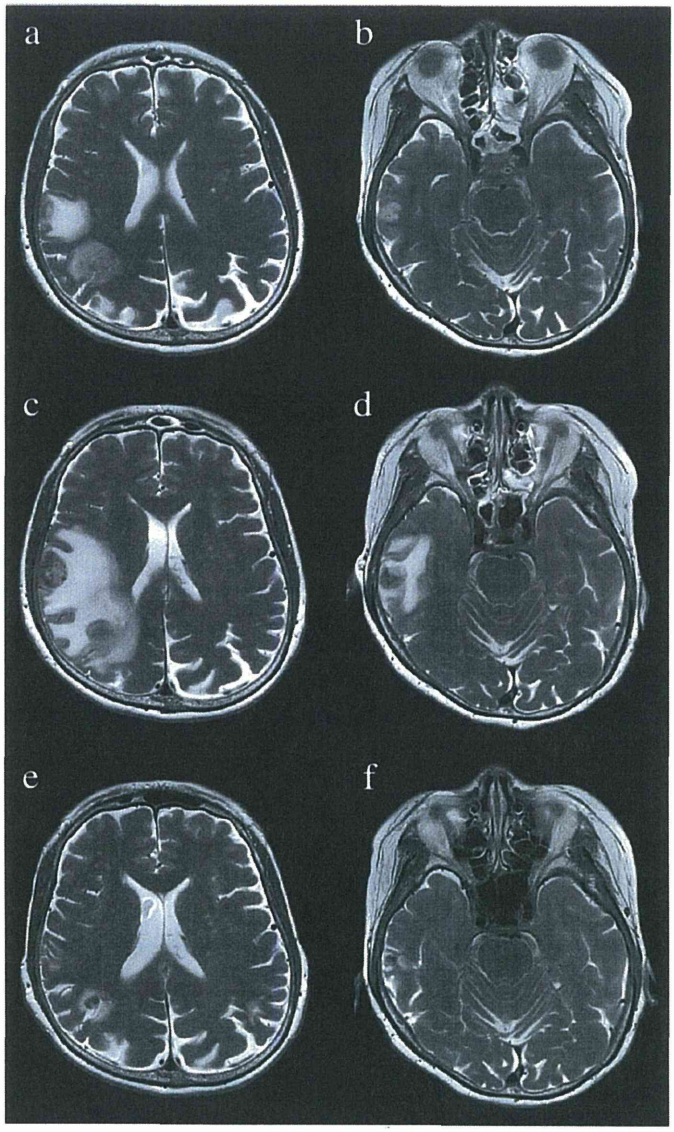

Fig. 1. Serial magnetic resonance (MR) findings. Initial MR T2-weighted images obtained on day 2, showing abnormally high signal intensity in the right lateral and parietal lobes (panels a and b). Lesions with abnormally high signal intensity on images obtained at the second MR examination, performed on day 23 (panels c and d). Third MR T2-weighted images obtained on day 200, showing that the abnormally high signal intensity had resolved (panels e and f).

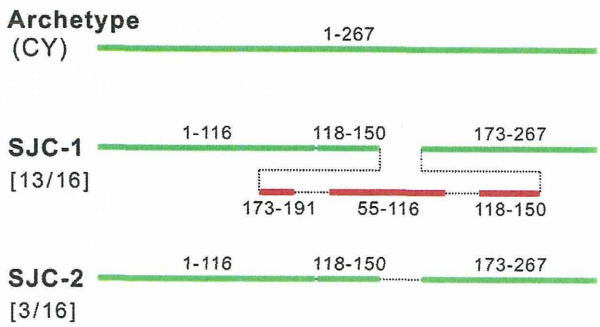

Fig. 2. Mutation of the JC virus genome detected in the CSF. The non-coding control region was cloned and sequenced, and the rearrangement patterns of each clone (designated as SJC-1 and SJC-2) were compared with the sequence of the archetype (nonpathogenic) CY strain. The green and red lines indicate the fragments identical to the archetype virus and the duplicated sequences, respectively. The nucleotide numbers corresponding to the archetype NCCR are shown above or below the solid lines. Frequencies of NCCR patterns are shown in square brackets.

and involuntary movements of the forearm progressed, and a second MRI scan on day 9 showed increased numbers of abnormal high-intensity signals (Fig. 1c and d). On day 10 after admission, MMF was discontinued. On day 25, cyclosporine was withdrawn, and the dose of prednisone was decreased to 5 mg/day. One month after the withdrawal of these drugs, the involuntary movements decreased, and the weakness had resolved. On day 200 after admission, the third MRI scan showed markedly decreased numbers of high-intensity signals (Fig. 1e and f), and the results of real-time PCR for JCV DNA in CSF became negative. In April 2013, the patient had no other neurological deficits. After that prednisone (5 mg/day) was continued. In November 2012, renal function deteriorated (serum creatinine: 3.6 mg/dl), and she received cyclosporine (10 mg/day), prednisone (60 mg/dl), and mizoribin (100 mg/day). In July 2013, neurological deterioration was not evident, but the CSF titer of JCV DNA on quantitative real-time PCR had increased to 1068 copies/ml. Both cyclosporine and mizoribin were withdrawn, and prednisone (5 mg/day) was continued. In August 2013, JCV DNA became negative. In November, she was reintroduced to hemodialysis, and no neurological deterioration occurred.

3. Discussion

To date, PML has been managed by mefloquine or highly active antiretroviral treatment (HAART) for HIV-positive cases or by the withdrawal of immunosuppressants, but the benefits of these measures have been limited. In our patient, the withdrawal of both cyclosporine and MMF and a decrease in the dose of prednisone reduced the severity of PML as well as cranial lesions on MRI, and the results of tests for JCV genome in CSF became negative.

Previously, six patients with PML after renal transplantation who had good outcomes have been reported (Table 1) [4,15,8,2,5,12]. The interval from renal transplantation to the onset of PML in our patient was 5 years, similar to previous patients (median, 4.25 years; range, 1 month to 10 years). This interval is longer than that of general cases of PML after renal transplantation (median, 30 months) [11]. The interval from the withdrawal of immunosuppressant therapy to the onset of clinical improvement was shorter in our patient (1 month) than in the other patients (median, 3 months; range, 1 to 11 months). The relatively prompt improvement in our patient might be ascribed to the fact that the immunosuppressant therapy was discontinued immediately after the early diagnosis of PML, performed using a highly sensitive quantitative PCR approach. MMF (n = 5), tacrolimus (2), cyclophosphamide (2), or cyclosporine (1) was withdrawn in the 7 reported patients including our patient [4,15,8,2,5,12]. The early withdrawal of MMF in most patients was probably related to the fact that current guidelines issued by the United States Food and Drug Administration recommend that MMF is initially withdrawn [14].

