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Clinicopathological features of neuropathy associated with lymphoma

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Lymphoma causes various neurological manifestations that might affect any part of the nervous system and occur at any stage of the disease. The peripheral nervous system is one of the major constituents of the neurological involvement of lymphoma. In this study we characterized the clinical, electrophysiological and histopathological features of 32 patients with neuropathy associated with non-Hodgkin's lymphoma that were unrelated to complications resulting from treatment for lymphoma. Nine patients had pathologically-proven neurolymphomatosis with direct invasion of lymphoma cells into the peripheral nervous system. These patients showed lymphomatous cell invasion that was more prominent in the proximal portions of the nerve trunk and that induced demyelination without macrophage invasion and subsequent axonal degeneration in the portion distal from the demyelination site. Six other patients were also considered to have neurolymphomatosis because these patients showed positive signals along the peripheral nerve on fluorodeoxyglucose positron emission tomography imaging. Spontaneous pain can significantly disrupt daily activities, as frequently reported in patients diagnosed with neurolymphomatosis. In contrast, five patients were considered to have paraneoplastic neuropathy because primary peripheral nerve lesions were observed without the invasion of lymphomatous cells, with three patients showing features compatible with chronic inflammatory demyelinating polyneuropathy, one patient showing sensory ganglionopathy, and one patient showing vasculitic neuropathy. Of the other 12 patients, 10 presented with multiple mononeuropathies. These patients showed clinical and electrophysiological features similar to those of neurolymphomatosis rather than paraneoplastic neuropathy. Electrophysiological findings suggestive of demyelination were frequently observed, even in patients with neurolymphomatosis. Eleven of the 32 patients, including five patients with neurolymphomatosis, fulfilled the European Federation of Neurological Societies/Peripheral Nerve Society electrodiagnostic criteria of definite chronic inflammatory demyelinating polyneuropathy. Some of these patients, even those with neurolymphomatosis, responded initially to immunomodulatory treatments, including the administration of intravenous immunoglobulin and steroids. Patients with lymphoma exhibit various neuropathic patterns, but neurolymphomatosis is the major cause of

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neuropathy. Misdiagnoses of neurolymphomatosis as chronic inflammatory demyelinating polyneuropathy are frequent due to a presence of a demyelinating pattern and the initial response to immunomodulatory treatments. The possibility of the concomitance of lymphoma should be considered in various types of neuropathy, even if the diagnostic criteria of chronic inflammatory demyelinating polyneuropathy are met, particularly in patients complaining of pain.

Keywords: chronic inflammatory demyelinating polyneuropathy; demyelination; lymphoma; neurolymphomatosis; neuropathy

Abbreviations: CIDP = chronic inflammatory demyelinating polyneuropathy; EFNS/PNS = European Federation of Neurological Societies/Peripheral Nerve Society; FDG = fluorodeoxyglucose; IVIg = intravenous immunoglobulin

Introduction

Lymphoma causes various neurological manifestations that might affect any part of the nervous system and occur at any stage of the disease (MacKintosh *et al.*, 1982; Giglio and Gilbert, 2006; Antoine and Camdessanché, 2007; Briani *et al.*, 2011; Baehring and Batchelor, 2012). Involvement of the PNS is one of the major constituents of the neurological disorders associated with lymphoma (Walsh, 1971), occurring in 5% of patients with lymphoma (Hughes *et al.*, 1994).

In addition to the direct invasion of lymphoma cells into the PNS (Baehring *et al.*, 2003; Grisariu *et al.*, 2010; Baehring and Batchelor, 2012), the causes of peripheral neuropathy in lymphoma include chemotherapy, radiation therapy, stem cell transplantation, malnutrition, infection, hyperviscosity, secondary amyloidosis, compression, and paraneoplastic syndrome (Correale *et al.*, 1991; Koike *et al.*, 2011*a*). The definitive diagnosis of neuropathy in patients with lymphoma enables the establishment of an appropriate treatment strategy for these patients. For patients not yet diagnosed with lymphoma, the appropriate diagnosis of neuropathy is also important because it enables the treatment for lymphoma to be initiated at an early stage of the disease. However, the conclusions regarding the clinical, electrophysiological and pathological features of neuropathy associated with lymphoma are not always definitive and have not enabled a detailed understanding of the pathophysiology of this condition. The types of neuropathy might include neuropathy associated with the infiltration of lymphoma cells, known as neurolymphomatosis; demyelinating neuropathy mimicking Guillain-Barré syndrome or chronic inflammatory demyelinating polyneuropathy (CIDP); sensory neuronopathy; vasculitic neuropathy; and neuropathy associated with paraproteinaemia (Bosch *et al.*, 2005; Kelly and Karcher, 2005; Viala *et al.*, 2008; Briani *et al.*, 2011). According to a previous study, polyneuropathy with demyelinating features was the most common form of neuropathy associated with lymphoma, and humoral factors might play an important role in this disease (Viala *et al.*, 2008). However, reports have also described the presence of demyelination in neurolymphomatosis (Kohut, 1946; Moore and Oda, 1962). Hence, the clinicopathological features of neuropathy associated with lymphoma, particularly the correlation of these features with lymphomatous cell invasion or the paraneoplastic condition, and their prognostic features have not yet been well characterized.

In the present study, we assessed the clinical, electrophysiological and histopathological findings of 32 patients with neuropathy associated with lymphoma and elucidated the clinicopathological features of neuropathy in these patients.

Materials and methods

Patients

We retrospectively investigated patients with neuropathy associated with lymphoma who were referred to the neurological department of our institutions from 1997 to 2012. The diagnosis of lymphoma was pathologically confirmed based on the 2001 and 2008 World Health Organization classification or the Revised European-American Lymphoma classification in all patients (Harris *et al.*, 1994; Campo *et al.*, 2011). The patients underwent clinical and neurological assessments, routine blood and urine studies, and CSF analysis. Neurological examinations were performed repetitively by at least two neurologists in each case. The presence of neuropathy was clinically defined through the presence of sensory and/or motor signs and the reduction or absence of deep tendon reflexes without pathological reflexes. Nerve conduction studies were used to confirm the presence of neuropathy in each case. Values that deviated from the mean ± two standard deviations (SD) of these controls were defined as abnormal. The muscle strength was assessed through manual muscle testing. Sensory examinations were performed to evaluate pinprick, temperature, light touch, vibratory, and joint position sensations. Autonomic involvement was characterized in terms of the abdominal, urinary, and orthostatic symptoms; pupillary responses; and sweating.

A detailed history of illness was obtained from each patient and the patient's family concerning the lifestyle, occupation, diet and amount of alcohol consumed daily. Patients with underlying diseases other than malignant lymphoma that might cause neuropathy, such as diabetes mellitus, renal failure, vitamin deficiency, thyroid dysfunction, cachexia, and autoimmune disease, were excluded from the study. Patients with Waldenström's macroglobulinaemia were excluded. Patients who were considered to have had treatment-induced neuropathy were also excluded (Windebank and Grisold, 2008; Koike *et al.*, 2011*a*). We excluded cases exhibiting neurological onset within 1 month of the initiation of treatment to exclude neuropathy associated with the side effects of chemotherapy or radiation. Clinical and pathological findings in one patient (Patient 19) were reported in a previous study (Kobayashi *et al.*, 2005). Informed consent was obtained from all patients. The study was approved through the Ethics Committee of Nagoya University Graduate School of Medicine.

Electrophysiological assessment

A nerve conduction study was performed on all patients. Motor and sensory conduction were measured in the median, ulnar, tibial and

sural nerves using a standard method with surface electrodes for stimulation and recording (Koike *et al.*, 2005, 2008*a*; Suzuki *et al.*, 2008). Motor conduction was investigated in the median, ulnar and tibial nerves through recordings obtained from the abductor pollicis brevis, abductor digiti minimi, and abductor hallucis brevis, respectively. The following nerve segments were used to calculate the motor conduction velocity: wrist to elbow for the median nerve, wrist to distally at the elbow for the ulnar nerve, and ankle to popliteal fossa for the tibial nerve. The sensory conduction was investigated in the median, ulnar and sural nerves using antidromic recordings from ring electrodes at the second and fifth digits for the median and ulnar nerves, respectively, and bar electrodes at the ankle for the sural nerve. The sensory conduction velocity was calculated for the distal segment. The amplitudes of the compound muscle action potential and sensory nerve action potential were measured from the baseline to the first negative peak. Waveforms were also analysed to assess temporal dispersion. A conduction block was defined according to the electrodiagnostic criteria of the European Federation of Neurological Societies/Peripheral Nerve Society (EFNS/PNS) (Joint Task Force of the EFNS and the PNS, 2010). The normal control values were based on previously published reports (Koike *et al.*, 2005; Suzuki *et al.*, 2008).

Pathological assessment

Details of the pathological assessments are described in Supplementary material. A sural nerve biopsy was performed in 20 patients as previously described (Sobue *et al.*, 1989; Koike *et al.*, 2003, 2010). The specimens were divided into two portions. The first portion was fixed in 2.5% glutaraldehyde in a 0.125 M cacodylate buffer (pH 7.4) and embedded in epoxy resin. The density of the myelinated fibres was assessed in toluidine blue-stained semi-thin sections as previously described (Sobue *et al.*, 1990*a*; Koike *et al.*, 2001, 2004). The density of unmyelinated fibres was assessed in uranyl acetate- and lead citrate-stained ultra-thin transverse sections as previously described (Koike *et al.*, 2003, 2007, 2008*b*). A fraction of the glutaraldehyde-fixed sample was processed for a teased-fibre study (Sobue *et al.*, 1989; Dyck *et al.*, 2005). The control values were based on a previous report (Koike *et al.*, 2008*b*).

The second portion of the specimen was fixed in a 10% formalin solution and embedded in paraffin. Sections were cut using routine methods and stained with haematoxylin and eosin and Congo red. Immunohistochemical assessments were performed using the peroxidase–antiperoxidase method in consecutive deparaffinized sections (Sobue *et al.*, 1990*b*; Asano *et al.*, 2005, 2006).

An autopsy was performed on five patients (Patients 1, 2, 7, 8 and 19) as previously described (Sobue *et al.*, 1989, 1990*a*; Koike *et al.*, 2011*b*).

Treatment and assessment of the response to the treatment

The functional status of the patients was assessed at the peak phase according to the modified Rankin scale (van Swieten *et al.*, 1988): 0 = no symptoms at all; 1 = no significant disability despite the presence of symptoms, demonstrated as the ability to perform all typical duties and activities; 2 = slight disability, demonstrated as the inability to perform all previous activities but the ability to perform self-care without assistance; 3 = moderate disability, demonstrated as requiring some help but being able to walk without assistance; 4 = moderately severe disability, demonstrated as the inability to walk without assistance and to attend to bodily needs without assistance; and 5 = severe disability, demonstrated as being bedridden and incontinent and requiring constant nursing care and attention.

The response to the treatment for neurological symptoms was defined using the following terms: effective (2+), i.e. upgraded according to the modified Rankin scale after treatment, and mildly effective (1+), i.e. a reduction in the neurological symptoms without an upgrade on the modified Rankin scale after treatment.

Statistical analyses

The quantitative data are presented as the means ± SD. The statistical analyses were performed using the χ^2 test, Mann-Whitney *U*-test, or Spearman's rank correlation analysis, as appropriate. $P < 0.05$ was considered to indicate significance.

