferrari S, Bazzoli E et al (2010) Progressive multifocal leukoencephalopathy in a patient with Good's syndrome. *Int J Infect Dis* 14:e444–e447
- Steiner I, Berger JR (2012) Update on progressive multifocal leukoencephalopathy. *Curr Neurol Neurosci Rep* 12:680–686
- Verma S, Cikurel K, Koralnik IJ et al (2007) Mirtazapine in progressive multifocal leukoencephalopathy associated with polycythemia vera. *J Infect Dis* 196:709–711
- Vulliemoz S, Lurati-Ruiz F, Borruat FX et al (2006) Favourable outcome of progressive multifocal leukoencephalopathy in two patients with dermatomyositis. *J Neurol Neurosurg Psychiatry* 77:1079–1082
- Weber T (2008) Progressive multifocal leukoencephalopathy. *Neurol Clin* 26:833–854
- Wüthrich C, Koralnik IJ (2012) Frequent infection of cortical neurons by JC virus in patients with progressive multifocal leukoencephalopathy. *J Neuropathol Exp Neurol* 71:54–65