In one patient with a favorable course, the JCV DNA load in serum was evaluated, and the viral genome was not detected in serum 6 weeks after stopping immunosuppressant therapy (Table 1); however, the viral load in CSF was not assessed [15]. We analyzed the JCV DNA load in CSF by quantitative PCR and diagnosed PML without the need for an invasive brain biopsy, and the JCV genome in CSF disappeared with a reduction in the severity of PML. A study of real-time PCR in patients with HIV-positive PML showed that JCV DNA levels in CSF turned out to be negative in 4 patients with benign or asymptomatic PML [3]. The JCV DNA level in our patient was 4.10 log copies/ml. A JCV DNA level exceeding 3.64 log copies/ml in CSF on real-time PCR has been associated with a shorter survival time in HIV-positive PML [1]. However, another study showed that viral load values of >4.68 log in CSF were associated with shorter survival in HIV-positive PML [6]. This difference might account for different proportions of subjects who received highly active antiretroviral therapy (HAART) in these studies (former vs. later study; 38/61, 62% vs. 5/12, 42%). HIV-negative PML is characterized by a very high rate of JCV replication in the CNS [3], and mortality is as high as 84% [7]. The good outcome in our patient may be attributed to

Table 1
Clinical features of previously reported cases of PML developing after renal transplantation in patients who had good outcomes.

	Patient 1 [15]	Patient 2 [2]	Patient 3 [8]	Patient 4 [12]	Patient 5 [5]	Patient 6 [4]
Age/sex	13–14/M	47/M	52/F	28/M	28/M	42/M
Immunosuppressant	MMF, PSL, rapamycin	MMF, PSL, FK-506	MMF, PSL	CPA, PSL	CPA, PSL	MMF, PSL, FK-506
Duration from renal transplantation to the onset of PML	3.5 yr	5 mo	20 yr	10 yr	10 yr	1 mo
Clinical features	Somnolence	Focal seizure, cognitive decline	Aphagia, hemiparesis, apraxia, cognitive decline	Hemiparesis, vague sensation, dysarthria	Disorientation, hemiparesis	Coma, seizure, cognitive decline
Diagnostic method for PML	PCR (serum)	Brain pathology	Brain pathology	Brain pathology	Brain pathology	Neuroimages
JCV DNA load (copies/ml)	4012 (serum)	ND	Not detected (CSF)	ND	ND	ND
Cranial MRI lesions	Thalamus, brainstem, parietal WM	Parietal WM, basal ganglia	Temporal, parietal, and frontal WM	Frontal, parietal, and occipital WM*	ND	Occipital WM
Therapy for PML	Withdrawal of MMF, cidofovir	Withdrawal of MMF, FK-506	Withdrawal of MMF, decreased dosage of PSL	Withdrawal of CPA and PSL, Ara-C	Withdrawal of CPA, Ara-C	Withdrawal of MMF, PSL and FK-506
Outcome						
Clinical features	Disappeared	Disappeared	Decreased	Decreased	Decreased	Disappeared
Cranial MRI lesions	Disappeared	Decreased	NA	ND	ND	Decreased
JCV DNA load (copies/ml)	Not detected	ND	ND	ND	ND	ND
Duration from PML therapy to clinical improvement	6 weeks	4 weeks	3 mo	11 mo	NA	4 mo

PML: progressive multifocal leukoencephalopathy, JCV: JC virus, PCR: polymerase chain reaction, MRI: magnetic resonance imaging, CSF: cerebrospinal fluids, WM: subcortical white matter, MMF: mycophenolate mofetil, PSL: prednisolone, CPA: cyclophosphamide, FK-506: tacrolimus, M: male, F: female, NA: not available, ND: not done, mo: months, yr: years and *: computed tomography.

the early withdrawal of immunosuppressant therapy before further JCV replication in the CNS. Withdrawal of immunosuppressant treatment has a beneficial effect on the course of PML after renal transplantation, and quantitative PCR may facilitate the immediate withdrawal of immunosuppressant agents.

Disclosure

The authors report no conflicts of interest related with our paper.

Financial disclosure

This work was partly supported by Grants-in-Aid for the Research on HIV/AIDS (H24-AIDS-Wakate-002) and the Research Committee of Prion Disease and Slow Virus Infection (H22-Nanchi-Ippan-013) from the Ministry of Health, Labour and Welfare of Japan.

References

[1] Bossolasco S, Calori G, Moretti F, Boschini A, Bertelli D, Mena M , et al. Prognostic significance of JC virus DNA levels in cerebrospinal fluid of patients with HIV-associated progressive multifocal leukoencephalopathy. Clin Infect Dis 2005;40:738–44.
[2] Clinton DC, Kymberly AG, Cinthia BD, Werner J, Morales RE, Hirsch HH, et al. Successful outcome of progressive multifocal leukoencephalopathy in a renal transplant patient. Am J Transplant 2005;5:1151–8.
[3] Delbue S, Elia F, Carloni C, Tavazzi E, Marchioni E, Carluccio S, et al. JC virus load in cerebrospinal fluid and transcriptional control region rearrangements may predict the clinical course of progressive multifocal leukoencephalopathy. J Cell Physiol 2012;227:3511–7.
[4] El Kabbaj D, Hassani M, Kadiri M, Mounach J, Ouhabi H, Haimeur C, et al. Mycophenolate mofetil associated with progressive multifocal leukoencephalopathy with successful outcome. Saudi J Kidney Dis Transpl 2012;23:790–3.
[5] Embrey JR, Silva FG, Helderman JH, Peters PC, Sagalowsky AI. Long-term survival and late development of bladder cancer in renal transplant patient with progressive multifocal leukoencephalopathy. J Urol 1988;139:580–1.
[6] García De Viedma D, Díaz Infantes M, Miralles P, Berenguer J, Marin M, Miralles P, Muñoz L, et al. JC virus load in progressive multifocal leukoencephalopathy: analysis of the correlation between the viral burden in cerebrospinal fluid, patient survival, and the volume of neurological lesions. Clin Infect Dis 2002;34:1568–75.
[7] Mateen FJ, Muralidharan R, Carone M, van de Beek D, Harrison DM, Aksamit AJ, et al. Progressive multifocal leukoencephalopathy in transplant recipients. Ann Neurol 2011;70:305–22.
[8] Manfro RC, Vedolin L, Cantarelli M, Oppitz P, Antunes AC, Rieder CR. Progressive multifocal leukoencephalopathy in a kidney transplant recipient after conversion to mycophenolic acid therapy. Transpl Infect Dis 2009;11:189–90.
[9] Nakamichi K, Kishida S, Tanaka K, Saganuma A, Sano Y, Sano H, et al. Sequential changes in the non-coding control region sequences of JC polyomaviruses from the cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. Arch Virol 2013;158:639–50.
[10] Neff RT, Hurst FP, Falta EM, Bohen EM, Lentine KL, Dharnidharka VR, et al. Progressive multifocal leukoencephalopathy and use of mycophenolate mofetil after kidney transplantation. Transplantation 2008;86:1474–8.
[11] Shitrit D, Lev N, Bar-Gil-Shitrit A, Kramer MR. Progressive multifocal leukoencephalopathy in transplant recipients. Transpl Int 2005;17:658–65.
[12] Saxton CR, Gailiunas Jr P, Helderman JH, Farkas RA, McCoy R, Diehl J, et al. Progressive multifocal leukoencephalopathy in a renal transplant recipient. Am J Med 1984;77:333–7.
[13] Tan CS, Koralnik IJ. Progressive multifocal leukoencephalopathy and other disorders caused by JC virus: clinical features and pathogenesis. Lancet Neurol 2010;9:425–37.
[14] US Food and Drug Administration. Communication about an ongoing safety review of CellCept (mycophenolate mofetil) and Myfortic (mycophenolic acid). Available at: http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/DrugSafetyInformationforHeathcareProfessionals/ucm072438.htm; April 10, 2008 . (Last Accessed on July 31, 2013).
[15] Weber SC, Uhlenberg B, Raile K, Querfeld U, Müller D. Polyoma virus-associated progressive multifocal leukoencephalopathy after renal transplantation: regression following withdrawal of mycophenolate mofetil. Pediatr Tranplant 2011;15:E19–24.

