other brain regions.[4–6] In previous studies of the dCJD case series, however, results were obtained without considering the dCJD pathological subtypes.[5 6]

There are two subtypes of dCJD: non-plaque type and plaque type. The non-plaque type is characterised by typical clinical features of CJD and synaptic-type PrP^{Sc} deposits without PrP^{Sc} plaques in the brain. In contrast, the plaque type is characterised by atypical clinical features and plaque-type PrP^{Sc} deposits in the brain.[7–9] These subtypes arise from two distinct prion strains.[7–9] Each prion strain has different characteristics of incubation period and neuropathological features when inoculated into defined inbred mice[8 10]; it was proposed that each prion strain must show a distinct propagation process in the human brain.[10] Therefore, the disease pathological subtypes (prion strains) should be taken into consideration when clinical features are analysed in dCJD cases. This study made use of the prospective prion disease surveillance in Japan to analyse clinical manifestations of dCJD cases taking into account not only their grafting sites but also their pathological subtypes.

MATERIALS AND METHODS

Patients

In Japan, the prospective surveillance of human prion disease by the CJD Surveillance Committee in Japan started in April 1999. Details of the Japanese surveillance system and case definition were reported previously.[11] Briefly, all patients suspected of having a prion disease were registered by the CJD Surveillance Committee and their diagnoses were judged. Prion diseases were classified into four categories: (1) sporadic CJD, (2) acquired prion diseases (iatrogenic CJD or variant CJD), (3) genetic prion diseases and (4) unclassified prion disease. Sporadic CJD was diagnosed according to the classical criteria established by Masters *et al*.[12] The WHO criteria (WHO, 1998)[13] were applied from April 2009. Regarding the patients with previous medical procedures which might be related to CJD, details of the information were collected and the diagnosis of iatrogenic CJD was decided carefully. Iatrogenic CJD was also diagnosed and categorised (definite, probable and possible) using the criteria for sporadic CJD. In patients with iatrogenic CJD, the diagnosis of dCJD was decided by confirmation of dura mater grafting. The medical records, information from each neurosurgeon or the autopsy findings were used to ensure the occurrence of dura mater grafting. Cases of dCJD were categorised into two pathological subtypes: the non-plaque type, which shows synaptic-type PrP^{Sc} deposits without PrP^{Sc} plaques; and the plaque type, which shows plaque-type PrP^{Sc} deposits.[7] In cases without pathological confirmation, we classified cases showing periodic sharp-wave complexes in the EEG within 12 months of disease onset as non-plaque type and cases showing no periodic sharp-wave complexes in the EEG within 12 months of disease onset as plaque type.[7] As the plaque-type dCJD patients without pathological confirmation never satisfied the criteria for probable case,[7] possible cases were included in this analysis in addition to definite and probable cases. We analysed all dCJD patients identified by the current surveillance system up to and including February 2012.

Written informed consent to participate in this study was obtained from the families of all the patients.

Clinical studies

We collected the following information regarding dura mater grafting: calendar year when the surgical operation was performed, the brand name of the dura mater graft and the site of the dural grafting. In addition to the grafting sites (supratentorial or infratentorial), we also analysed the following parameters: sex; age at dural grafting; incubation period; age at CJD onset; information about initial manifestation and symptoms which appeared during their clinical course, including cerebellar signs, psychiatric features, dementia, visual disturbances, myoclonus, extrapyramidal signs and pyramidal signs. Since several patients developed more than one initial manifestation, we counted each manifestation separately. Information about PrP gene polymorphisms at codons 129 and 219 was also collected from patients who underwent genetic analysis. Moreover, we analysed the initial symptoms and clinical manifestations that emerged during their clinical course in each pathological subtype, and compared the supratentorial grafting cases with the infratentorial grafting cases.

Statistical analysis

Any difference in age at dural grafting, incubation period and age at CJD onset between the supratentorial group and the infratentorial group was assessed using the Mann-Whitney U test. Pathological subtype classification, sex, initial symptoms, clinical manifestations and PrP gene polymorphisms were assessed using a χ^2 test or Fisher's exact probability test. Statistical significance was defined as $p<0.05$. Statistical analyses were performed using SPSS V.19 (IBM, Armonk, New York, USA).

RESULTS

The CJD Surveillance Committee in Japan identified 84 patients with dCJD between April 1999 and February 2012. Fifty-eight patients with dCJD had already been reported by previous investigations[9 11]; therefore, the total number of dCJD cases was 142.[2] The surgeries with dura mater grafts were performed between 1975 and 1993. The brand name of grafted dura mater was identified in 74 cases (88%). Lyodura (B Braun, Melsungen, Germany) was transplanted in all proven cases. The grafting site was confirmed in 77 of 84 cases (92%), 36 in supratentorial regions, 39 in infratentorial regions and 2 in spinal cord regions (table 1). An autopsy was performed in 32 cases (38%).