Results

Background and laboratory features

The background and laboratory features are shown in Table 1. The patient cohort included 21 males and 11 females. The age at neuropathy onset was 64.9 ± 13.1 years and ranged from 30–86 years. All patients had non-Hodgkin's lymphoma. Twenty-six patients had B cell lymphoma, and six patients had T cell lymphoma (Patients 5, 7, 16, 18, 31 and 32). In the patients with B cell lymphoma, the most common type was diffuse large B cell lymphoma, observed in 20 of 26 patients. The age at onset was significantly older in patients with B cell lymphoma than in those with T cell lymphoma (68.1 ± 11.1 versus 51.0 ± 12.9 years, respectively, $P < 0.01$). Twenty-three of the 32 patients manifested a focality of the distribution of neuropathic symptoms, as indicated by a multifocal mononeuropathic pattern in the extremities and/or unilateral cranial nerve involvement, whereas the other nine patients were characterized as having a symmetrical polyneuropathy pattern (Fig. 1, Table 2 and Supplementary Fig. 1). Fifty-five per cent of the patients (14 patients with B cell lymphoma and three patients with T cell lymphoma) were referred for the first time because of neuropathic symptoms, and the presence of lymphoma was not diagnosed at the time of the first referral. The other 45% (11 patients with B cell lymphoma and three patients with T cell lymphoma) were diagnosed as having lymphoma before the neurological symptoms appeared, and the duration from the appearance of lymphoma to that of neuropathy was 41.3 ± 37.7 months. Thirteen of these patients had received chemotherapy, and the chemotherapy was finished within 1 year of the diagnosis of lymphoma, with no neurological symptoms at the time of the cessation of chemotherapy.

An abnormal elevation of the serum soluble interleukin 2 receptor was observed in 17 of the 24 (71%) examined patients (range 343–30 500 U/ml; mean ± SD, 2868 ± 6128; normal 220–530). The CSF was examined in 29 patients, and an elevated cell count was observed in 12 patients (range 0–318/mm^3; mean ± SD, 21.0 ± 59.9), whereas protein abnormality was observed in 20 patients (range 26–466 mg/dl; mean ± SD, 116 ± 99). These values were not significantly different between the patients with B cell lymphoma and those with T cell lymphoma. The cytology of the CSF revealed mononuclear cells with an atypical nuclear appearance

Table 1 Clinical features and laboratory data of neuropathy associated with lymphoma

Patients	Sex	Age	Type of lymphoma	Duration of lymphoma until neuropathic onset (months)	Serum sIL-2R (U/ml)*	CSF findings**			FDG-PET
						Cell (no./mm^3)	Protein (mg/dl)	Cytology	
Neurolymphomatosis***									
1	M	56	DLBCL	−12	343	1	47	−	ND
2	F	73	DLBCL	1	7020	1	30	+	ND
3	F	45	DLBCL	−11	ND	25	124	+	ND
4	M	70	DLBCL	29	467	1	125	−	ND
5	M	56	T cell	−9	ND	318	166	+	ND
6	M	64	DLBCL	−7	1860	ND	ND	ND	ND
7	M	51	ATLL	−20	1590	1	35	−	ND
8	M	69	BL	1	529	0	94	−	ND
9	F	62	DLBCL	5	924	1	65	−	Negative
10	F	82	DLBCL	72	509	1	30	−	Brachial plexus
11	F	75	DLBCL	12	1009	2	30	−	Brachial plexus, adrenal gland, abdominal muscle
12	F	75	DLBCL	6	ND	0	55	−	Lumbar plexus
13	M	63	B cell	−6	441	42	328	+	Lumbar plexus
14	M	76	DLBCL	−12	356	16	34	−	Brachial plexus, abdominal lymph node
15	M	58	DLBCL	−12	915	3	39	−	Brachial plexus
Paraneoplastic neuropathy									
CIDP-type									
16	M	65	AILT	44	ND	4	120	−	ND
17	M	61	LPL	−12	635	1	191	−	Iliopsoas muscle
18	M	61	MF	132	ND	ND	ND	ND	ND
Sensory ganglionopathy									
19	M	63	DLBCL	−72	1200	25	116	−	ND
Vasculitic neuropathy									
20	F	74	DLBCL	24	839	0	26	−	ND
Unclassified group									
Multiple mononeuropathy									
21	M	42	DLBCL	−20	558	0	35	−	Testis
22	M	55	DLBCL	−3	1630	6	153	ND	ND
23	M	86	DLBCL	−2	ND	5	145	−	ND
24	M	73	DLBCL	−6	2350	2	33	−	ND
25	F	78	FL	65	2410	1	133	−	Negative
26	M	72	LPL	51	4230	25	110	−	ND
27	F	84	DLBCL	76	ND	ND	ND	ND	ND
28	F	80	DLBCL	−3	2548	52	212	+	ND
29	M	69	DLBCL	−6	5491	10	112	−	Adrenal gland
30	M	66	MCL	ND	ND	6	226	−	ND
Polyneuropathy									
31	F	30	PTCL-U	−21	484	45	466	−	ND
32	M	43	T cell	60	30500	14	94	+	Bone

ATLL = adult T cell leukaemia/lymphoma; AITL = angio-immunoblastic T cell lymphoma; B cell = unclassifiable B cell lymphoma; BL = Burkitt lymphoma; DLBCL = diffuse large B cell lymphoma; FDG = fluorodeoxyglucose; FL = follicular lymphoma; LPL = lymphoplasmacytic lymphoma; MCL = mantle cell lymphoma; MF = mycosis fungoides; ND = not determined; PTCL-U = peripheral T cell lymphoma unspecified; sIL-2R = soluble interleukin-2 receptor; T cell = unclassifiable T cell lymphoma; + = positive − = negative.

*Normal range, 220-530 U/ml.

**The level of protein and cell count were those at the first examination. The cytology was performed twice in Patients 1, 13 (positive at second examination). and 19, three times in Patients 2 (positive at the first examination), 24, and 31, and 14 times in Patient 32 (positive at the first examination). Flow cytometry was. performed in Patients 5, 13, 28 and 32, and revealed the findings corresponding to each specific diagnosis of lymphoma.

***Patients 1 to 9 were pathologically-proven neurolymphomatosis, whereas Patients 10 to 15 were FDG-PET assessed neurolymphomatosis.

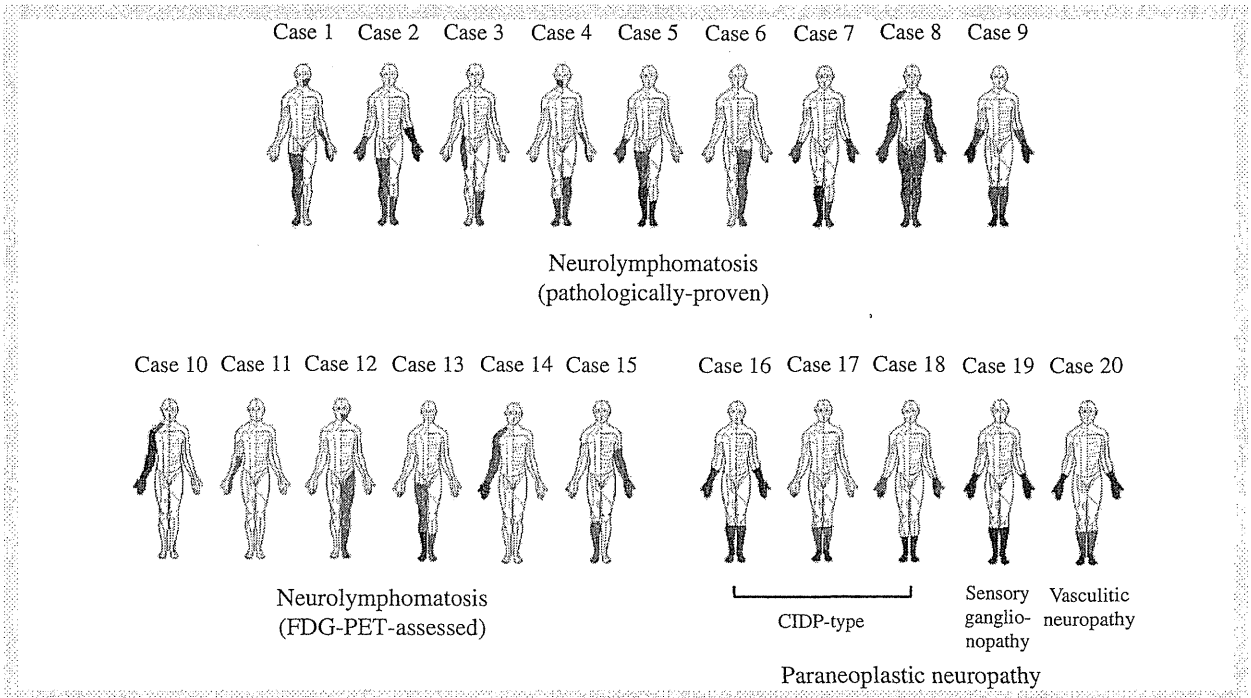

Figure 1 Distribution of sensory deficits in representative cases. Cases 1 to 9 and 10 to 15 represent pathologically-proven and FDG-PET-assessed neurolymphomatosis, respectively. All cases in this category, except for Cases 8 and 9, showed multiple mononeuropathy patterns, whereas Cases 8 and 9 manifested a symmetrical polyneuropathy pattern. Cases 16 to 20 were pathologically-proven paraneoplastic neuropathy. All patients manifested a symmetrical polyneuropathy pattern. Cases 16 to 18 were diagnosed as chronic inflammatory demyelinating polyneuropathy-type neuropathy, Cases 19 was diagnosed as sensory ganglionopathy, and Case 20 was diagnosed as vasculitic neuropathy. The red areas represent those of mild to moderate sensory deficits, while the black areas are where there is severe sensory deficits.

in six of the 28 examined patients. Onconeural antibodies were screened in seven patients (Patients 3, 7, 8, 15, 16, 20 and 31), and anti-Hu, anti-Ri, ANNA-3, anti-Yo, anti-PCA-2, anti-PCA-Tr, anti-CV2, amphiphysin, anti-striatal, anti-P/Q type calcium channel, N-type calcium channel, and anti-ganglionic acetylcholine receptor antibodies were negative in all seven patients. The sera of these patients were assessed based on an indirect immunofluorescence assay at Mayo Medical Laboratories. In Patient 19, the serum IgM antibody against GD1b was positive (× 1000) in an ELISA. Whole-body fluorodeoxyglucose (FDG)-PET was performed in 12 patients, and lymphoma was detected in five of six patients before the clinical discovery of lymphoma (Table 1).

Classification of neuropathy

Neuropathy associated with lymphoma can be classified broadly into neurolymphomatosis, which represents the direct invasion of lymphoma cells into the PNS, and paraneoplastic neuropathy, which represents damage remote from the site of lymphoma (Koike *et al.*, 2011*a*). Nine patients (Patients 1–9) were considered to have pathologically-proven neurolymphomatosis with lymphomatous cell invasion into the PNS. The direct invasion of lymphoma cells into the PNS was confirmed through biopsy or autopsy (Figs 3 and 4; Supplementary Tables 1 and 2). Additionally, six other patients (Patients 10–15) were considered to have neurolymphomatosis based on the FDG-PET study. FDG accumulation along the peripheral nerves was detected using PET, and the results strongly suggested the presence of neurolymphomatosis in these patients (Fig. 2A and Supplementary Fig. 2).