Jpn. J. Infect. Dis., 67, 307-310, 2014

Short Communication

Stability of JC Virus DNA in Cerebrospinal Fluid Specimens Preserved with Guanidine Lysis Buffer for Quantitative PCR Testing

Kazuo Nakamichi*, Chang-Kweng Lim, and Masayuki Saijo

Department of Virology 1, National Institute of Infectious Diseases, Tokyo 162-8640, Japan

(Received September 25, 2013. Accepted January 8, 2014)

SUMMARY: Quantitative PCR testing for JC virus (JCV) DNA in the cerebrospinal fluid (CSF) is one of the diagnostic standards for progressive multifocal leukoencephalopathy (PML). The present study was conducted to examine its reliability using CSF specimens that had been preserved with guanidine lysis buffers in commercial nucleic acid extraction kits under different conditions. When CSFs were mixed with guanidine buffers, JCV DNA levels were not statistically reduced even after storage for 1 month at room temperature or for 3 months at −80°C, compared with the control samples. In addition, the JCV DNA level was not decreased in a mixture of CSF and guanidine thiocyanate buffer incubated for 3 days at 56°C. These data suggest that CSF specimens mixed with commercial guanidine buffers can be stored without refrigeration, more safely handled, and directly subjected to JCV DNA testing for PML.

The JC virus (JCV) is a small DNA virus belonging to the family *Polyomaviridae* and genus *Polyomavirus* (1-3). It is the causative agent of progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease of the central nervous system (1-3). PML is mainly diagnosed in patients with immunodeficiency, including those with HIV infection (1-3). The detection of JCV DNA in the cerebrospinal fluid (CSF) by PCR is a reliable and less-invasive diagnostic marker of PML, particularly when combined with typical magnetic resonance imaging patterns (1-3). The amount of JCV DNA in CSF has been reported to be associated with the severity of PML (4,5). Thus, JCV DNA testing in CSF using a sensitive and quantitative PCR technique has become one of the current diagnostic standards (6).

Real-time PCR testing for viral DNA in CSF is generally performed on DNA extracts prepared by either manual or automated methods using commercially available extraction kits, most of which contain guanidinium salts as one of their components (7). Guanidinium salts such as guanidine hydrochloride (Gdn-HCl) and guanidine thiocyanate (Gdn-SCN) are widely used for cell lysis and protein solubilization (7). These compounds inhibit nuclease activities (8,9) or inactivate infectious pathogens (10-14). If viral DNA in CSF is stable when mixed with a solution containing guanidine such as Gdn-HCl and Gdn-SCN, the specimen could be stored and transported without cooling and directly used for DNA extraction. Furthermore, CSF mixed with a guanidine solution can be considered to be a noninfectious material. The present study was undertaken to examine the stability of JCV DNA in CSF specimens preserved with guanidine lysis buffer.

The study was approved by the Ethical Committee for Biomedical Science in the National Institute of Infectious Diseases, Tokyo, Japan (application no. 340). All the experiments were conducted in accordance with the ethical standards of the Declaration of Helsinki. All the CSF specimens were collected by lumbar puncture from patients suspected of having PML and were transferred from the respective hospitals to the Department of Virology 1, National Institute of Infectious Diseases, for real-time PCR testing for JCV DNA (15). Because all the specimens had been frozen without centrifugation at the hospitals, they were considered to contain leukocytes. The specimens were stored at −80°C until further analyses. Total DNAs were extracted from aliquots of the CSF specimens using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and were subjected to real-time PCR testing for JCV DNA, as described previously (15-17).

To prepare a CSF pool for the experiments, JCV-negative CSF specimens from 71 patients were combined in a single tube (approximately 0.5 mL/sample). The median values of CSF cell counts and protein levels in the samples were 3.0 cells/μL (range, 0-95.0 cells/μL) and 48.0 mg/dL (range, 23.0-226.0 mg/dL), respectively. A JCV-positive CSF sample from an AIDS-related PML patient was diluted by more than 100-fold with JCV-negative CSF. In the JCV-positive patient, although the CSF cell count was normal (1 leukocyte/μL), a relatively high level of the JCV genome (2.7×10^6 copies/mL CSF) was detected (15). Thus, it was thought that cell-free virions were dominant in this CSF pool. The copy number of JCV DNA in this CSF pool was adjusted to 2.0×10^4 copies/mL, which is similar to the median JCV load in CSFs of 69 PML patients in Japan (15). The CSF pools were aliquoted (200 or 140 μL) into 1.5-mL screw-cap plastic microcentrifuge tubes (Ina-Optica Co. Ltd., Osaka, Japan) and frozen at −80°C until use.