Table 1 Clinical features of the dura mater graft-associated Creutzfeldt-Jakob disease cases for all cases, the supratentorial group and the infratentorial group

	Total (n=84)*	Supratentorial group (n=36)	Infratentorial group (n=39)	p Value†
Type classification‡				
Non-plaque type (%)	53 (63)	20 (56)	28 (72)	ns
Plaque type (%)	18 (21)	9 (25)	8 (21)	ns
Male/female	35/49	19/17	10/29	0.015
Age at dural grafting§ (years)	45 (1–65)	45 (1–60)	51 (7–65)	ns
Incubation period§ (years)	15 (6–30)	15 (8–30)	14 (6–25)	ns
Age at onset§ (years)	61 (15–80)	61 (15–79)	66 (24–80)	ns
Initial manifestations¶ (%)	(n=63)	(n=30)	(n=26)	
Unsteady gait	30 (48)	16 (53)	11 (42)	ns
Dementia	16 (25)	8 (27)	6 (23)	ns
Vertigo	9 (14)	1 (3)	8 (31)	0.007
Behavioural abnormality	7 (11)	5 (17)	2 (8)	ns
Ataxia	7 (11)	4 (13)	1 (4)	ns
Diplopia	4 (6)	0 (0)	4 (15)	0.041
Sensory disturbance	4 (6)	2 (7)	2 (8)	ns
Visual disturbance	3 (5)	0 (0)	1 (4)	ns
Extrapyramidal signs	2 (3)	1 (3)	1 (4)	ns
Others**	5 (8)	5 (17)	0 (0)	—
Manifestations during clinical course (%)				
Cerebellar signs	62/82 (76)	23/36 (64)	32/37 (87)	0.024
Psychiatric feature	51/79 (65)	20/32 (63)	27/38 (71)	ns
Dementia	82/84 (98)	35/36 (97)	38/39 (97)	ns
Visual disturbance	36/81 (44)	16/35 (46)	18/37 (49)	ns
Myoclonus	71/82 (87)	31/36 (86)	34/37 (92)	ns
Extrapyramidal signs	53/82 (65)	25/36 (69)	26/37 (70)	ns
Pyramidal signs	58/81 (72)	28/35 (80)	25/37 (68)	ns
PrP gene polymorphisms	(n=58)	(n=25)	(n=27)	
Codon 129	MM 56, MV 2	MM 25	MM 25, MV 2	ns
Codon 219	EE 52, EK 3	EE 21, EK 3	EE 26	ns

*Total includes two cases with spinal cord regions and seven cases with uncertain grafting regions.
†p Value was assessed between the supratentorial group and the infratentorial group.
‡Thirteen cases of type classification were unclear.
§Median.
¶Twenty-two cases of the total, 9 cases of the supratentorial group and 8 cases of the infratentorial group developed more than one initial manifestation.
**Others include individual cases of hemiparesis, dysarthria, incontinence, hearing disturbance and nystagmus.
EE, glutamine homozygote; EK, glutamine/lysine heterozygote; MM, methionine homozygote; MV, methionine/valine heterozygote; ns, not significant; PrP, prion protein.

The 84 dCJD cases were classified into 53 cases (63%) of the non-plaque type and 18 cases (21%) of the plaque type. It was not possible to classify the pathological subtype in 13 cases (16%) due to an inadequacy of clinical or pathological information (table 1). There were 18 of 53 non-plaque-type cases (34%) proven by autopsy and 14 of 18 plaque type (78%) cases proven by autopsy.

The clinical features of dCJD for all patients, the supratentorial group and the infratentorial group are summarised in table 1. The proportion of women in the infratentorial group was larger than that in the supratentorial group (p=0.015). Age at dural grafting, incubation period or age at CJD onset showed no significant difference between the two groups. Regarding initial manifestations, vertigo (31% and 3%; p=0.007) and diplopia (15% and 0%; p=0.041) were more frequently observed in the infratentorial group than in the supratentorial group. Dementia and behavioural abnormality suggesting dysfunction of the cerebrum demonstrated no significant difference between the groups. In the infratentorial group, eight cases (31%) developed dementia or behavioural abnormalities. The incubation periods of cases developing dementia or behavioural abnormalities in the supratentorial group and the infratentorial group, reported as the median (range), were 15 (11–30) years and 16 (10–25) years, respectively (p=0.847). During the clinical course, the infratentorial group showed cerebellar signs (87% and 64%; p=0.024) more frequently than did the supratentorial group. There was no significant difference in the proportion of the PrP genotype or the type classification of dCJD between the two groups. In addition, two cases with spinal cord region grafting developed dementia, diplopia or unsteady gait.

Table 2 Clinical manifestations of non-plaque-type cases

	Total (n=53)*	Supratentorial group (n=20)	Infratentorial group (n=28)	p Value†
Pathologically confirmed cases (%)	18 (34)	9 (45)	7 (25)	ns
Initial manifestations‡ (%)	(n=39)	(n=16)	(n=19)	
Unsteady gait	12 (31)	4 (25)	7 (37)	ns
Dementia	11 (28)	6 (38)	5 (26)	ns
Vertigo	6 (15)	0 (0)	6 (32)	0.017
Behavioural abnormality	6 (15)	4 (25)	2 (11)	ns
Ataxia	6 (15)	3 (19)	1 (5)	ns
Diplopia	5 (13)	0 (0)	4 (21)	0.074
Sensory disturbance	2 (5)	1 (6)	1 (5)	ns
Extrapyramidal signs	1 (3)	1 (6)	0 (0)	ns
Visual disturbance	1 (3)	0 (0)	1 (5)	ns
Others§	3 (8)	3 (19)	0 (0)	—
Manifestations during clinical course (%)				
Cerebellar signs	35/51 (69)	10/20 (50)	22/26 (85)	0.014
Psychiatric feature	32/50 (64)	11/17 (65)	19/28 (68)	ns
Dementia	51/53 (96)	19/20 (95)	27/28 (96)	ns
Visual disturbance	21/51 (41)	8/19 (42)	12/27 (44)	ns
Myoclonus	50/52 (96)	20/20 (100)	25/26 (96)	ns
Extrapyramidal signs	30/51 (59)	12/20 (60)	17/26 (65)	ns
Pyramidal signs	40/51 (78)	18/20 (90)	19/26 (73)	ns