Five patients (Patients 16–20) were considered to have paraneoplastic aetiologies. These patients exhibited pathological findings suggesting CIDP, sensory ganglionopathy, or vasculitic neuropathy without lymphomatous cell invasion (Supplementary Tables 1 and 2). All five of these patients exhibited a symmetrical polyneuropathic pattern with respect to symptom manifestation (Fig. 1, Table 2 and Supplementary Fig. 1). Three patients (Patients 16–18) manifested subacute to chronic progressive, sensorimotor symmetrical polyneuropathy. The sural nerve biopsy specimens revealed extensive segmental demyelination without lymphomatous cell invasion in these patients (Supplementary Table 1). Another patient (Patient 19) manifested sensory ganglionopathy as previously described (Kobayashi *et al.*, 2005). The autopsy revealed a loss of neurons in the dorsal root ganglia with the preservation of motor neurons in the spinal cord. Patient 20 exhibited vasculitis that was pathologically confirmed through a sural nerve biopsy specimen, although the patient manifested a symmetrical polyneuropathy pattern. Vasculitis in this patient was observed in the epineurial blood vessels, and the infiltrating cells did not present an atypical appearance with a mixture of CD3- and CD20-positive cells, suggesting that the vasculitis was

Table 2 **Neuropathic features of neuropathy associated with lymphoma**

Patients	Progression*	Type of neuropathy	Cranial nerve involvement	Muscle weakness	Sensory disturbance		Spontaneous pain	Autonomic failure	EFNS/PNS CIDP electropiagnostic criteria**
					Superficial sensation	Deep sensation			
Neurolymphomatosis***									
1	Subacute	MM	V	3+	2+	2+	3+	–	Possible
2	Subacute	MM	VI	2+	1+	3+	3+	–	
3	Chronic	MM	III VI VII	3+	2+	0	1+	Adie pupil	Possible
4	Subacute	MM	V VII	3+	2+	2+	–	–	Definite
5	Chronic	MM	–	2+	2+	3+	–	–	Possible
6	Chronic	MM	–	3+	1+	0	3+	–	
7	Chronic	MM	XII	3+	3+	2+	1+	–	Possible
8	Subacute	PN	–	1+	2+	3+	–	–	Definite
9	Chronic	PN	–	3+	3+	3+	1+	–	Definite
10	Chronic	MM	–	3+	3+	3+	2+	–	Definite
11	Chronic	MM	–	3+	1+	1+	1+	–	Probable
12	Chronic	MM	V	3+	2+	0	1+	–	
13	Subacute	MM	VII	3+	2+	3+	3+	–	
14	Chronic	MM	–	3+	3+	3+	2+	–	
15	Chronic	MM	VII	3+	2+	1+	3+	–	Definite
Paraneoplastic neuropathy									
CIDP-type									
16	Subacute	PN	–	3+	1+	3+	–	–	Possible
17	Chronic	PN	–	3+	1+	3+	–	–	Definite
18	Subacute	PN	–	3+	3+	3+	–	–	Definite
Sensory ganglionopathy									
19	Chronic	PN	–	1+	1+	3+	–	–	Definite
Vasculitic neuropathy									
20	Subacute	PN	–	2+	3+	3+	1+	–	
Unclassified									
Multiple mononeuropathy									
21	Chronic	MM	–	3+	1+	1+	3+	–	Possible
22	Subacute	MM	X	3+	3+	2+	1+	–	Definite
23	Subacute	MM	–	3+	1+	2+	–	–	
24	Chronic	MM	IX X XII	3+	2+	2+	2+	–	Definite
25	Subacute	MM	–	2+	2+	3+	–	–	Definite
26	Chronic	MM	–	1+	1+	3+	1+	–	Possible
27	Subacute	MM	–	3+	3+	3+	–	–	
28	Subacute	MM	VI VII IX	3+	0	2+	–	–	
29	Chronic	MM	VII IX	3+	1+	2+	1+	–	
30	Acute	MM	III IV VI VII IX	3+	0	3+	–	–	Possible
Polyneuropathy									
31	Acute	PN	–	2+	0	3+	1+	–	Possible
32	Acute	PN	–	2+	0	1+	–	–	

MM = multiple mononeuropathy; PN = polyneuropathy; III = oculomotor nerve; IV = trochlear nerve; V = trigeminal nerve; VI = abducens nerve; VII = facial nerve. IX = glossopharyngeal nerve; X = vagus nerve; XII = hypoglossal nerve; 0 = absent; + = present.
1+, 2+, 3+ represent minimal, moderate, and severe involvement for muscle weakness and sensory disturbance.
*Acute = within 4 weeks; subacute = 4 weeks to 3 months; chronic = more than 3 months.
**Based on the European Federation of Neurological Societies/Peripheral Nerve Society Guideline on management of.
chronic inflammatory demyelinating polyradiculoneuropathy (Joint Task Force of the EFNS and the PNS, 2010).
***Patients 1 to 9 were pathologically-proven neurolymphomatosis, whereas Patients 10 to 15 were fluorodeoxyglucose-PET assessed neurolymphomatosis.

not caused by lymphomatous cells. These forms of neuropathies have been described as paraneoplastic neuropathies (Graus *et al.*, 2004; Koike *et al.*, 2011*a*), and thus, these patients were considered as having 'paraneoplastic neuropathy'.

The other 12 patients were not specifically classified as having neurolymphomatosis or paraneoplastic neuropathy, as the diagnostic pathological or radiological findings described above were not obtained. These patients were assigned to an 'unclassified' group. Ten of these patients (Patients 21–30) exhibited multiple mononeuropathy, whereas the other two patients (Patients 31 and 32) exhibited signs of symmetrical polyneuropathy (Supplementary Fig. 1).

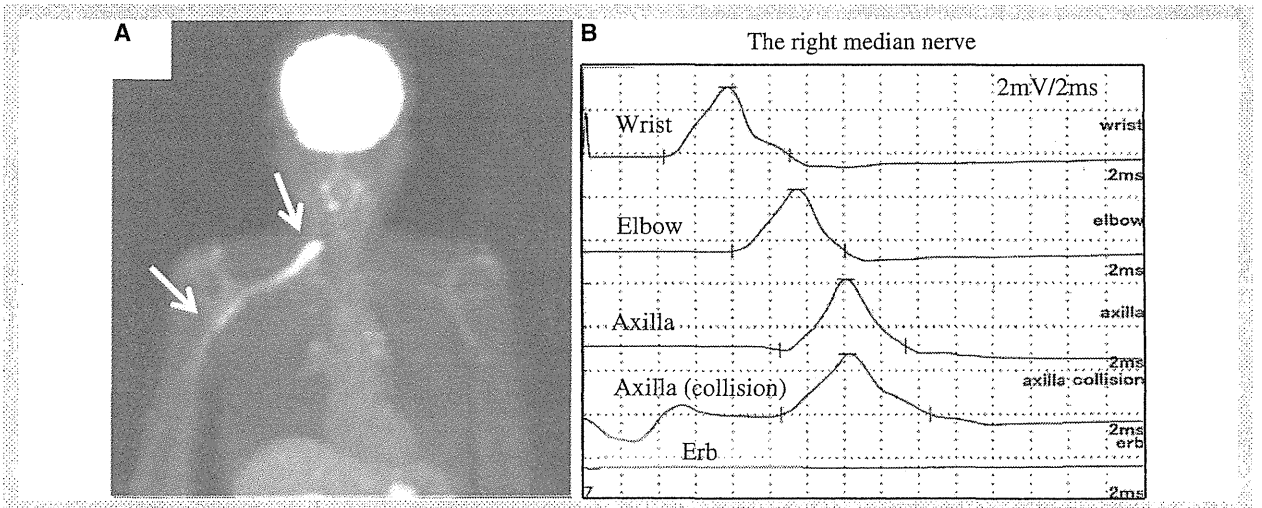

Figure 2 Findings of whole-body FDG-PET and nerve conduction studies in Patient 10, who had diffuse large B cell lymphoma. (**A**) Accumulation of FDG along the right brachial plexus (arrow) was observed. (**B**) A conduction block was present between the erb and the axilla, which was located between the proximal and distal portion of the nerve segments, with increasing FDG uptake.

Features of neurolymphomatosis

Pathologically-proven neurolymphomatosis

Nine patients (Patients 1–9) were pathologically confirmed as having neurolymphomatosis. The lymphoma in this group was primarily of the B cell type, as only two patients (Patients 5 and 7) exhibited the T cell type (Table 1). The most common type was diffuse large B cell lymphoma, which was present in six of nine patients. The mode of progression was subacute to chronic in these patients. The neuropathic features included local peripheral nerve involvement, such as multiple mononeuropathy in the extremities or cranial nerves in seven patients (Patients 1–7). The other two patients (Patients 8 and 9) manifested symmetrical polyneuropathy (Fig. 1 and Table 2). Spontaneous pain in the affected extremities was reported in six of nine patients. Three of these patients (Patients 1, 2 and 6) complained of severe pain that disrupted routine activities.

Nerve conduction studies revealed some degree of reduced compound muscle and sensory nerve action potentials in all of these patients (Supplementary Table 3), and demyelinating features were also concomitantly observed in some patients. Three of these patients (Patients 4, 8 and 9) met the EFNS/PNS electrodiagnostic criteria of definite CIDP (Joint Task Force of the EFNS and the PNS, 2010) (Table 2).

Sural nerve biopsy specimens were obtained from seven of these patients (Patients 1, 3, 4, 5, 7, 8 and 9) (Supplementary Table 1). These specimens showed a reduction of myelinated fibres to varying extents. A mild to moderate reduction of the unmyelinated fibre density was also observed in these specimens. Teased-fibre preparations revealed axonal degeneration with only minor demyelinating changes. Lymphomatous cell invasion was observed in five of these patients (Patients 3, 4, 5, 7 and 9). The regions of lymphomatous cell invasion were primarily observed in and around the perineurium, particularly the subperineurium, with extension into the inner space of the endoneurium. Epineurial lymphomatous cell invasion was also observed in one patient (Patient 9). In Patient 6, a biopsy of the tumour in the left sciatic nerve was performed, and the patient was diagnosed with lymphoma.

Autopsied specimens were obtained from four patients (Supplementary Table 2). An invasion of lymphomatous cells was observed in all of these patients (Figs 3 and 4) and was conspicuous in the proximal portions of the PNS, such as the nerve roots and proximal portions of the nerve trunks. The mode of lymphomatous cell invasion into the nerve was similar to that in the sural nerve biopsy specimens, as cell infiltration was prominent in and around the perineurium, particularly the subperineurium, with extension into the inner space of the endoneurium. Lymphomatous cells were observed along the pia mater of the spinal cord (Patient 1), but no invasion of these cells into the parenchyma was observed. The invasion of lymphomatous cells was not observed in the dorsal root ganglia, thoracic sympathetic ganglia, or spinal cord (Patients 2, 7 and 8).

Invading lymphomatous cells in the PNS of this group showed an obvious atypical cellular appearance (Fig. 3B), leading to a diagnosis of neurolymphomatosis. The diagnoses were also supported by evidence from additional immunohistochemical studies for each specific diagnosis of lymphoma (Supplementary Table 4). *In situ* hybridization of small ribonucleic acids of Epstein-Barr virus was performed in five of six cases with diffuse large B cell lymphoma (Asano *et al.*, 2009), and the results were negative (Supplementary Table 4).