The column-based nucleic acid extraction kits and guanidine lysis buffers were all purchased from Qiagen. A 200-μL aliquot of the frozen CSF pool was thawed at 37°C in a block incubator (CTU-Mini; Taitec, Saitama, Japan), left untreated or mixed with an equal volume of

*Corresponding author: Mailing address: Department of Virology 1, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan. Tel: +81-3-5285-1111 ext. 2530, Fax: +81-3-5285-2115, E-mail: nakamich@nih.go.jp

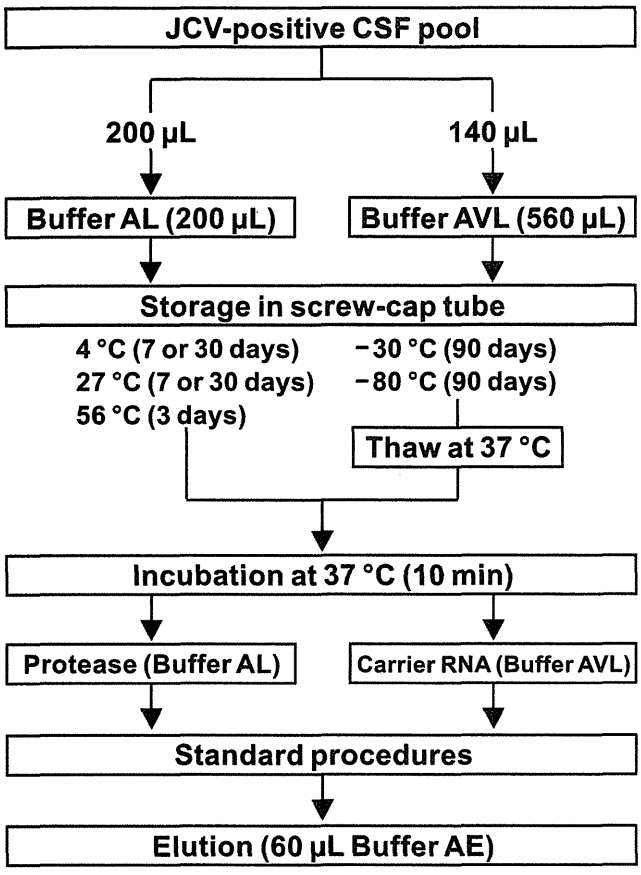

Fig. 1. Schematic representation of the handling procedures for DNA extraction and the storage conditions of the mixtures of CSF and guanidine. Aliquots of the JCV-positive CSF pool were incubated with either Buffer AL or Buffer AVL. After incubation at different temperatures and for different durations, total DNA was extracted using a QIAamp DNA Blood Mini Kit or QIAamp Viral RNA Mini Kit as described in the text.

lysis buffer containing Gdn-HCl (Buffer AL), and stored with protection from exposure to light. Whether DNA recovery is decreased by visible light during storage in Buffer AL or Buffer AVL remains unclear. However, because the laboratory equipment was routinely irradiated with ultraviolet light to prevent DNA contamination before and after examination, we placed samples in lightproof boxes for long storage periods.

The handling procedures of the CSF guanidine solutions and storage conditions are summarized in Fig. 1. The frozen samples were thawed at 37°C and mixed thoroughly after storage. All the samples were incubated at 37°C for 10 min and then vortexed for 1 min. Subsequently, protease solution was added to the mixture, and total DNA was extracted using the QIAamp DNA Blood Mini Kit according to the manufacturer's protocol. Alternatively, 140-μL aliquots of the frozen CSF pool were thawed at 37°C and were left untreated or mixed with 560 μL of lysis buffer containing Gdn-SCN (Buffer AVL) and subsequently incubated under similar conditions. Total DNA was then extracted using the QIAamp Viral RNA Mini Kit according to the manufacturer's protocol, with the exception that carrier RNA was added to the lysate after incubation at 37°C for 10 min and that DNA was eluted in 60 μL of Buffer AE. Although Buffer AL and Buffer AVL were supplied in the QIAamp DNA Blood Mini Kit and QIAamp Viral RNA Mini Kit, respectively, both the kits used the same extraction columns and wash buffers. Thus, after the lysates were passed through the columns, DNAs could be extracted using a common procedure. In the control experiments without additional incubation, the frozen aliquots of the CSF pool were thawed at 37°C just before use, and total DNAs were immediately extracted by standard procedures using the QIAamp DNA Blood Mini Kit and QIAamp Viral RNA Kit. The extraction kits were routinely checked for their quality by adding plasmid DNA solution to the lysis buffer (control experiments). The contamination of control plasmid DNA into test samples can be detected using specific primers and probe (15).

The JCV DNA copy number in each sample was determined in duplicate using real-time PCR targeting the JCV large T gene, as described previously (15–17). The absence of DNA contamination was confirmed by means of negative controls. The absolute copy numbers of JCV DNA in each sample were determined. The experiments were repeated six times, and the amounts of JCV DNA in the extracts under the respective conditions were statistically compared using the nonparametric Steel test for multiple comparisons. All P-values less than 0.05 were considered to be statistically significant.

The absolute copy numbers of JCV DNA in the CSF pool, as determined by the standard procedures for the QIAamp DNA Blood Mini Kit and QIAamp Viral RNA Kit, were 1.92×10^4 and 3.6×10^4 copies/mL, respectively. When CSFs were mixed with Buffer AL containing Gdn-HCl, JCV DNA levels were statistically reduced after incubation at 56°C for 3 days (Fig. 2A). However, there were no statistically significant differences between the control and Buffer AL-incubated samples stored under other conditions. In CSFs mixed with Buffer AVL containing Gdn-SCN, the amounts of JCV DNA were not statistically reduced after incubation under all conditions tested, although the viral DNA level was statistically lower when the specimens were incubated without lysis buffer at 27°C for 1 week and DNA was extracted by the standard procedure (Fig. 2B). No thermal deposition of guanidinium salt was observed in any lysate during incubation at 4°C for 1 month.