*Total includes two cases with spinal cord regions and three cases with uncertain grafting regions.
†p Value was assessed between the supratentorial group and the infratentorial group; ns, not significant.
‡Thirteen cases of the total, 5 cases of the supratentorial group and 8 cases of the infratentorial group developed more than one initial manifestation.
§Others include individual cases of hemiparesis, dysarthria and nystagmus.

Results from the analysis of the non-plaque-type cases were similar to those of the sample population as a whole (table 2). Vertigo was more frequently observed as an initial manifestation in the infratentorial group than in the supratentorial group (32% and 0%; p=0.017). There was a trend in the increase of diplopia frequency in the infratentorial group (21% and 0%; p=0.074). Dementia and behavioural abnormalities demonstrated no significant difference between the two groups. In the infratentorial group, seven cases (37%) demonstrated dementia or behavioural abnormalities as initial manifestations. Similar to the analysis of the sample population as a whole, the median incubation period of cases developing dementia or behavioural abnormalities showed no significant difference between the supratentorial and infratentorial groups (data not shown). Cerebellar signs were realised significantly more often in the infratentorial group during their clinical course than in the supratentorial group (87% and 50%; p=0.041). In contrast, there was no significant difference between the supratentorial group and the infratentorial group concerning initial manifestations or manifestations during their clinical course in the analysis of the plaque-type cases (table 3).

DISCUSSION

In this study of dCJD cases, we have reported that infratentorial grafting cases in not only the sample population as a whole but also the non-plaque-type cases developed manifestations related to dysfunction of the brain stem and cerebellum more frequently than did the supratentorial grafting cases. Moreover, cerebellar signs appeared more frequently in the infratentorial group during their clinical course. In contrast, plaque-type cases showed no significant difference between the supratentorial and the infratentorial groups.

These results suggest that the non-plaque-type PrP^{Sc} strain would propagate directly from the grafted dura mater to the adjacent brain. In experimental studies, PrP^{Sc} has the ability to spread from cell to cell.[3] Mice experiments with PrP^{Sc} inoculated directly into the brain showed that PrP^{Sc} accumulated at the site of initial inoculation and spread around that area.[14–16] A mouse model of dCJD, in which a small collagen sheet absorbing prion-infected brain homogenates was transplanted onto the brain surface, also disclosed spongiform changes and accumulation of PrP^{Sc} in the transplanted cortical areas.[17] Concerning the plaque-type prion strain, there was no significant difference between the infratentorial grafting and supratentorial grafting groups. The plaque-type PrP^{Sc} strain may have a propagation process that is distinct from the non-plaque-type prion strain. A case series study of dCJD demonstrated that plaque-type patients were likely to develop gait disturbance.[7] In this study, three of four plaque-type patients developed unsteady gait after supratentorial grafting (table 3). These results suggest that the plaque-type PrP^{Sc} strain, which must have a distinct nature from the non-plaque-type PrP^{Sc} strain, might damage specific brain regions causing gait disturbance during the early stage of the disease process in spite of the difference in

Table 3 Clinical manifestations of plaque-type cases

	Total (n=18)*	Supratentorial group (n=9)	Infratentorial group (n=8)	p Value†
Pathologically confirmed cases (%)	14 (78)	7 (78)	6 (75)	ns
Initial manifestations‡ (%)	(n=14)	(n=8)	(n=5)	
Unsteady gait	9 (64)	6 (75)	2 (40)	ns
Dementia	2 (14)	1 (13)	1 (20)	ns
Vertigo	3 (21)	1 (13)	2 (40)	ns
Diplopia	1 (7)	0 (0)	0 (0)	ns
Sensory disturbance	1 (7)	0 (0)	1 (20)	ns
Others§	2 (14)	2 (25)	0 (0)	—
Manifestations during clinical course (%)				
Cerebellar signs	16/18 (89)	8/9 (89)	7/8 (88)	ns
Psychiatric feature	10/16 (63)	4/8 (50)	5/7 (71)	ns
Dementia	18/18 (100)	9/9 (100)	8/8 (100)	ns
Visual disturbance	11/18 (61)	5/9 (56)	5/8 (63)	ns
Myoclonus	14/18 (78)	8/9 (89)	6/8 (75)	ns
Extrapyramidal signs	14/18 (78)	8/9 (89)	6/8 (75)	ns
Pyramidal signs	8/17 (47)	5/8 (63)	3/8 (38)	ns

*Total includes one case with an uncertain grafting region.
†p Value was assessed between the supratentorial group and the infratentorial group; ns, not significant.
‡Four cases of the total, two cases of the supratentorial group and one case of the infratentorial group developed more than one initial manifestation.
§Others include individual cases of incontinence and hearing disturbance.

grafting sites, possibly through transportation of PrP^{Sc} to the specific brain regions. An animal study proved that the plaque-type dCJD could be caused by cross-sequence transmission of sporadic CJD VV2 prions to individuals that are methionine homozygotes at codon 129 of the PrP gene.[8] Furthermore, another animal study showed that sporadic CJD MV2 prions could also induce plaque-type dCJD pathology (TK unpublished data). CJD VV2 and CJD MV2, which might cause plaque-type prions, are well known as ataxic forms of CJD.[18] Meanwhile, the small number of plaque-type cases might influence these results, resulting in no significant difference between the supratentorial and the infratentorial groups.