Demyelination was observed at sites of lymphomatous cell invasion (Fig. 4). Nerves with demyelination were not in direct contact with lymphomatous cells. The direct attachment of lymphomatous cells to the Schwann cells of myelinated and

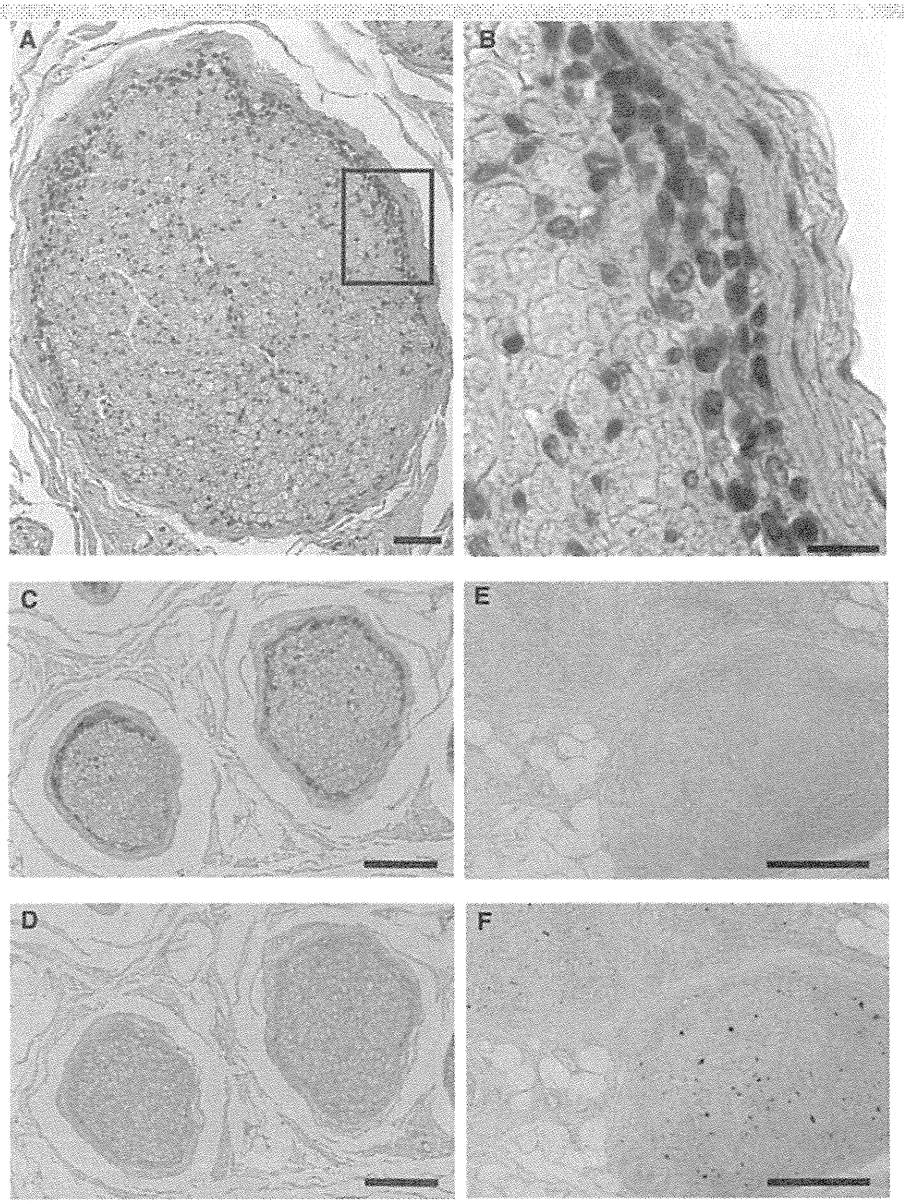

Figure 3 Sciatic nerve from Patient 1 with diffuse large B cell lymphoma. (**A**) Invasion of lymphomatous cells was primarily observed in the subperineurium through haematoxylin-eosin staining. Cross section. (**B**) In a high power view, invading cells exhibited atypical cellular appearance. (**C**) Lymphomatous cell invasion was observed in the proximal portion of the sciatic nerve after immunostaining with CD20 as a marker of B cell lymphoma. (**D**) Macrophages were not observed after immunostaining with CD68 in the proximal portion of the sciatic nerve with lymphomatous cell invasion. (**E**) Lymphomatous cell invasion was not observed after immunostaining with CD20 in the distal portion of the sciatic nerve with axonal degeneration. (**F**) Macrophages were abundantly observed after immunostaining with CD68 in the distal portion of the sciatic nerve, where axonal degeneration was observed. Scale bars: **A** = 50 μm; **B** = 20 μm; **C–F** = 200 μm.

unmyelinated fibres was not observed. Macrophages were not observed at demyelinating sites (Figs 3C–F and 4). In contrast, axonal degeneration was conspicuous in the distal portions of the nerve trunk. Axonal degeneration was observed without lymphomatous cell invasion in the nerve trunk distal from the sites of the lymphomatous cell invasion and demyelination. Rather, macrophages were abundantly present in the regions of the nerves where the axonal degeneration was conspicuous (Fig. 3F). These findings suggest that demyelination in lesions with lymphomatous cell invasion is not associated with macrophage infiltration but, rather, induces axonal degeneration with the infiltration of macrophages in the distal portion. These findings were similar in all four autopsied cases examined. A representative case is described in the Supplementary material.

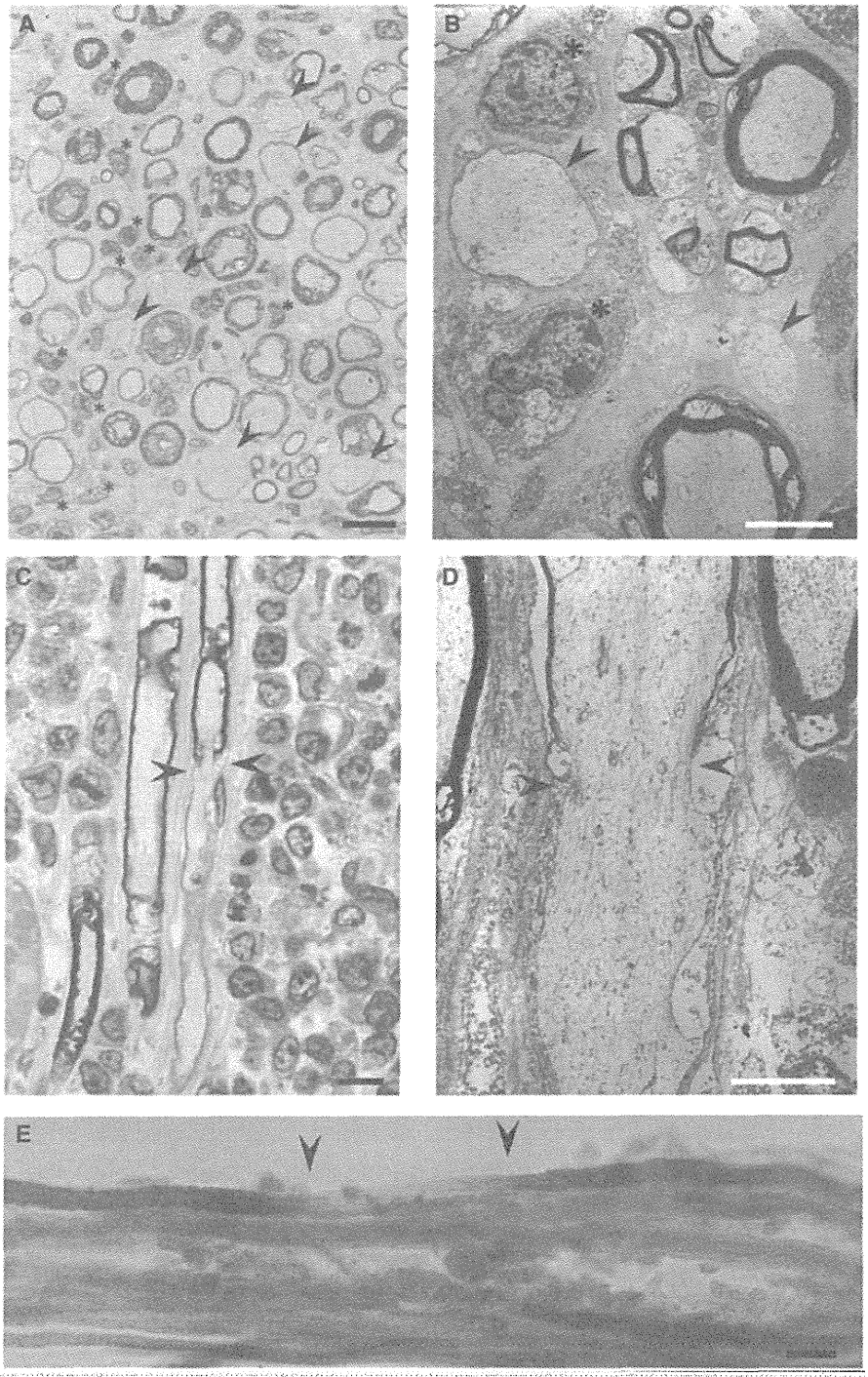

Figure 4 L4 anterior root from Patient 8 with Burkitt's lymphoma. (**A**) Demyelination (arrowhead) was observed near the infiltration of lymphomatous cells (asterisk) in toluidine blue-stained semi-thin cross sections. (**B**) Demyelination (arrowhead) near the infiltration of lymphomatous cells (asterisk) was confirmed through electron microscopy. Uranyl acetate and lead citrate stain. (**C**) The myelin sheath disappeared at the arrowhead in semi-thin longitudinal sections. Toluidine blue stain. Mononuclear cells with an atypical cellular appearance were abundant around the site of demyelination. (**D**) The disappearance of the myelin sheath was confirmed through electron microscopy (arrowhead). Uranyl acetate and lead citrate stain. (**E**) Demyelination was also observed at the site of lymphomatous cell infiltration in the teased-fibre study (arrowhead). Scale bars: **A** and **C** = 10 μm; **B** and **D** = 5 μm; **E** = 20 μm.

Neurolymphomatosis assessed through fluorodeoxyglucose positron emission tomography

All six patients (Patients 10–15) who exhibited an accumulation of FDG along the peripheral nerves on FDG-PET imaging had B cell lymphoma, and five patients had diffuse large B cell lymphoma (Table 1). The accumulation of FDG was observed at the brachial plexus in four patients (Patients 10, 11, 14 and 15) and at the lumbar plexus in two patients (Patients 12 and 13) (Table 1, Fig. 2A and Supplementary Fig. 2). In Patient 10, a conduction block was present between the erb and the axilla (Fig. 2B), located between the proximal and distal portions of nerve segments with increasing FDG uptake. This finding indicates that lymphoma cell invasion was present in the brachial plexus and that segmental demyelination was present at this lesion.

Neuropathic features were characterized according to the locality of the peripheral nerve involvement represented as multiple mononeuropathy in the extremities in all patients (Fig. 1 and Table 2). Three patients also manifested unilateral cranial nerve involvement. The mode of progression was subacute to chronic. Spontaneous pain in the affected extremities was reported in all patients. Nerve conduction studies revealed some degree of axonal features in all patients, but demyelinating features were also conspicuously observed in two of these patients (Patients 10 and 15), fulfilling the EFNS/PNS electrodiagnostic criteria of definite CIDP (Joint Task Force of the EFNS and the PNS, 2010) (Supplementary Table 3). The clinical and electrophysiological features were similar to those of pathologically-proven neurolymphomatosis. A representative case is described in the Supplementary material.