The results of the present study demonstrated that if CSF specimens are mixed with commercially available guanidine lysis buffers, they can be used for quantitative PCR testing for JCV DNA even after storage for 1 month at room temperature. In addition, it was also shown that lysates could be frozen to meet longer storage requirements. The target fragments of JCV DNA were demonstrated to be stable in the presence of Gdn-HCl or Gdn-SCN and other buffer components. However, JCV DNA levels were impaired in CSFs incubated with Buffer AL at 56°C for 3 days. This temperature is recommended for the proteinase K digestion step in the QIAamp DNA Blood Mini Kit protocol (56°C for 10 min) and is used to examine the stability of JCV DNA at a higher temperature. The present data suggest that JCV DNA in Buffer AL tends to be degraded at relatively high temperatures. It is important to examine the stability of purified viral DNA under more precise conditions in future studies. It was also found that JCV

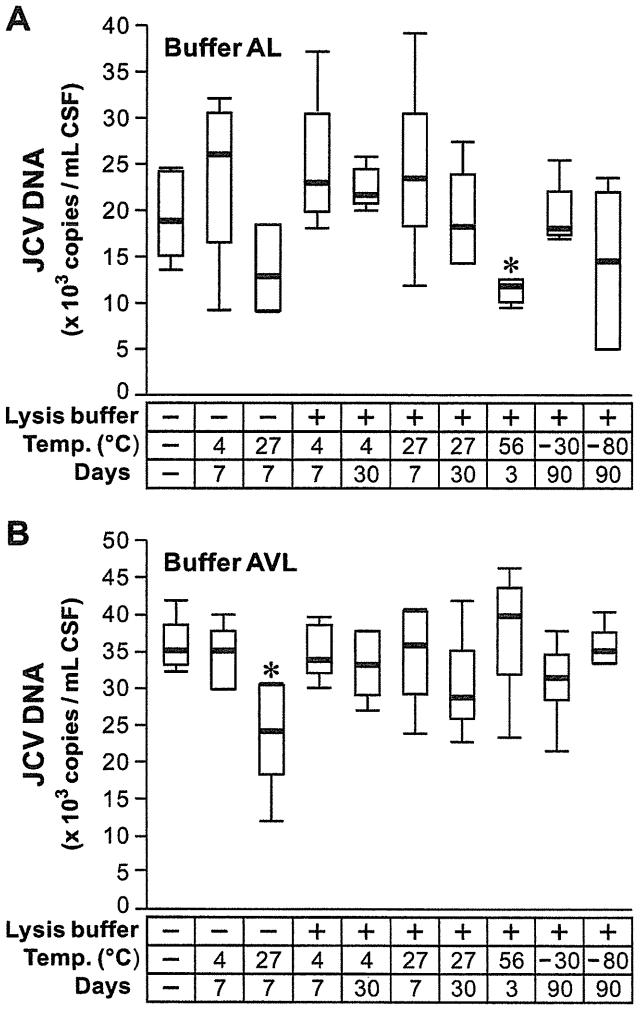

Fig. 2. Availability of CSF specimens preserved with guanidine-containing lysis buffers for the quantitative PCR testing for JCV DNA detection. Aliquots of the JCV-positive CSF pool were incubated with (+) or without (−) either Buffer AL (A) or Buffer AVL (B) under the different conditions, and total DNA was extracted as described in the legend to Fig. 1. When CSFs were incubated without lysis buffer, the samples were mixed with lysis buffer after time periods and were subjected to DNA extraction. The absolute copy numbers of the JCV genome in each DNA extract were determined using a real-time PCR method. The experiments were repeated independently 6 times, and the results are shown in box-and-whisker plots. The thick horizontal line within each box is the median; the lower and upper boundaries are the 25th and 75th percentiles, respectively; vertical whiskers extend over the range; and no outliers were detected. Statistically significant differences ($P < 0.05$) are indicated by asterisks (comparison to the first box from the left).

DNA in CSF can be extracted using the QIAamp Viral RNA Kit designed for RNA extraction from liquid samples. The data obtained in the present study suggest that the QIAamp Viral RNA Kit containing Buffer AVL can be used to extract JCV DNA from liquid materials according to the manufacturer's instructions.

Compared with the results presented in Fig. 2A and 2B, it seems likely that the median copy numbers of JCV DNA in Buffer AVL have less variation under different conditions than those in Buffer AL. These results may be related to the higher initial amount of the obtained JCV DNA in Buffer AVL (3.6×10^4 copies/mL) than that in Buffer AL (1.92×10^4 copies/mL), probably because of the addition of carrier RNA in the procedure. To examine the copy number of JCV DNA for PML diagnosis, it is important to obtain the amount of JCV DNA with less variation from samples containing the same amounts of JCV DNA under various conditions. Based on the above-mentioned findings, it seems likely that Buffer AVL is more applicable to the JCV DNA sample in CSF.

In addition, the current protocol using Buffer AVL is advantageous not only for sample storage as a part of routine JCV testing but also for the transportation of CSF specimens from outlying clinics to laboratories, without the need for a refrigerant. Considering the ability of Gdn-SCN to inactivate infectious agents, it is thought that the use of guanidine lysis buffer can be advantageous in decreasing the infectivity of pathogens. The results of the present study are thought to demonstrate that the use of a guanidine lysis buffer is a safe and convenient method for storing and handling CSF specimens. Furthermore, the preservation and storage of cell-free JCV suspension with guanidine solution may be useful for the in vitro high-throughput quantification of JCV DNA in a large number of samples.

Acknowledgments The authors are grateful to Dr. Toshio Shimokawa (University of Yamanashi) for his support with the statistical analyses. The authors are grateful to all study participants and physicians who contributed to the present study.

This work was partly supported by Grants-in-Aid for the Research on HIV/AIDS (H24-AIDS-Wakate-002), the Research for Intractable Infectious Diseases in Organ Transplant Recipients (H21-Shinko-Ippan-009), and the Research Committee of Prion Disease and Slow Virus Infection (H22-Nanchi-Ippan-013) from the Ministry of Health, Labour and Welfare of Japan.

Conflict of interest None to declare.

REFERENCES

1. Brew BJ, Davies NWS, Cinque P, et al. Progressive multifocal leukoencephalopathy and other forms of JC virus disease. Nat Rev Neurol. 2010;6:667-79.
2. Tan CS, Koralnik IJ. Progressive multifocal leukoencephalopathy and other disorders caused by JC virus: clinical features and pathogenesis. Lancet Neurol. 2010;9:425-37.
3. Shishido-Hara Y. Progressive multifocal leukoencephalopathy and promyelocytic leukemia nuclear bodies: a review of clinical, neuropathological, and virological aspects of JC virus-induced demyelinating disease. Acta Neuropathol. 2010;120:403-17.
4. Bossolasco S, Calori G, Moretti F, et al. Prognostic significance of JC virus DNA levels in cerebrospinal fluid of patients with HIV-associated progressive multifocal leukoencephalopathy. Clin Infect Dis. 2005;40:738-44.
5. García De Viedma D, Díaz Infantes M, Miralles P, et al. JC virus load in progressive multifocal leukoencephalopathy: analysis of the correlation between the viral burden in cerebrospinal fluid, patient survival, and the volume of neurological lesions. Clin Infect Dis. 2002;34:1568-75.
6. Major EO. Progressive multifocal leukoencephalopathy in patients on immunomodulatory therapies. Annu Rev Med. 2010;61:35-47.
7. Espy MJ, Uhl JR, Sloan LM, et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin Microbiol Rev. 2006;19:165-256.
8. Pommer AJ, Kuhlmann UC, Cooper A, et al. Homing in on the role of transition metals in the HNH motif of colicin endonucleases. J Biol Chem. 1999;274:27153-60.
9. Eggert LS, Maldonado JE, Fleischer RC. Nucleic acid isolation from ecological samples-animal scat and other associated materials. Methods Enzymol. 2005;395:73-87.