Our study suggests that generally brain tissue near the grafting site was damaged earlier and more severely through direct propagation of PrP^{Sc} from the grafts. However, some cases have also suggested that there were different patterns of PrP^{Sc} propagation. For instance, 31% of all infratentorial grafting cases and 37% of the non-plaque type with infratentorial grafting cases developed dementia or behavioural abnormalities, indicating initial dysfunction of the cerebrum. Moreover, two cases of spinal cord grafts did not develop symptoms related to spinal cord dysfunction. Interestingly, the cerebellar signs throughout the clinical course in the supratentorial group were demonstrated less frequently than those in the infratentorial group in all cases and in the non-plaque-type cases.

These results may suggest the presence of different propagation pathways of PrP^{Sc} in addition to the direct invasion of brain tissue, via the cerebrospinal fluid, bloodstream or lymphatic drainage from the CNS. Recently, it has been suggested that lymphatic systems could play an important role in the PrP^{Sc} infection of the brain.[19] In most cases of prion infection regarding variant CJD, the point of PrP^{Sc} entry can be outside the nervous system.[19] After the infection of the organs outside the nervous system, PrP^{Sc} moves into the blood and lymphoid fluids and replicates in the lymphoid organs. PrP^{Sc} is then transported to the brain through the peripheral nervous system.[19 20] In addition to variant CJD cases, PrP^{Sc} was detected in the muscles, intramuscular nerve fibres and dorsal root ganglia in sporadic CJD, in which causes of the disease are uncertain.[21–23] PrP^{Sc} contamination of dural grafts may have a similar process of indirect infection of the CNS in addition to the direct invasion of the adjacent brain tissue. Although little data regarding PrP^{Sc} deposition in tissues other than the CNS in dCJD are available, PrP^{Sc} was detected in the peripheral nerves in cases of dCJD in an immunohistochemical study and western blot analysis.[24] In this study, no significant difference in the incubation period was revealed between the patients with suggested direct infection and the patients with suggested indirect infection. Further examination regarding PrP^{Sc} accumulation in other organs is necessary to confirm this indirect infection of PrP^{Sc} into the CNS in dCJD cases.

In addition to a hypothesis of indirect propagation of PrP^{Sc} into the CNS, various combinations of clinical manifestations and brain lesions may influence the symptoms of dCJD patients. Ataxia is a common manifestation stemming from cerebellar or brain stem dysfunction; however, lesions involving cerebral areas and fibres connecting to the cerebellum also cause ataxia.[25] Moreover, several case reports presented patients showing rotational vertigo in the clinical course of stroke in the cerebral hemisphere.[26]

It would be difficult to determine focal brain lesions by PrP^{Sc} accumulation and subsequent neuronal damage from only information about the clinical manifestations in each case. Therefore, analyses with imaging techniques, including MRI, single-photon emission tomography and positron emission tomography, are necessary to confirm the relationship between the grafting site and PrP^{Sc} propagation in human CNS.

In conclusion, our results indicate that PrP^{Sc} of non-plaque-type dCJD tends to spread from the grafted sites to the adjacent brain, although different propagation pathways may be present in some cases.

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Short communication

Failure of mefloquine therapy in progressive multifocal leukoencephalopathy: Report of two Japanese patients without human immunodeficiency virus infection

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ABSTRACT

Although progressive multifocal leukoencephalopathy (PML) cases showing responses to mefloquine therapy have been reported, the efficacy of mefloquine for PML remains unclear. We report on the failure of mefloquine therapy in two Japanese patients with PML unrelated to human immunodeficiency virus. One of the patients was a 47-year-old male who had been treated with chemotherapy for Waldenström macroglobulinemia, and the other was an 81-year-old male with idiopathic $CD4^+$ lymphocytopenia. Diagnosis of PML was established based on MRI findings and increased JC virus DNA in the cerebrospinal fluid in both patients. Mefloquine was initiated about 5 months and 2 months after the onset of PML, respectively. During mefloquine therapy, clinical and radiological progression was observed, and JC virus DNA in the cerebrospinal fluid was increased in both patients. Both patients died about 4 months and 2 months after initiation of mefloquine, respectively. Further studies are necessary to clarify the differences between mefloquine responders and non-responders in PML.

1. Introduction

Progressive multifocal leukoencephalopathy (PML) is a brain disorder caused by JC polyomavirus, which causes death in one-half of patients within 1 year [1]. Primary infection usually occurs during childhood and is often asymptomatic. The initial site of JC virus (JCV) infection is thought to be the tonsils, and it is then carried by lymphocytes to the kidneys and bone marrow. Reactivation of JCV occurs due to severe cellular immunodeficiency, and the virus crosses the blood–brain barrier (BBB) and infects oligodendrocytes, causing widespread demyelinating lesions. A recent study revealed promyelocytic leukemia nuclear bodies as an intranuclear target of JCV [2].