Features of paraneoplastic neuropathy

CIDP-type neuropathy

Three patients (Patients 16–18) categorized as 'paraneoplastic CIDP-type' showed subacute to chronic sensorimotor polyneuropathy with a symmetrical manifestation (Fig. 1 and Table 2). In contrast to the patients suspected as having neurolymphomatosis, none of these patients had diffuse large B cell lymphoma, and two of the three patients had T cell lymphoma (Table 1). The electrophysiological features revealed a marked prolongation of the distal latency and a reduction of the conduction velocity (Supplementary Table 3). Two patients (Patients 17 and 18) fulfilled the EFNS/PNS electrodiagnostic criteria of definite CIDP (Joint Task Force of the EFNS and the PNS, 2010). In addition, a reduction of compound muscle action potential was observed in these patients. The sural nerve biopsy findings of these patients revealed extensive segmental demyelination in the teased-fibre preparations (21, 31 and 34% for Patients 16, 17 and 18, respectively) without lymphomatous cell invasion (Supplementary Table 1). Axonal degeneration with a reduction of the myelinated fibre density was also conspicuous in Patients 16 and 17 (58 and 43%, respectively). However, the reduction of unmyelinated fibres was not conspicuous relative to the loss of myelinated fibres. FDG-PET was performed in Patient 17, and no invasion into the PNS was observed. A representative case is described in the Supplementary material.

Sensory ganglionopathy

A patient (Patient 19) with diffuse large B cell lymphoma manifested sensory ataxia in the extremities arising from proprioceptive and kinaesthetic sensory loss. The electrophysiological features revealed a reduction of sensory nerve action potentials (Supplementary Table 3). The sural nerve biopsy findings revealed a predominant large-fibre loss (Supplementary Table 1). In addition, motor nerve conduction studies revealed findings suggestive of demyelination, consistent with previous reports (Camdessanché *et al.*, 2002). The autopsy revealed a loss of neurons in the dorsal root ganglia with the preservation of motor neurons in the spinal cord and an absence of lymphomatous cell invasion into the PNS (Supplementary Table 2).

Vasculitic neuropathy

A patient (Patient 20) with diffuse large B cell lymphoma, categorized as having 'paraneoplastic vasculitic neuropathy', showed subacute symmetrical sensorimotor polyneuropathy (Fig. 1 and Table 2). The electrophysiological features suggested predominant axonal neuropathy (Supplementary Table 3). The sural nerve biopsy findings revealed an occlusion of small vessels in the epineurium with inflammatory cellular infiltration without an atypical cellular appearance (Supplementary Table 1).

Features of unclassified group

Multiple mononeuropathy

All 10 patients (Patients 21–30) in this group had B cell lymphoma, and seven patients had diffuse large B cell lymphoma (Table 1). Neuropathic features were characterized by the locality of the peripheral nerve involvement, as represented by multiple mononeuropathy in the extremities or unilateral cranial nerve involvement (Table 2 and Supplementary Fig. 1). Spontaneous pain in the affected extremities was reported in five patients. Patient 21 complained of severe pain significantly disrupting daily activities, which is similar to the pain experienced by patients with neurolymphomatosis. In addition, the cytology of the CSF was positive in Patient 28. Nerve conduction studies revealed some degree of reduction of compound muscle action potentials and sensory nerve action potentials in all of these patients (Supplementary Table 3), and demyelinating features were also concomitantly observed in some patients. Three patients (Patients 22, 24 and 25) fulfilled the EFNS/PNS electrodiagnostic criteria of definite CIDP (Joint Task Force of the EFNS and the PNS, 2010). Clinical and electrophysiological features of patients in this group were more similar to those of neurolymphomatosis than those of paraneoplastic neuropathy, except for Patient 30, who manifested acutely progressive sensorimotor neuropathy compatible with the definition of Guillain-Barré syndrome (Asbury and Cornblath, 1990).

Sural nerve biopsy specimens were obtained from seven of 10 patients (Supplementary Table 1). These specimens revealed a reduction of myelinated fibres to varying extents. A moderate to severe reduction of unmyelinated fibre density was also observed. Teased-fibre preparations revealed axonal degeneration with few demyelinating changes. Lymphomatous cell invasion was not

observed in these cases. In Patient 25, several fibres with segmental demyelination at consecutive nodes of Ranvier were observed. Considering the concomitant observation of axonal degeneration, demyelination in these fibres was considered secondary to axonal atrophy rather than primary demyelination (Dyck *et al.*, 1981). A representative case is described in the Supplementary material.

Polyneuropathy

These patients (Patients 31 and 32) had T cell lymphoma (Table 1). Both patients showed a symmetrical polyneuropathy pattern, and their electrophysiological features were characterized by axonal involvement (Table 2, Supplementary Fig. 1 and Supplementary Table 3). The mode of neuropathy progression was acute in both patients, and Patient 31 became unable to walk within 1 month, mimicking the course of Guillain-Barré syndrome. Paraneoplastic aetiology was suspected in this patient, whereas the invasion of lymphomatous cells may have been present in Patient 32, as the cytology of the CSF was positive.

Treatments and outcomes

The details of treatments and outcomes are listed in Table 3. Immunomodulatory treatment was administered in nine of 15 patients (Patients 1–3, 5, 7, 9 and 13–15) diagnosed as having neurolymphomatosis. As the diagnosis of lymphoma was delayed in seven patients (Patients 1, 3, 5, 7 and 13–15), immunomodulatory treatment for neuropathy was performed before antineoplastic therapies were administrated. In Patients 2 and 9, immunomodulatory treatments were performed after chemotherapy. Intravenous immunoglobulin (IVIg; 400 mg/kg/day for 5 days) and high-dose intravenous methylprednisolone (1000 mg/day for 3 days) were administered alone or in combination. One of seven patients responded to the IVIg therapy, whereas six of the seven patients responded to some degree to the intravenous methylprednisolone. Plasma exchange was performed in Patient 7, which was slightly effective. However, the effects of these treatments were only partial and transient; therefore, repetitive treatments were needed, and most patients eventually deteriorated despite the multiple treatments.

Among patients diagnosed as having paraneoplastic neuropathy, two of the three patients, with features of CIDP (Patients 17 and 18), were administered an immunomodulatory treatment, and these patients responded well. One patient (Patient 17) was treated with IVIg, and the other (Patient 18) was treated with intravenous methylprednisolone. A patient with sensory ganglionopathy (Patient 19) also responded temporarily to immunomodulatory treatments.

For the 10 patients categorized as having 'unclassified multiple mononeuropathy', IVIg or intravenous methylprednisolone was administered alone or in combination. Two of five patients treated with IVIg therapy responded to the treatment, and two of five patients treated with intravenous methylprednisolone therapy responded to the treatment. Among the patients categorized as having 'unclassified polyneuropathy', the response to immunomodulatory treatment was favourable in Patient 31, although the recurrence of similar episodes was frequent (four times within 25 months).

Overall, the response to the immunomodulatory treatments was better in patients with paraneoplastic neuropathy than in those with neurolymphomatosis. The response of the patients with 'unclassified multiple mononeuropathy' appeared to be similar to that of patients with neurolymphomatosis rather than those with paraneoplastic neuropathy.

The effect of chemotherapy on neuropathy was assessed in 21 patients. In 13 of the 21 patients treated with chemotherapy, the neurological deficits improved after the chemotherapy for lymphoma. The effect was positive in eight of the 14 patients with neurolymphomatosis, in one of the two patients with 'paraneoplastic CIDP-type', and in two of the three patients with 'unclassified multifocal mononeuropathy'. Intrathecal chemotherapy was administered in eight patients (Patients 2, 5, 8, 11 to 13, 24 and 32). The 5-year overall survival rate was 48%. In the patients with diffuse large B cell lymphoma, the 5-year overall survival rate was 39%.

Spontaneous pain was reported particularly in patients diagnosed as having neurolymphomatosis. Six patients (Patients 1, 2, 6, 13, 15 and 21) complained of severe pain that significantly disrupted daily activities. Symptomatic therapies for pain included the oral administration of antiepileptic drugs or tricyclic antidepressants, such as carbamazepine, clonazepam, gabapentin, or imipramine, which showed only partial effects. Sacral root block and epidural block were performed in Patients 1 and 21, respectively. Opiates were used in four patients (Patients 2, 6, 13 and 15).

Discussion

The pathogenesis of neuropathy in the patients with lymphoma is considered diverse. Neuropathies associated with lymphoma are broadly divided into neurolymphomatosis and paraneoplastic neuropathies (Vital *et al.*, 1990; Vallat *et al.*, 1995; Viala *et al.*, 2008; Grisariu *et al.*, 2010; Briani *et al.*, 2011; Baehring and Batchelor, 2012). Thus far, in patients showing multiple mononeuropathies, distal axonopathy arising from the direct invasion of lymphoma cells into the nerve trunk or vasculitis associated with paraneoplastic syndrome has been postulated to cause neuropathy, whereas demyelinating changes arising from humoral factors have been suspected as the major cause of neuropathy in patients showing a symmetric polyneuropathy pattern (Viala *et al.*, 2008). However, the electrophysiological study presented here indicated a more complex mixture of demyelinating and axonal changes in both the multiple mononeuropathy and symmetric polyneuropathy patterns.

In our study, cases with multiple mononeuropathy were more frequent than those with symmetrical polyneuropathy, constituting 23 of 32 (72%) cases. In these cases, 13 patients (57%) were diagnosed as having neurolymphomatosis. Among the other 10 patients manifesting multiple mononeuropathy, the similarities of the clinical and electrophysiological features to those diagnosed as neurolymphomatosis might suggest that the mechanisms underlying neurolymphomatosis cases play a major role in these patients, although we cannot completely rule out the possibility of the participation of paraneoplastic mechanisms in these patients. Patients with pathologically-proven neurolymphomatosis were also included in

Table 3 Treatments and outcomes

Patients	Effect of immunotherapy on neuropathy			Therapy for lymphoma		Effect of chemotherapy on neuropathy	mRS			Follow up duration (months)
	IVIg	Steroid	PE	Before neuropathy	After neuropathy		At admission	Afer treatment	Long-term outcome	
Neurolymphomatosis*										
1	ND	1+	ND		CHOP	1+	3	3	Dead	14
2	–	1+	ND	CHOP	Rituximab CHOP MTX** CHASE	–	3	3	Dead	7
3	2+	2+	ND		R-CHOP CODOX-M MTX	2+	5	4	4	75
4	ND	ND	ND	MTX R-CHOP	MTX AraC PSL Radiation	2+	3	2	Dead	65
5	–	ND	ND		AraC** MTX** PSL**	ND	4	4	Dead	30
6	ND	ND	ND		CHOP R-DeVIC THP-COP	2+	3	1	1	108
7	–	1+	1+		VP16	2+	4	3	Dead	91
8	ND	ND	ND	CVAD	MTX** AraC** Rituximab CPM Dexamethasone**	–	3	3	Dead	7
9	–	ND	ND	R-CHOP	IMVP-16/CBDCA CHASER	–	4	4	Dead	36
10	ND	ND	ND	R-CHOP	Rituximab DeVIC	2+	3	2	2	84
11	ND	ND	ND	R-CHOP	R-DeVIC MTX** PSL**	2+	3	2	1	69
12	ND	ND	ND	EPOCH rituximab	MTX*	–	4	4	Dead	12
13	ND	2+	ND		MTX** AraC** Dexamethasone** Radiation	–	3	2	Dead	9
14	–	2+	ND		R-CHOP	2+	3	2	2	29
15	–	–	ND		R-CHP CHASER MTX DeVIC	–	3	4	Dead	33
Paraneoplastic neuropathy CIDP type										
16	ND	ND	ND	CHOP VP16 MST16 PCZ	MST16 VP16 PSL L-PAM	–	4	5	Dead	80
17	2+	ND	ND		Rituximab CPM PSL	2+	4	3	3	46
18	ND	2+	ND	INFγ PUVA Vorinostat		ND	4	3	Dead	132
Sensory ganglionopathy										
19	2+	2+	2+			ND	5	3	Dead	92