10. Schlegel A, Immelmann A, Kempf C. Virus inactivation of plasma-derived proteins by pasteurization in the presence of guanidine hydrochloride. Transfusion. 2001;41:382-9.
11. Lifson JD, Rossio JL, Piatak M Jr, et al. Evaluation of the safety, immunogenicity, and protective efficacy of whole inactivated simian immunodeficiency virus (SIV) vaccines with conformationally and functionally intact envelope glycoproteins. AIDS Res Hum Retroviruses. 2004;20:772-87.
12. Roberts PL, Lloyd D. Virus inactivation by protein denaturants used in affinity chromatography. Biologicals. 2007;35:343-7.
13. Tateishi J, Tashima T, Kitamoto T. Practical methods for chemical inactivation of Creutzfeldt-Jakob disease pathogen. Microbiol Immunol. 1991;35:163-6.
14. Rutala WA, Weber DJ. Creutzfeldt-Jakob disease: recommendations for disinfection and sterilization. Clin Infect Dis. 2001;32:1348-56.
15. Nakamichi K, Mizusawa H, Yamada M, et al. Characteristics of progressive multifocal leukoencephalopathy clarified through internet-assisted laboratory surveillance in Japan. BMC Neurol. 2012;12:121.
16. Nakamichi K, Kurane I, Saijo M. Evaluation of a quantitative real-time PCR assay for the detection of JC polyomavirus DNA in cerebrospinal fluid without nucleic acid extraction. Jpn J Infect Dis. 2011;64:211-6.
17. Nakamichi K, Kishida S, Tanaka K, et al. Sequential changes in the non-coding control region sequences of JC polyomaviruses from the cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. Arch Virol. 2013;158:639-50.

J Neurol
DOI 10.1007/s00415-014-7500-y

ORIGINAL COMMUNICATION

Usefulness of ^{11}C-methionine-positron emission tomography for the diagnosis of progressive multifocal leukoencephalopathy

Shinichi Shirai · Ichiro Yabe · Takahiro Kano · Yuka Shimizu · Toru Sasamori · Kazunori Sato · Makoto Hirotani · Takayuki Nonaka · Ikuko Takahashi · Masaaki Matsushima · Naoya Minami · Kazuo Nakamichi · Masayuki Saijo · Kanako C. Hatanaka · Tohru Shiga · Shinya Tanaka · Hidenao Sasaki

Received: 25 April 2014 / Revised: 8 September 2014 / Accepted: 9 September 2014

Abstract Progressive multifocal leukoencephalopathy (PML) is a subacute demyelinating disease of the brain caused by the JC virus that occurs mainly in immunocompromised patients. The prognosis is very poor. As the lesion looks like non- specific leukoencephalopathy, making a diagnosis at the early stage is very difficult. We report three PML cases in which there was a mismatch between ^{11}C-methionine-positron emission tomography (MET-PET) uptake and ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET) uptake. All three cases demonstrated the hyper-uptake of MET around the white matter lesions and hypo-uptake of FDG inside the lesions. We speculate that the infection had ended inside the white matter lesions of these patients, while JC virus infection was ongoing around the lesions, resulting in the increase of methionine metabolism, and the glucose metabolism was reduced or intermediate because inflammatory cells infiltrate PML lesions rarely. Two patients who were diagnosed and treated with mefloquine while the JC virus was at a low level in the cerebrospinal fluid are still alive. We suggest the usefulness of MET-PET for the early diagnosis of PML and early treatment with mefloquine.

Keywords Progressive multifocal leukoencephalopathy · ^{11}C-methionine-positron emission tomography · ^{18}F-fluorodeoxyglucose-positron emission tomography · Mefloquine

S. Shirai · I. Yabe (✉) · T. Kano · Y. Shimizu · T. Sasamori · K. Sato · M. Hirotani · I. Takahashi · M. Matsushima · H. Sasaki
Department of Neurology, Hokkaido University Graduate School of Medicine, N15 W7, Kita-ku, Sapporo 060-8638, Japan
e-mail: yabe@med.hokudai.ac.jp

T. Nonaka · N. Minami
Department of Neurology, Hokkaido Medical Center, Sapporo, Japan

K. Nakamichi · M. Saijo
Department of Virology 1, National Institute of Infectious Diseases, Tokyo, Japan

K. C. Hatanaka
Department of Surgical Pathology, Hokkaido University Hospital, Sapporo, Japan

T. Shiga
Department of Nuclear Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan

S. Tanaka
Department of Cancer Pathology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Introduction

Progressive multifocal leukoencephalopathy (PML) is a subacute demyelinating disease of the brain caused by the JC virus (JCV) and occurs mainly in immunocompromised patients. The prognosis is very poor. As the lesion resembles non-specific leukoencephalopathy, it is difficult to make a diagnosis at the early stage of the disease. We report three PML cases in which there was a mismatch between ^{11}C-methionine-positron emission tomography (MET-PET) uptake and ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET) uptake in and around the lesions. We suggest this mismatch may be useful for the early diagnosis of PML.