A study of 9675 cases of PML between 1998 and 2005 showed that 82% of patients had human immunodeficiency virus (HIV), 8.4% hematologic malignancies, 2.83% solid organ cancers, and 0.44% rheumatologic diseases [3]. Recently, a new category of PML patients has emerged among patients treated with immunomodulatory medications including natalizumab, rituximab, and efalizumab. PML may also occur in patients with minimal or occult immunosuppression including idiopathic $CD4^+$ lymphocytopenia [4]. In Japan, the proportion of hematological malignancies or rheumatologic diseases as underlying diseases is relatively high, whereas that of HIV infection is low [5,6].

The estimated probability of survival at 1 year is reported to be 52% in HIV related PML [1] and variable in PML unrelated to HIV among reports. Some patients with PML do survive for extended periods of time after diagnosis [7,8]. Survival in PML is influenced by the presence of JCV-specific cytotoxic T-lymphocytes, $CD4^+$ cell counts, or JCV DNA levels [1,9]. One study reported that estimated 1-year survival was 48% in patients with HIV related PML with $CD4^+$ cell counts $<200/\mu l$ at PML diagnosis compared to 67% in those with $CD4^+$ cell counts $>200/\mu l$ [1]. Another study showed that JCV DNA levels >4365 copies/ml of cerebrospinal fluid (CSF) correlated significantly with shorter survival in patients with HIV related PML not receiving highly active antiretroviral therapy (HAART) [9].

To date, although antiviral drugs such as cytarabine and cidofovir show activity against JCV *in vitro* [10,11], large clinical studies have failed to establish the efficacies of these drugs in the treatment of PML [12–14]. The reason for this may be that these drugs are not able to cross the BBB and accumulate throughout the entire brain parenchyma at a dose sufficient to suppress JCV proliferation [15].

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In 2008, mefloquine, an anti-malarial drug, was reported to show activity against JCV *in vitro* [15]. Since then, there have been at least 5 reported cases of PML in which mefloquine was effective [16–20]. In contrast, a recent mefloquine trial of 24 patients with PML (21 HIV-positive and 3 HIV-negative) reported failure in reducing JCV DNA levels in the CSF [21], although it is pending publication. Because there have been no reports describing the details of PML patients demonstrating mefloquine treatment failure, we report two HIV-negative patients with PML in whom mefloquine was not effective.

2. Case reports

2.1. Case 1

A 47-year-old male presented with progressive left hemiparesis. The patient had been treated with chemotherapy including rituximab for Waldenström macroglobulinemia for six years in our hospital. The interval between the last administration of rituximab and occurrence of hemiparesis was about 1 month. Diffusion weighted images (DWI) of brain MRI about 3 months after the onset of hemiparesis demonstrated high intensity areas with internal low intensity areas in the white matter of the right frontal lobe. The apparent diffusion coefficient (ADC) values of the lesion were increased. Because serum IgM had been prominently elevated (around 5000 mg/dl) in association with Waldenström macroglobulinemia, we presumed that the hyperviscosity syndrome resulted in brain infarction.

About 4 months after the onset of hemiparesis, the patient was admitted to our hospital because a convulsion occurred in the left upper and lower limbs. At that time, the patient did not receive any immunosuppressive therapy. On admission, neurological examination revealed upper limb-dominant left hemiparesis, and Babinski's sign and Chaddock's reflex on the left. MRI on admission demonstrated lesion expansion and extension to the right parietal and insular white matter, right putamen, right internal capsule, right thalamus, corpus callosum, left frontal white matter, and midbrain. There was no edema or gadolinium-enhanced lesions. Peripheral blood tests showed white blood cell count (WBC): 3790/μl (normal range: 4500–9000), hemoglobin: 10.4 g/dl (normal range: 13–16), and platelet count: 3.7×10^4/μl (normal range $15–30\times10^4$), indicating pancytopenia. C-reactive protein (CRP) was below 0.1 mg/dl. Testing for HIV was negative. On the next day of admission, a nasogastric feeding tube was inserted because of dysphagia. Four days after admission, CSF examination demonstrated cell count: 1 cell per 3 μl, total protein: 97 mg/dl, and glucose: 67 mg/dl. PCR was positive for JCV DNA in the CSF and detected 1200 copies/ml of DNA. A diagnosis of PML was established based on MRI findings and increased JCV DNA in the CSF.

After diagnosis, the patient developed right hemiparesis and apraxia of speech. Brain MRI 18 days after admission demonstrated lesion expansion and extension to the left insular white matter and left putamen (Fig. 1A). The JCV DNA copy number in the CSF was increased to 4300 copies/ml. $CD4^+$ cell count of the peripheral blood was 219/μl (normal range: 500–1300). Nineteen days after admission, about 5 months after the onset of PML, mefloquine was initiated at a dose of 275 mg/day orally for 3 days, followed by 275 mg once a week [17]. We used Mephaquin Hisamitsu tablets (Hisamitsu Pharmaceutical, Tosu, Japan), which show maximum concentration (C_{max}) of 3.1 μM, time at which C_{max} is observed (T_{max}) of 5.2 h, and terminal half-life (T1/2) of 400.1 h when 1100 mg of drug is once administered. Treatment with mefloquine was approved by the Ethics Committee in our hospital. We obtained written, informed consent from the patient's family. We also used 1 mg/day of risperidone, a 5HT2A receptor blocker at the same time. After initiation of mefloquine, we observed no symptoms suggestive of mefloquine neurotoxicity such as nausea, dizziness, sleep disturbances, anxiety, and psychosis [22]. Eight days after initiation of mefloquine, the JCV DNA copy number in the CSF was increased to 150,000 copies/ml, and the dose of mefloquine was returned to 275 mg/day for 3 days per week (Fig. 2).