(continued)

Table 3 Continued

Patients	Effect of immunotherapy on neuropathy			Therapy for lymphoma		Effect of chemotherapy on neuropathy	mRS			Follow up duration (months)
	IVIg	Steroid	PE	Before neuropathy	After neuropathy		At admission	Afer treatment	Long-term outcome	
Vasculitic neuropathy										
20	ND	ND	ND	CHOP		ND	4	ND	ND	24
Unclassified										
Multiple mononeuropathy										
21	1+	1+	ND		CODOX-M/IVAC R-CHOP CHASER PBSCT	–	2	2	Dead	39
22	2+	ND	ND			ND	5	4	Dead	11
23	–	ND	ND			ND	3	4	Dead	3
24	ND	1+	ND		MTX** AraC** Dexamethasone** R-CHOP	1+	3	3	Dead	41
25	–	–	ND	Rituximab THP-COP	Rituximab	ND	4	4	4	68
26	ND	ND	ND	R-CHOP F-ara-A MIT		ND	4	ND	ND	58
27	ND	–	ND	CHOP		ND	4	4	5	70
28	ND	ND	ND			ND	3	5	Dead	7
29	–	–	ND			ND	4	5	Dead	5
30	ND	ND	ND		CHOP	1+	ND	ND	ND	2
Polyneuropathy										
31	2+	2+	ND		MTX PCZ VCR AraC CHOP PBSCT	2+	3	2	2	129
32	1+	1+	ND		MTX** AraC** PSL** R-CHOP BMT	2+	2	1	1	120

AraC = Cytarabine; BMT = bone marrow transplantation; CDOX-M = Cyclophosphamide, Vincristine, Doxorubicin, Methotrexate; CDOX-M/IVAC = CDOX-M + Etoposide, Ifosfamide, Cytarabine; CHASE = Cyclophosphamide, Cytarabine, Etoposide, Dexamethasone; CHASER = CHASE + Rituximab; CHOP = Cyclophosphamide, Doxorubicin, Vincristine, Prednisolone; CPM = Cyclophosphamide; CVAD = Cyclophosphamide, Vincristine, Doxorubicin, Dexamethasone; DeVIC = Dexamethasone, Etoposide, Ifosfamide, Carboplatin; EPOCH = Etoposide, Prednisolone, Vincristine, Cyclophosphamide, Doxorubicin; F-ara-A = Fludarabine; INFγ = Interferon γ; IMVP-16/CBDCA = Ifosfamide, Methotrexate, Etoposide, Carboplatin; L-PAM = Melphalan; PE = plasma exchange; MIT = Mitoxantrone; MST16 = Perazolin; MTX = Methotrexate; ND = not done; PBSCT = peripheral blood stem cell transplantation; PCZ = Procarbazine; PUVA = psoralen + ultraviolet A; PSL = Prednisolone; R-CHOP = Rituximab + CHOP; R-CHP = Rituximab, Cyclophosphamide, Doxorubicin, Prednisolone; R-DeVIC = Rituximab + DeVIC; THP-COP = Pirarubicin, Cyclophosphamide, Vincristine, Prednisolone; VCR = Vincristine; VP16 = Etoposide.

1+ and 2+ represent slight and good response to treatment, respectively.

*Patients 1 to 9 were pathologically-proven neurolymphomatosis, whereas Patients 10 to 15 were FDG-PET assessed neurolymphomatosis.

**Therapy was done intrathecally.

two of the nine cases manifesting symmetrical polyneuropathy. Therefore, patients who showed asymmetrical features in the early phase might develop the symmetrical polyneuropathy type as the disease advances. In this context, neurolymphomatosis might play a more important role in neuropathy associated with lymphoma than previously suggested. Sural nerve biopsy is limited for the diagnosis of neuropathy associated with lymphoma, as biopsy assesses only a distal portion of the PNS (van den Bent *et al.*, 1999). Notably, only one patient with sensory ganglionopathy, which is the most common form of paraneoplastic neuropathy (Oki *et al.*, 2007; Koike *et al.*, 2011*a*), was included among five patients with pathologically-proven paraneoplastic neuropathy. However, because this is a retrospective study and only patients who were referred to the neurological department of our institute were included, there was a sample bias.

The characteristic findings that explain the cause of neuropathy in most patients included lymphoma cell invasion-associated demyelination and axonal degeneration of the distal portion of the nerve trunk. The invasion of lymphomatous cells into the nerve trunk has been designated as neurolymphomatosis and is reported as a characteristic feature of neuropathy associated with lymphoma (Guberman *et al.*, 1978). In our cases, lymphomatous cells invaded in and around the perineurium, particularly the subperineurium. Because the lymphatic flow is preferentially present in the subperineurial space, this area has an affinity for lymphomatous cells. In addition, the lymphomatous cell invasion continued into the endoneurium. Demyelination was observed at the site of lymphoma cell invasion, and axonal degeneration occurred distal from the site of lymphoma cell invasion. Therefore, demyelination at the site of neurolymphomatosis might be the primary lesion, with axonal degeneration occurring secondarily in most patients with lymphoma-associated neuropathy. This view is supported through electrophysiological findings showing a mixture of demyelinating and axonal changes and sural nerve biopsy findings showing predominant axonal degeneration in the distal portion of the nerves. The demyelination observed in the present study was different from that in acute inflammatory demyelinating polyneuropathy or CIDP, as macrophage-mediated demyelination was not observed in our patients (Prineas and McLeod, 1976). Because lymphomatous cells do not directly contact myelinated fibres or Schwann cells, humoral factors produced by the lymphoma cells close to the myelinated fibres likely play an important role. Future studies are needed to identify the humoral factors that mediate this demyelination. In addition to the reduction of the myelinated fibres, a reduction of unmyelinated fibres was observed. Therefore, primary axonal changes might also occur, to some extent, even in patients with neurolymphomatosis. Considering the fairly well-preserved nerve fibres at the site of demyelination, most axonal changes might secondarily occur distally from the site of the lymphomatous cell invasion. Interestingly, proximal demyelination with distal axonal changes, which is considered as the most common pathogenesis of neuropathy associated with lymphoma, was observed in both B cell and T cell types of lymphoma, although most of the patients had B cell lymphoma. It is also interesting that even patients with diffuse large B cell lymphoma, which was the most common type of lymphoma in the present study, showed a diverse neuropathic presentation, including neurolymphomatosis as well as paraneoplastic demyelination and vasculitis.

The difficulty of the diagnosis of lymphoma-associated neuropathy deserves attention. Neuropathy can occur at any stage of lymphoma, and neuropathic symptoms preceded the detection of lymphoma in approximately half of our patients. Therefore, elucidating the characteristics of neuropathy associated with lymphoma is important because it will enable the early detection of lymphoma and the immediate initiation of therapy for both the lymphoma and the neuropathy itself. The diagnostic pitfalls elucidated in this study include the scarcity of laboratory evidence suggestive of the presence of lymphoma, demyelinating features in the electrophysiological study, and the possible response to immunomodulatory therapies. These features resulted in the physicians recalling the diagnosis of Guillain-Barré syndrome or CIDP in some of the patients, as described in the case presentation (Supplementary material). Indeed, 11 patients in the present study fulfilled the EFNS/PNS electrodiagnostic criteria of definite CIDP (Joint Task Force of the EFNS and the PNS, 2010). However, patients with neurolymphomatosis in our study were characterized by a focality of neuropathic involvement even though the electrophysiological features fulfilled the EFNS/PNS criteria for CIDP, which is considered atypical (Joint Task Force of the EFNS and the PNS, 2010). In addition, the frequent presence of spontaneous pain in our patients would be suggestive of diseases other than CIDP, as pain is not common in CIDP (Nasu *et al.*, 2012). Pain has also been frequently reported in patients with neurolymphomatosis (Grisariu *et al.*, 2010; Baehring and Batchelor, 2012).

Currently, FDG-PET is the most sensitive and specific imaging technique available for patients with lymphoma (Cheson, 2011). In our study, a whole-body FDG-PET was useful for the detection of lymphoma in five of the six patients with lymphoma not observed through conventional means. With respect to patients previously diagnosed as having lymphoma, five of the six patients were positive in the FDG-PET study. Therefore, we should consider that FDG-PET does not necessarily reveal lymphoma in all patients. Case reports suggested the usefulness of this technique in the detection of neurolymphomatosis even in patients with negative MRI, CSF, or bone marrow analysis findings (Salm *et al.*, 2012). However, the specificity of FDG-PET study in the diagnosis of neurolymphomatosis (e.g. differentiation from neurolymphomatosis to CIDP) has not yet been established. In our study, FDG-PET was performed in a patient with paraneoplastic CIDP-type neuropathy (Patient 17). In this patient, no accumulation of FDG was observed in the PNS, although the presence of lymphoma was detected in the iliopsoas muscle.

Serum-soluble interleukin-2 receptor levels offer a rapid, reliable and non-invasive measure of disease activity and response to therapy in a broad spectrum of conditions associated with B or T cell immune activation, including lymphoma (Rubin and Nelson, 1990). Therefore, interleukin-2 receptor is used as an indicator for lymphoma in clinical practice, although it is not specific. Seventeen of the 24 examined patients (71%) showed elevated levels of serum soluble interleukin-2 receptor. Therefore, a high level of serum-soluble interleukin-2 receptor might lead to a suspicion of the concomitance of lymphoma in a patient with

neuropathy, although a normal level does not exclude the possibility of lymphoma.

Immunomodulatory treatment was more or less effective, particularly in the early phase of neuropathy, even in patients with neurolymphomatosis, potentially leading to the misdiagnosis of the cause of neuropathy. Because antineoplastic therapy is essential for the treatment of lymphoma, early diagnosis is necessary for neuropathy associated with lymphoma. The presence of the high degree of axonal loss irrespective of the type of neuropathy might also support this view. However, the response to the immunomodulatory treatments appeared to be better in patients with paraneoplastic neuropathies than in those with neurolymphomatosis; thus, immunomodulatory treatment before, during, or after antineoplastic therapy might also be beneficial for paraneoplastic neuropathy. The outcome determined by the 5-year survival rate in patients with non-Hodgkin's lymphoma with CNS involvement is generally poor, ranging from 14–20% (Hollender *et al.*, 2000; Kridel and Dietrich, 2011). In contrast, the outcome of neuropathy associated with lymphoma was better in our study, although the outcome was poorer than that for lymphoma overall (The Non-Hodgkin's Lymphoma Classification Project, 1997; Feugier *et al.*, 2005).