Published online: 16 September 2014

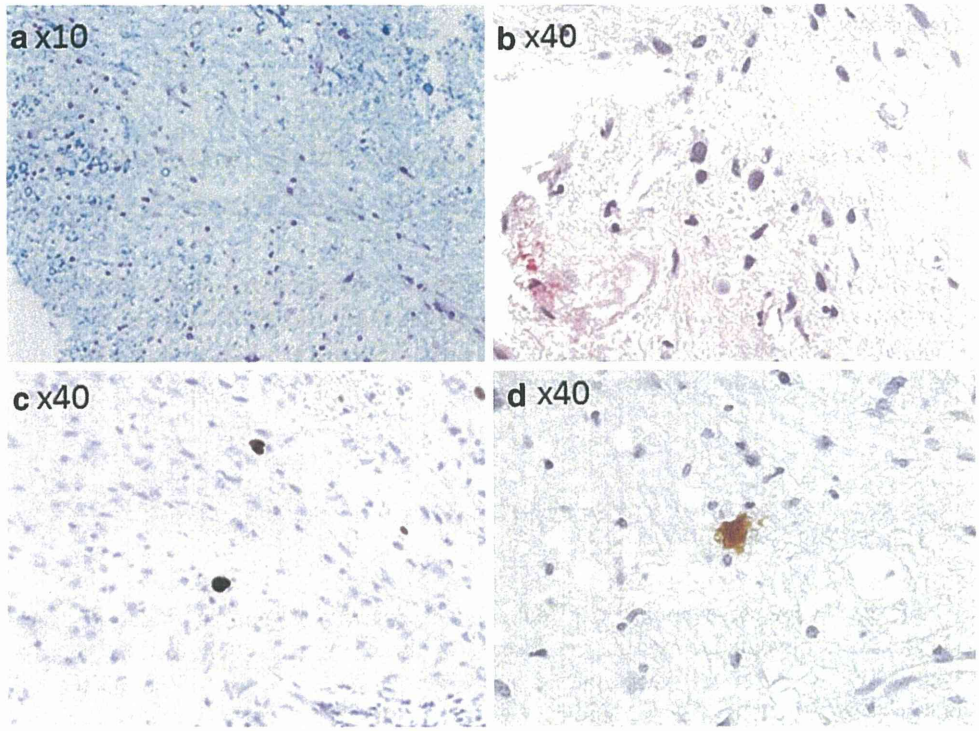

Fig. 1 The histopathological findings of case 1. **a** Klüver-Barrera staining (×10) showing demyelination. **b** Hematoxylin-eosin staining (×40) showing swollen oligodendrocytes. **c** Anti-agnoprotein staining (×40); and **d** anti Vp1 antibody staining (×40); the oligodendrocytes were positive, which is consistent with PML

Subjects and methods

Case series

We report three PML cases without human immunodeficiency virus (HIV) infection from July 2012 to December 2013.

Case 1

A 51-year-old man was admitted to our hospital in July 2012 because of gait impairment. He reported dizziness and gait impairment from April 2012, when he was followed for chronic hepatitis B infection and hepatic cell carcinoma. Ataxia of the four extremities was detected and a cerebellar lesion was observed on magnetic resonance imaging (MRI). His positive neurological findings were slurred speech, left spastic hemiparesis (MMT 4), and ataxia of the four extremities. He could not stand by himself. He had a slight hepatic function disorder and was positive for the hepatitis B virus surface (HBs) and hepatitis B virus e (HBe) antigens. Hepatitis C virus (HCV) and HIV antibodies were negative. His cerebrospinal fluid (CSF) showed a cell count of 2 cells/μL, protein level of 27 mg/dL, and glucose level of 60 mg/dL. The polymerase chain reaction (PCR) for JCV was negative. A brain biopsy from the left middle cerebellar peduncle resulted in a diagnosis of glioma. The pathological findings revealed swollen oligodendrocytes on hematoxylin-eosin staining, demyelination on Klüver-Barrera staining, and staining using anti-agnoprotein and anti-VP1 antibodies was positive, which was consistent with PML (Fig. 1a–d). Mefloquine therapy was initiated. His symptoms improved and he was able to ride in a wheelchair in December 2013.

Case 2

An 80-year-old male was admitted to our hospital because of right hemiparesis, aphasia, and dysarthria. He underwent chemotherapy including rituximab for primary thyroid MALT lymphoma in June 2011. He had slight weakness of his right upper and lower extremities in October 2012. His symptoms worsened and a brain MRI showed white matter lesions of the right frontal and parietal lobes. He was admitted to our hospital in February 2013 because the white matter lesion enlarged and his symptoms worsened further.

His positive neurological findings on admission were motor aphasia, right unilateral spatial neglect, dysphagia, and right hemiparesis. He had a slight elevation of serum soluble interleukin 2 (IL-2) receptor (654 U/mL). He was negative for the HBs antigen and HCV and HIV antibodies. His CSF showed a cell count of <1 cell/μL, protein level of 31 mg/dL, and glucose level of 85 mg/dL. He was diagnosed as probable PML because PCR for JCV in the CSF was positive (5.08×10^5 copies/mL). Although mefloquine therapy was initiated, his symptoms worsened and

the amount of JCV in the CSF increased to 5.91×10^6 copies/mL. He died on the 97th hospital day.

Case 3

A 66-year-old female was admitted to our hospital because of left hemiparesis in June 2013. She had been treated with prednisolone and azathioprine since 1991 for overlap syndrome of systemic lupus erythematosus, dermatomyositis, and systemic sclerosis. The weakness of the left extremity occurred in April 2013. No abnormalities were found on brain MRI. Her symptoms worsened and she had difficulty in walking in May 2013. Brain MRI revealed a white matter lesion in the right frontal lobe. Her symptoms and MRI findings worsened further in June 2013, so she was admitted to our hospital. Her positive neurological findings were left hemiparesis and mild left unilateral spatial neglect. She had a slight elevation of serum soluble IL-2 receptor (520 U/mL). She was negative for HBs antigen and HCV and HIV antibodies. Her CSF showed a cell count of 1 cell/μL, protein level of 35 mg/dL, and glucose level of 50 mg/dL. She was diagnosed as probable PML because PCR for JCV in the CSF was positive (6.23×10^3 copies/mL). Mefloquine and mirtazapine therapy was initiated. Her hemiparesis improved and JCV became undetectable in the CSF.

Methods

In addition to brain MRI and FDG-PET, we assessed the MET-PET findings of each case, which have been used to evaluate low-grade glioma and cerebral inflammatory lesions [1, 2]. The MET-PET images were acquired with an EXACT HR + scanner (Asahi-Siemens Medical Technologies LTD., Tokyo, Japan).