However, the JCV DNA copy number in the CSF 22 days after initiation of mefloquine was increased to 850,000 copies/ml. Because of severe aspiration pneumonia, tracheotomy was performed 37 days after initiation of mefloquine. Brain MRI 38 days after initiation of mefloquine demonstrated lesion expansion and extension to the right temporal and occipital white matter and pons (Fig. 1B). The JCV DNA copy number in the CSF 50 days after initiation of mefloquine increased to 3,700,000 copies/ml. Changes in the JCV DNA load are shown in Fig. 2. Brain MRI about 3 months after initiation of mefloquine demonstrated lesion expansion and extension to the left temporal and parietal white matter, left internal capsule, left thalamus, and medulla oblongata (Fig. 1C). The patient died of respiratory failure about 4 months after initiation of mefloquine. The total clinical course of PML was about 9 months. Autopsy could not be performed.

2.2. Case 2

An 81-year-old male with a three-week history of gait disturbance presented with muscle cramp in the bilateral upper limbs and was taken to another hospital by ambulance. Past medical history included hypertension, hyperuricemia, chronic heart failure, and chronic renal failure due to renal sclerosis. A diagnosis of brain infarction of the

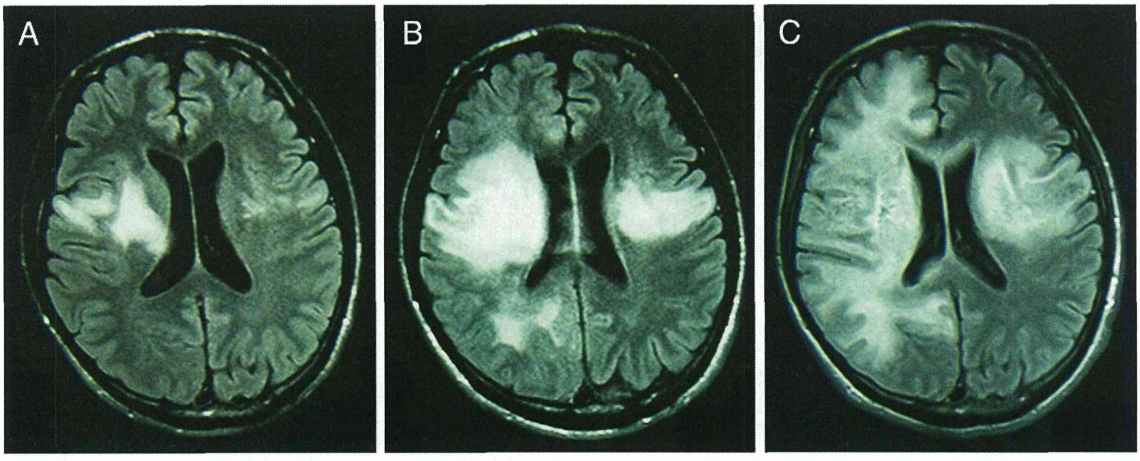

Fig. 1. A. Fluid-attenuated inversion recovery (FLAIR) sequence of brain MRI before initiation of mefloquine demonstrated high intensity areas in the white matter of the bilateral frontal lobes. B, C. FLAIR sequence of brain MRI 38 days (B) and about 3 months (C) after the initiation of mefloquine showed lesion expansion.

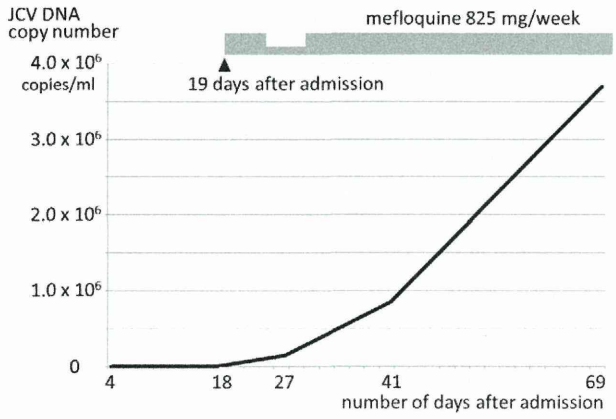

Fig. 2. Changes in the JCV DNA load of case 1 are shown. The JCV DNA copy number in the CSF was increased even after initiation of mefloquine.

subacute phase and the worsening of renal failure was made in the emergency room and the patient was transferred to our hospital.