In conclusion, patients with lymphoma can manifest various neuropathic patterns, and neurolymphomatosis is the major cause of neuropathy. Demyelination unrelated to macrophages at the site of lymphomatous cell invasion and axonal degeneration distal from the site of the lymphomatous cell invasion were the most prominent pathologies of this type of neuropathy. The misdiagnosis of neurolymphomatosis as CIDP is frequent due to the presence of a demyelinating pattern and the initial response to immunomodulatory treatments in patients with neurolymphomatosis. The possibility of the concomitance of lymphoma should be actively considered in various types of neuropathy even if the neuropathy meets the diagnostic criteria of CIDP, particularly in patients complaining of pain.

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Supplementary material

Supplementary material is available at *Brain* online.

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RESEARCH PAPER

Spreading of amyotrophic lateral sclerosis lesions—multifocal hits and local propagation?

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ABSTRACT

Objective To investigate whether or not the lesions in sporadic amyotrophic lateral sclerosis (ALS) originate from a single focal onset site and spread contiguously by prion-like cell-to-cell propagation in the rostrocaudal direction along the spinal cord, as has been hypothesised (the 'single seed and simple propagation' hypothesis).

Methods Subjects included 36 patients with sporadic ALS and initial symptoms in the bulbar, respiratory or upper limb regions. Abnormal spontaneous activities in needle electromyography (nEMG)—that is, fibrillation potentials, positive sharp waves (Fib/PSWs) or fasciculation potentials (FPs)—were compared among the unilateral muscles innervated by different spinal segments, especially between the T10 and L5 paraspinal muscles, and between the vastus medialis and biceps femoris. Axon length and the proportion of muscle fibre types, which are both related to motoneuronal vulnerability in ALS, are similar in the paired muscles.

Results Fourteen of 36 patients showed a non-contiguous distribution of nEMG abnormalities from the onset site, with skipping of intermediate segments. In eight of them, the non-contiguous pattern was evident between paired muscles with the same motoneuronal vulnerability. The non-contiguously affected lumbosacral lesions involved motoneuron columns horizontally or radially proximate to one another, appearing to form a cluster in four of the eight patients. FPs, known to precede Fib/PSWs, were shown more frequently than Fib/PSWs in all the lumbosacral segments but L5, suggesting that 2nd hits occur at L5 and then spread to other lumbosacral segments.

Conclusions In sporadic ALS, the distribution of lower motoneuron involvement cannot be explained by the 'single seed and simple propagation' hypothesis alone. We propose a 'multifocal hits and local propagation' hypothesis instead.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an incurable progressive neurodegenerative disease in which both the upper (UMN) and lower motoneurons (LMN) are diffusely involved at the end. Recent biological studies have demonstrated the remarkable concept of 'prion-like propagation' of pathogenic proteins, such as tau or α-synuclein, in neurodegenerative diseases.[1 2] According to this hypothesis, the pathogenic proteins are transferred from diseased cells to neighbouring healthy cells; this intercellular transfer then leads to spreading of the lesion. In ALS, in vitro studies have indicated that newly formed aggregates of SOD1, TDP-43 or toxic RNA conformation can act as templates for the subsequent misfolding of the respective native proteins,[3–5] and that aggregated SOD1 can be intercellularly transferred in cultured cells.[6] These suggest that the mechanism of prion-like cell-to-cell propagation also underlies the progression of ALS.

The clinical symptoms of most ALS patients start focally, which had already been confirmed both electrophysiologically[7] and pathologically.[8 9] As we have reviewed in the previous article,[10] recent clinical observations have demonstrated that the clinical symptoms spread contiguously from the onsets into the following broadly divided body regions: the bulbar region, upper limbs, trunk and lower limbs.[11–14] This has prompted us to suppose that ALS lesions simply propagate from a single 'seed' to adjacent cells in a domino-like manner (ie, the 'single seed and simple propagation' hypothesis). Alternatively, it can rest on anatomical proximity with the spreading of ALS lesion from the onset site by diffusion of soluble toxic factors in the extracellular matrix.[15] On the other hand, up to about 30% of sporadic ALS patients have also been found to show non-contiguous spread of symptoms from the bulbar region to the lower limbs or vice versa, skipping the upper limbs and trunk.[14 16] However, compensatory re-innervation by the remaining motoneurons can mask the manifestation of clinical signs until more than one-third of the LMNs for a given muscle are lost.[17] Therefore, whether the lesions actually spread non-contiguously among the spinal segments remains unclear.

Needle electromyography (nEMG) can sensitively detect LMN involvement from each segment separately, even in the presymptomatic stage. For this reason, it is a powerful method for investigating whether or not ALS lesions spread contiguously along the spinal segments. In this study, we used nEMG in the early stage of ALS to demonstrate that LMN involvement cannot be necessarily explained by the 'single seed and simple propagation' hypothesis. We then propose a hypothesis of 'multifocal hits and local propagation.'

SUBJECTS AND METHODS

Subjects

We designed this study to investigate whether LMN involvement in sporadic ALS spreads contiguously in the rostrocaudal direction from the onset site. Therefore, of 66 consecutive patients with suspected ALS referred to our hospitals from March 2011 to April 2012, 14 patients with lower limb onset were excluded. One patient with a family history of ALS was also excluded. Forty-two of the remaining 51 patients met the revised El Escorial criteria[18] for clinically definite, clinically probable or clinically probable laboratory-supported ALS, although two patients were excluded because their MRIs indicated lumbar spinal disease, which can influence the results of nEMG. Thus, 40 sporadic ALS patients with bulbar, upper limb, or respiratory symptoms at onset were ultimately included in this study. None of these 40 patients had diabetes or any other complicating neuropathies, which were confirmed by nerve conduction studies (performed on their unilateral median, ulnar, tibial, peroneal and sural nerves).

Selection of muscles to be examined

Motoneurons with longer axons,[19 20] larger motoneurons[9] and fast-fatigable motoneurons[21] have been described as more vulnerable to damage from ALS. If the pathological process begins at the same time in individual motoneurons with different degrees of vulnerability, then motoneurons that are more vulnerable will degenerate faster than those that are more resistant. Thus the pattern of nEMG abnormalities should be influenced by differences in motoneuronal vulnerability. Therefore, to establish adequate milestones for lesion spreading, we selected two pairs of muscles innervated from different spinal segments but with similar degrees of motoneuronal vulnerability; that is, the length of the innervating motor axons and the ratio of type I muscle fibres differ little between the paired muscles (see online supplementary figure S1). One pair—T10 paraspinalis (T10PS; type I fibre ratio: 62.0% in men, 67.8% in women) and L5 paraspinalis (L5PS; 63.6–65.0%)—was selected from the trunk.[22] The other pair—the deep layer of the vastus medialis (VM) (innervating segment: L3/4; type I fibre ratio: 61.5%) and the long head of the biceps femoris (BF; L5/S1, mainly S1; 66.9%)—was selected from the thigh.[23–27]

If a focal ALS lesion spreads contiguously in the rostrocaudal direction along the spinal segments, nEMG abnormalities in the paired muscles should be found in the muscle innervated by the rostral segment earlier than in the muscle innervated by the caudal segment (the 'contiguous pattern' in online supplementary figure S1). On the other hand, if the abnormalities are observed only in the muscle innervated by the caudal segment while the muscle of the rostral segment remains intact (the 'non-contiguous (skipping) pattern' in online supplementary figure S1), the results cannot be attributed to differences in motoneuronal vulnerability. We also examined the first dorsal interosseous (FDI; mainly innervating segment: C8), L3 paraspinalis (L3PS), rectus femoris (RF; L3/4), tibialis anterior (TA; L4/5, mainly L5) and medial head of the gastrocnemius (GC; S1/2, mainly S1).[23–26]

Needle electromyography

Spontaneous EMG activities were detected with a conventional concentric needle electrode (recording surface area: 0.3 mm^2) in the above-mentioned muscles on the ipsilateral side of symptom onset in the upper limb onset patients and on the right side in the patients with bulbar or respiratory onset. For evaluation of paraspinal muscles, we examined the multifidus muscles, which are innervated by a single segment.[28]

Fibrillation potentials and positive sharp waves (Fib/PSWs) were explored at 10 different sites in each muscle. Fib/PSWs were diagnosed to be pathological only when they were identified at more than two different sites within the muscle. The fasciculation potential (FP) was defined as a potential that was similar in shape to the motor unit potential (MUP) and fired in a highly irregular pattern, often with a clustering of discharges. We identified FPs only when potentials of the same shape appeared at least twice. To detect FPs, we observed spontaneous activity at one site in each muscle for 60–90 s, which is sufficiently long enough to confirm the reproducibility of FPs.[29] Any persistence of voluntary MUPs was considered to render the identification of FPs impossible. We considered the examined muscles to be involved if Fib/PSWs, FPs or both were observed. Considering their higher objectivity beyond multicentre and burdens of patients, only spontaneous activities were adopted to prove LMN involvements in this study.

All EMG examinations were performed by proficient electromyographers with at least 5 years of professional EMG experience (TS, TK, KS and YN).

Data analysis

Frequencies of the presence of abnormal spontaneous EMG activity were compared among the examined muscles by performing multiple comparisons with Fisher's exact probability test and the p value adjustment method of Holm. p Values less than 0.05 were considered to be significant.

Standard protocol approvals, registrations and patient consents

The local ethics committees of Tokyo Medical and Dental University School of Medicine, Chiba University Graduate School of Medicine, Kyoto Prefectural University of Medicine, Musashino Red Cross Hospital, Kanto Central Hospital and Nakano General Hospital approved this study. All patients gave us informed consents for the procedures.

RESULTS

Of the 40 patients with sporadic ALS included in this study, we ultimately analysed data from 36 patients (23 men, 13 women) because sufficient data for the paired paraspinal and thigh muscles were not obtained in 4 patients. The ages of 36 patients ranged from 41 years to 79 years (mean 63.3). The diagnoses were definite ALS in 8 patients, probable in 14 and probable-laboratory-supported in 14 according to the revised El Escorial criteria. Symptom onset occurred in the bulbar region in 10 patients, in the upper limb in 25 patients and as respiratory symptoms in 1 patient. The mean duration from symptom onset to the nEMG study was 16.9 months (range 3–84).