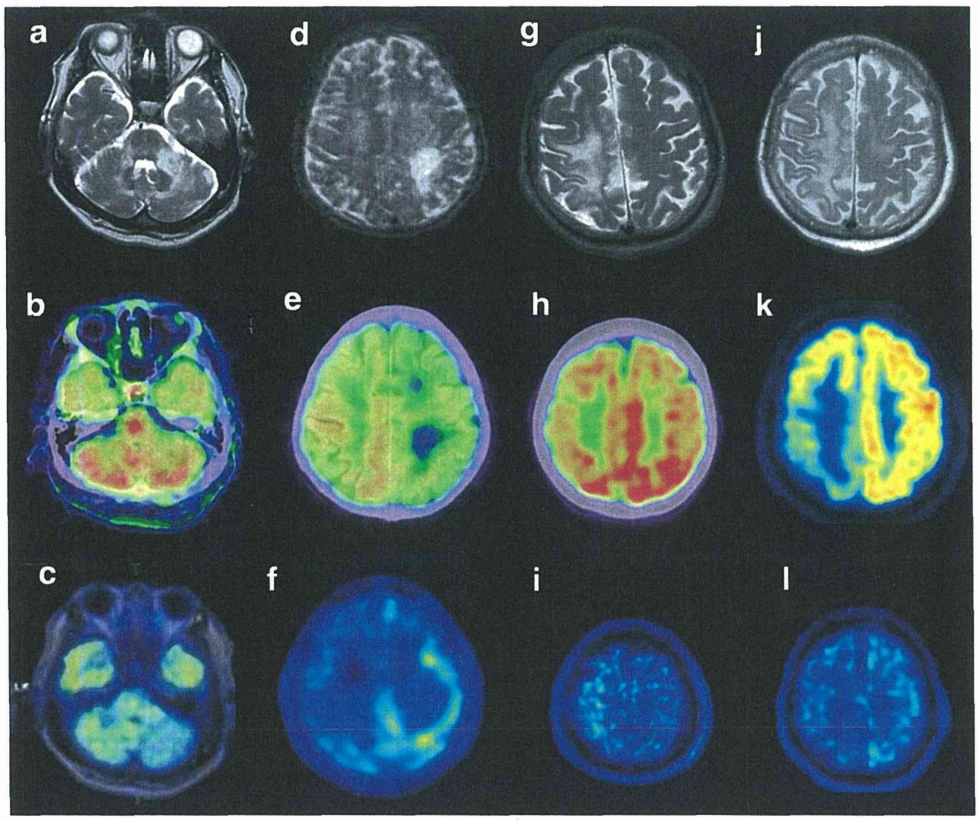

Fig. 2 Neuroradiological findings Neuroradiological findings of case 1 (**a–c**). **a** Brain MRI image on admission (T2-weighted image, TR = 4,438 ms, TE = 100 ms) showing leukoencephalopathy of the left middle cerebellar peduncle. **b** FDG-PET on admission. FDG uptake is decreased inside the lesion. **c** MET-PET on admission. Methionine uptake is increased around the lesion. Neuroradiological findings of case 2 (**d–f**). **d** Brain MRI image on admission (T2-weighted image, TR = 4,540 ms, TE = 96 ms) showing leukoencephalopathy of the left parietal lobe. **e** FDG-PET on admission. FDG uptake is decreased inside the lesion. **f** MET-PET on admission. Methionine uptake is increased around the lesion. Neuroradiological findings of case 3 (**g–l**). **g** Brain MRI image on admission (T2-weighted image, TR = 4,540 ms, TE = 96 ms) showing leukoencephalopathy of the right frontal lobe. **h** FDG-PET on admission. FDG uptake is decreased inside the lesion. **i** MET-PET on admission. Methionine uptake is increased around the lesion. **j** Brain MRI image on admission (T2-weighted image, TR = 4,160 ms, TE = 85 ms) still showing leukoencephalopathy of the right frontal lobe. **k** FDG-PET after the initiation of treatment. FDG uptake is decreased inside the lesion, similar to that observed on admission. **l** MET-PET after the initiation of treatment. The hyper-uptake of methionine is reduced

Table 1 Summary of our 3 cases

	Case 1 51-year-old male	Case 2 80-year-old male	Case 3 66-year-old female
Diagnosis	Definite PML	Probable PML	Probable PML
Background	HBV	MALT lymphoma	SLE + DM + SSc
Immunosuppressant	None	Chemotherapy including rituximab	PSL + AZT
CSF JC virus			
On diagnosis	Under the detection limit	5.08×10^5 copies/mL	6.23×10^3 copies/mL
After treatment	Unexamined	5.91×10^6 copies/mL	Under the detection limit
Treatment	Mefloquine mPSL	Mefloquine	Mefloquine Mirtazapine
Modified Rankin-Scale			
Before treatment	5	5	5
After treatment[a]	4	6	4

mPSL methylprednisolone, *AZT* azathioprine, *SLE* systemic lupus erythematosus, *DM* dermatomyositis, *SSc* systemic sclerosis

[a] We were able to initiate mefloquine therapy in the survivors when the amount of JC virus in the CSF was low

This study was approved by the Institutional Review Board of Hokkaido University.

Results

Case 1

Brain MRI showed a left middle cerebellar peduncle lesion (Fig. 2a). FDG-PET showed low uptake inside the lesion (Fig. 2b) and MET-PET showed hyper-uptake around the lesion (Fig. 2c).

Case 2

Brain MRI showed leukoencephalopathy in the white matter of the left parietal lobe (Fig. 2d). FDG-PET showed low uptake inside the lesion (Fig. 2e) and MET-PET showed hyper-uptake around the lesion (Fig. 2f).

Case 3

Brain MRI showed leukoencephalopathy in the white matter of the right frontal and parietal lobes (Fig. 2g). FDG-PET showed low uptake inside the lesion (Fig. 2h) and MET-PET showed hyper-uptake around the lesion (Fig. 2i). After treatment was initiated, the white matter lesion enlarged on brain MRI (Fig. 2j) and FDG-PET (Fig. 2k). However, the hyper-uptake of methionine decreased (Fig. 2l).

Discussion

Progressive multifocal leukoencephalopathy is an infectious multifocal demyelinating disease of the brain caused by JCV infection of oligodendrocytes. No definitive treatment has been established, so the prognosis is very poor. The underlying disorders are various, such as HIV infections, hematopoietic neoplasms, autoimmune diseases, and monoclonal antibodies [3]. Nowadays, the number of monoclonal antibodies related to PML, such as in case 2, is increasing. Especially, neurologists are aware of the risk of PML when immunosuppressive therapy including natalizumab is used to treat multiple sclerosis [4]. Cases in which mefloquine therapy was effective have been reported [5, 6].

A summary of our three cases is presented in Table 1. In the surviving cases, we were able to initiate mefloquine therapy when the amount of JCV in the CSF was low.

All three cases indicated the hyper-uptake of MET around the white matter lesions and hypo-uptake of FDG inside the lesions. Only one case report from Japan has described the hyper-uptake of MET in a patient with PML [7]. We suggest that the infection had ended inside the white matter lesions of our cases, while JCV infection was ongoing around the lesions, resulting in the increase of methionine metabolism, and glucose metabolism was reduced or intermediate because inflammatory cells infiltrate PML lesions rarely. In case 3, only the MET-PET abnormality improved after treatment. We hypothesize that the degeneration might have ended in the areas where methionine uptake was increased, so the T2 intensity increased. Moreover, methionine uptake was prevented and PCR for JCV in the CSF was negative. We could not follow up the PET findings of the treated patient (case 1) and the untreated patient (case 2), which is a limitation of this study. We speculate that the reduction of the amount of JCV in case 3 resulted in the improvement of the MET-PET findings.

We reported 3 cases of PML without HIV infection. The neurological impairments of such patients may improve