Physical examination on admission demonstrated muscle cramp in the bilateral upper limbs and face, and right hemiparesis. Consciousness was slightly disturbed, but the orientation to time and place was preserved. Peripheral blood tests showed WBC: 5240/μl, hemoglobin: 7.8 g/dl, platelet count: 17.6×10^4/μl, albumin: 2.7 g/dl (normal range: 3.9–4.9), blood urea nitrogen (BUN): 147 mg/dl (normal range: 8–20), creatinine: 7.83 mg/dl (normal range: 0.6–1.1), creatinine kinase (CK): 445 IU/l (normal range 50–200), CRP: 0.3 mg/dl (normal range <0.1), and glucose: 103 mg/dl. Testing for HIV was negative. Hemodialysis was started on the next day of admission.

DWI of brain MRI 3 days after admission demonstrated high intensity areas in the white matter of the left frontal and parietal lobes and right parietal lobe. ADC values of the lesions were increased. Right hemiparesis progressed after admission, and 18 days after admission, the left hemiparesis emerged. Because of dysphagia, a nasogastric feeding tube was inserted 19 days after admission. CSF examination 20 days after admission demonstrated cell count: 6 cells per 3 μl, total protein: 35 mg/dl, and glucose: 60 mg/dl. PCR was positive for JCV DNA in the CSF, and detected 2223 copies/ml of DNA. A diagnosis of PML was established based on MRI findings and increased JCV DNA in the CSF.

Brain MRI 32 days after admission demonstrated lesion expansion and extension to the corpus callosum and right frontal white matter. Thirty six days after admission, the patient manifested akinetic mutism. Thirty eight days after admission, about 2 months after the onset of PML, mefloquine was initiated at a dose of 275 mg/day orally for 3 days per week. Treatment with mefloquine was approved by the Ethics Committee in our hospital. We obtained written, informed consent from the patient's family. At that time, the JCV DNA copy number in the CSF was increased to 2,790,000 copies/ml. The $CD4^+$ cell count of the peripheral blood was 294/μl. Because whole body CT demonstrated no mass lesions or abnormal lymph node swelling, underlying diseases causing immunodeficiency remained unclear in this patient.

After initiation of mefloquine, we observed no acute neurological deterioration suggesting mefloquine neurotoxicity. Brain MRI 15 days after initiation of mefloquine demonstrated lesion expansion and extension to the bilateral temporal and occipital white matter (Fig. 3A). Twenty nine days after initiation of mefloquine, the JCV DNA copy number in the CSF was increased to 24,075,000 copies/ml. Changes in the JCV DNA load are shown in Fig. 4. Brain MRI 31 days after initiation of mefloquine demonstrated lesion expansion (Fig. 3B). Thirty three days after initiation of mefloquine, hemodialysis was discontinued because of hypotension. The patient died 19 days later. The total clinical course of PML was about 4 months. Autopsy could not be performed.

3. Discussion

Because there is no known specific antiviral agent against JCV, we treated PML in the two HIV-negative patients with mefloquine based on case reports describing the efficacy of mefloquine for PML [16–20]. However, during mefloquine therapy, clinical and radiological progression was observed, and JCV DNA in the CSF was increased in both patients.

Our case 1 had been treated with chemotherapy including rituximab for Waldenström macroglobulinemia. The interval between the last administration of rituximab and diagnosis of PML was about 6 months. Although it is difficult to exclude the possibility that the immunodeficiency due to Waldenström macroglobulinemia itself was related to the occurrence of PML [23], rituximab is well known to cause PML [7]. Rituximab is an anti-CD20 monoclonal antibody that targets human B cells. The pathogenesis of rituximab in PML is considered to decrease B cells in the cerebral perivascular spaces, resulting in decreased antigen presentation to T cells and subsequent alterations in the cellular immune response [7]. One study reported that a median $CD4^+$ cell count was 216/μl in 25 patients who received rituximab [24]. The interval between the last administration of rituximab and

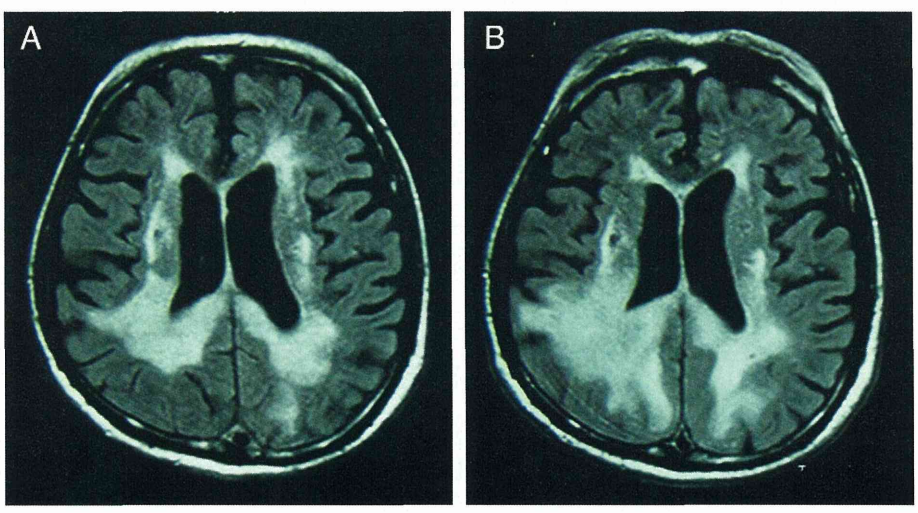

Fig. 3. A. FLAIR sequence of brain MRI 15 days after initiation of mefloquine demonstrated abnormal high intensity areas in the bilateral temporal and occipital white matter. B. FLAIR sequence of brain MRI 31 days after the initiation of mefloquine showed lesion expansion.