The full nEMG data for the 36 patients are shown in figure 1, online supplementary figure S2A,B. Abnormal spontaneous EMG activity was present in the FDI of all 36 patients. The distribution patterns of nEMG abnormalities among the spinal segments could be divided into three types: diffuse, contiguous and non-contiguous (skipping) patterns. The diffuse pattern was observed in 19 patients (53%); of these, 13 (patients 1–13) showed abnormal nEMG findings at all examined muscles and 6 (patients 14–19) showed abnormalities at every examined spinal segment, although not at all muscles. The contiguous pattern was found in three patients (8.3%; patients 20–22) in whom abnormal findings were detected in all examined segments except S1—the most remote segment from the onset site. One

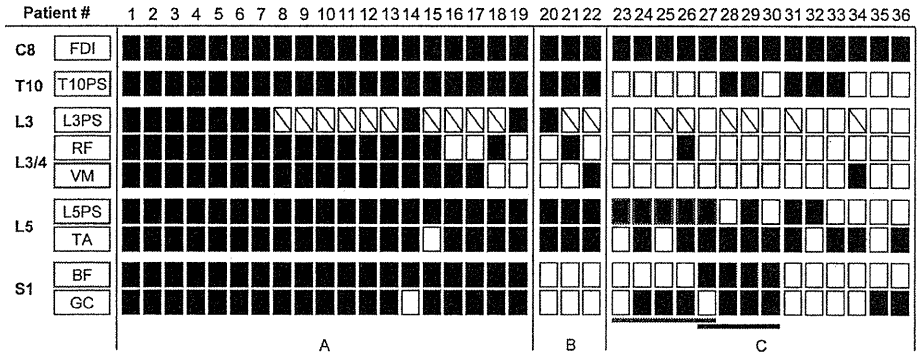

Figure 1 Distribution patterns of needle electromyography (nEMG) abnormality in all patients. Closed squares: abnormal spontaneous EMG activity present. Open squares: abnormal spontaneous EMG activity absent. Squares with oblique line: data not available. (A) Diffuse pattern. (B) Contiguous pattern. (C) Non-contiguous (skipping) pattern. Note that the rostrally absent and caudally present spontaneous activity pattern between paired muscles with the same motoneuronal vulnerability is evident in the paraspinal muscle pair (green, patients 23–27) and the thigh muscle pair (red, patients 27–30) in 8 of 14 patients with the skipping pattern. The non-contiguous (skipping) pattern was present in both muscle pairs in patient 27.

of these three patients (patient 22) also showed the contiguous pattern in the thigh muscle pair; that is, an abnormality was evident in VM but not in BF. The non-contiguous (skipping) pattern was found in 14 patients (39%; patients 23–36), in whom abnormal spontaneous activities were detected from C8 to more caudal segments with skipping of intermediate segments such as T10 or L3/4. Representative nEMG findings of the non-contiguous pattern in a patient with bulbar onset (patient 27) are shown in figure 2.

Eight of the 14 patients also exhibited the non-contiguous pattern in the paired muscles; of these, five (patients 23–27) showed the pattern in the paraspinal muscle pair (involvement of L5PS with skipping of T10PS) (table 1A), four (patients 27–30) showed the pattern in the thigh muscle pair (involvement of BF innervated by S1 with skipping of VM innervated by L3/4) (table 1B). One of the eight patients (patient 27) showed this pattern in both pairs.

In order to consider whether there is a local propagation of the non-contiguously affected lumbosacral lesion, we used schematics to examine the anatomical distributions of the involved motoneuron pools of the lumbosacral muscles in the eight patients who exhibited the skipping pattern in the paired muscles (figure 3).[23–26 30 31] The involved motoneuron pools were located in close horizontal or radial proximity to one another in five patients (patients 26–30) and appeared to form a cluster in four patients (patients 27–30). By contrast, the involved motoneuron pools were not horizontally contiguous in two patients (patients 24–25). The one remaining patient (patient 23) had only one lesion in the lumbosacral muscles.

Excluding FDI, which was involved in all patients, the percentage of patients with nEMG abnormalities was the highest in TA (13/17, 76.5%) and L5PS (11/17, 64.7%) and was the lowest in RF and VM (2/17, 11.8%) (figure 4). Pairwise comparisons among the muscles showed statistically significant differences in proportions between the muscles innervated by L3/4 and L5: RF and TA (p=0.01), RF and L5PS (p=0.03), VM and TA (p=0.01), and VM and L5PS (p=0.03). There were no

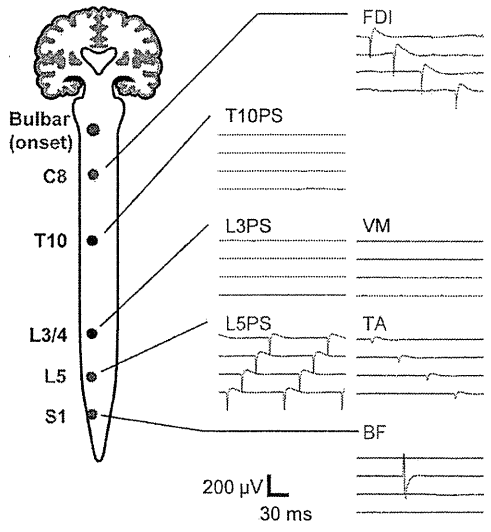

Figure 2 Representative needle electromyography (nEMG) finding in the patient with non-contiguous pattern. nEMG finding of patient 27 whose onset was bulbar symptoms. Positive sharp waves (first dorsal interosseous (FDI), L5PS and tibialis anterior (TA)) or a fasciculation potential (biceps femoris (BF)) are present with skipping of the muscles innervated by intermediate segments. Note that the non-contiguous (skipping) distribution pattern is evident between the muscles of the paraspinal (T10PS and L5PS) and thigh (vastus medialis (VM) and BF) pairs. Red type indicates nEMG abnormalities.

Table 1 Frequencies of nEMG abnormality patterns in paired muscles among 14 patients with a non-contiguous (skipping) pattern

(A) The patterns in the paraspinal muscle pairs				
FDI (C8)	+	+	+	+
T10PS	–	+	+	–
L5PS	–	–	+	+
Number of patients	4	2	3	5
(B) The patterns in the thigh muscle pairs				
FDI (C8)	+	+	+	+
VM (L3/4)	–	+	+	–
BF (S1)	–	–	+	+
Number of patients	9	1	0	4

+, abnormal spontaneous EMG activities present; –, abnormal spontaneous activities absent; BF, biceps femoris; FDI, first dorsal interosseous; T10PS, T10 paraspinalis; L5PS, L5 paraspinalis; VM, vastus medialis.

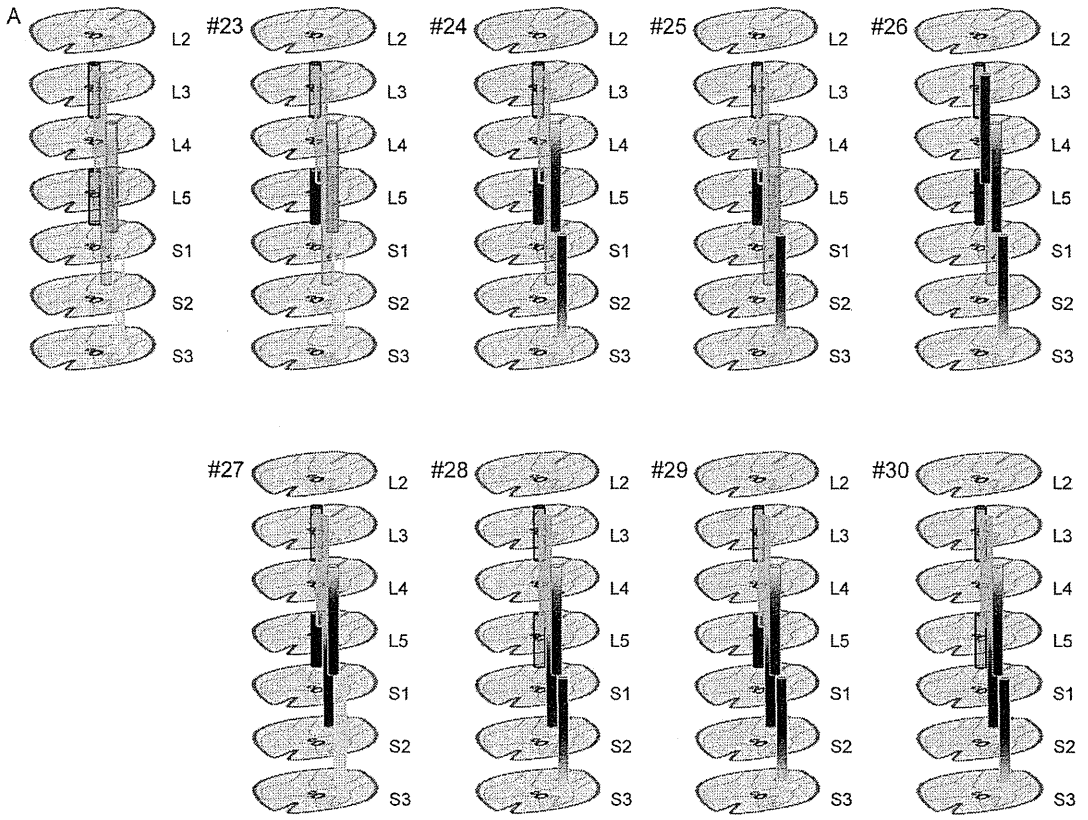

Figure 3 Schematic diagrams of motoneuron pools of the examined muscles in the lumbosacral cord (A) and their patterns of involvement in eight patients showing the non-contiguous (skipping) pattern in the paired muscles (patients 23–30). The locations of motoneuron pools innervating each muscle were taken from refs [23–26 30] and [31]. Note that the involved motoneuron pools (darkly shaded) appear to neighbour one another in 3-dimensional anatomy, and appear to form a cluster for four patients (patients 27–30) in particular. VM (orange column), vastus medialis deep layer; RF (orange column), rectus femoris; L3 PS (upper red column), paraspinal muscle at L3 level; TA (pink column), tibialis anterior; L5 PS (lower red column), paraspinal muscle at L5 level; BF (green column), biceps femoris long head; GC (yellow column), gastrocnemius medial head.

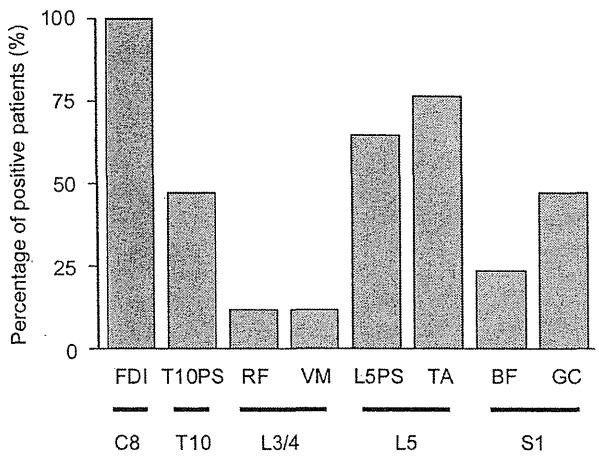

Figure 4 Frequency of needle electromyography abnormality of each muscle in the patients with contiguous and non-contiguous distribution patterns. With the exception of first dorsal interosseous (FDI) which, as the most rostral muscle, was affected in all patients, the highest frequencies were found in the muscles innervated by L5 and the lowest in the muscles innervated by L3/4. The differences were statistically significant ($p<0.05$, Fisher's exact probability test using the p value adjustment method of Holm). Note that the frequencies are almost same between L5PS and tibialis anterior (TA).

statistically significant differences in other pairs of muscles except those including FDI.

We also investigated and compared the frequency of Fib/PSWs and that of FPs in every muscle of all included patients (figure 5). Fib/PSWs were more frequently observed than FPs in FDI (C8), which was the onset region in most of the included patients. To the contrary, FPs were dominantly observed than Fib/PSWs in RF or VM (L3/4) and BF or GC (S1), away from the onset region. However, TA and L5PS, both of which are innervated by L5, showed Fib/PSWs less rarely than FPs.

DISCUSSION

We investigated whether the involvement of LMNs in sporadic ALS spreads contiguously in the rostrocaudal direction from the onset site. If prion-like propagation underlies the progression of ALS and the disease pathology in the first focal lesion propagates to adjacent cells in a cell-to-cell domino-like manner (the 'single seed and simple propagation' hypothesis) (see online supplementary figure S3A), involved LMNs should be distributed contiguously from the onset site.

Our nEMG study revealed that more than 50% of patients showed diffuse patterns. They showed weakness or muscle atrophy in lumbosacral regions more frequently than the rest (79% vs 29%). Therefore, they seemed to be in later stages of the disease.