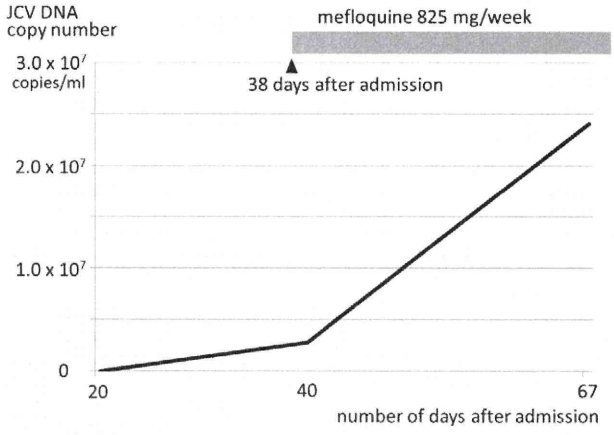

Fig. 4. Changes in the JCV DNA load of case 2 are shown. The JCV DNA copy number in the CSF was increased even after initiation of mefloquine.

diagnosis of PML has been reported to be 5.5 months [25]. Considering that 90% of patients with PML after rituximab therapy die [25], the unfavorable clinical course of our case 1 may be associated with the use of rituximab. In case 2, while $CD4^+$ lymphocytopenia was documented, there were no underlying diseases causing immunodeficiency. However, as PML may occur in patients with minimal or occult immunosuppression [4], idiopathic $CD4^+$ lymphocytopenia may be associated with the occurrence of PML in this patient.

Mefloquine is an anti-malarial drug used both for prophylaxis and treatment of chloroquine resistant *Plasmodium falciparum*. Because mefloquine is highly lipophilic and has a long terminal half-life of more than 1 week [26], a single dose of 15–25 mg/kg is used for treatment and 250 mg/week for prophylaxis. Among subjects administered 250 mg weekly, blood concentrations vary between 1 μM to 5 μM [27]. Mefloquine readily crosses the BBB, where active efflux by the P-glycoprotein membrane transporter prevents its accumulation in the brain [27].

In 2008, mefloquine was reported to show activity against JCV *in vitro* [15]. Brickelmaier et al. showed that mefloquine inhibits viral DNA replication, using quantitative PCR to quantify the number of viral copies in cultured cells. In this study, mefloquine reduced the number of infected cells by 50% or more at a concentration of 3.9 μM [15]. Brickelmaier et al. presumed that efficacious concentrations of mefloquine for PML are achieved in the brains of patients receiving approved doses of the drug [15].

Since the publication by Brickelmaier et al. [15], there have been at least 5 reported cases of PML in which mefloquine was effective [16–20]. The underlying diseases or conditions included sarcoidosis [16], umbilical cord blood transplant [17], HIV infection [18], and systemic lupus erythematosus [19]. $CD4^+$ cell counts in the peripheral blood of patients were described in 3 reports, and were 187/μl [18], 419/μl [17], and 420/μl [16], respectively. JCV DNA loads in the CSF before mefloquine therapy were available in these reports, and were 33,700 copies/ml [16], 535,500 copies/ml [18], and 911,175 copies/ml [17], respectively. The intervals between symptom onset and initiation of mefloquine therapy were about 3 months [17,19], 5 months [18], and 6 months [16,20], respectively. In 4 reports [16–19], the authors stated that PCR for JCV in the CSF became negative after mefloquine therapy. At present, the patients' background or laboratory data common among these cases showing responses to mefloquine therapy is unclear.

In contrast to these cases, a recent mefloquine trial of 24 patients with PML (21 HIV-positive and 3 HIV-negative) reported failure in reducing JCV DNA levels in the CSF [21]. Participants took 250 mg of mefloquine 4 times daily, followed by 250 mg weekly. The failure of this trial and the poor outcome of our patients raise the possibility that the improvement observed in mefloquine therapy in reported PML patients [16–20] may actually reflect the natural favorable course of those patients.

At present, we cannot tell the difference in patient backgrounds or laboratory data between patients showing responses to mefloquine [16–20] and our patients. Regarding the presence of both mefloquine responders and non-responders in PML, Nevin stated that responses to mefloquine may correlate with polymorphisms in the *MDR1* gene coding for P-glycoprotein that affect drug efflux across the BBB [28]. In cases of unsuccessful treatment of PML, active efflux as a result of drug induced upregulation of P-glycoprotein expression in the BBB may be preventing therapeutic concentrations of mefloquine [28]. From this point of view, co-administration of P-glycoprotein inhibitors or substrates such as risperidone may be recommended in the treatment of PML [27]. On the other hand, considering the failure of the mefloquine trial and the poor outcome of our patients, re-evaluation of the anti-JCV activity of mefloquine may be required. If the anti-JCV activity of mefloquine is verified again, further studies are necessary to clarify whether the response to mefloquine in PML is influenced by the presence of HIV infection, $CD4^+$ cell counts, JCV DNA levels in the CSF, blood concentration of mefloquine, interval between disease onset and initiation of therapy, or *MDR1* polymorphism.

Conflict of interest statement

The authors have no conflicts of interest.

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