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RESEARCH PAPER

Neck weakness is a potent prognostic factor in sporadic amyotrophic lateral sclerosis patients

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ABSTRACT

Objective To clarify the emergence of muscle weakness in regions of the body that affect survival, and deterioration in activities of daily living (ADL) in amyotrophic lateral sclerosis (ALS) patients.

Methods We conducted a multicentre-based prospective cohort study of patients with ALS. We enrolled 401 sporadic patients with ALS. Death or the introduction of invasive ventilation was defined as the primary endpoint, and the time to five clinical markers of ADL deterioration associated with bulbar paralysis or limb weakness were defined as ADL milestones. Muscle weakness was assessed in the neck flexor muscles; the bilateral abductors of the shoulders; the bilateral wrist extensor muscles; the bilateral flexor muscles of the hips; and the bilateral ankle dorsiflexion muscles. We performed Cox proportional hazards regression analyses for the primary endpoint and the five ADL milestones, adjusting for known covariate prognostic factors for ALS.

Results The Medical Research Council (MRC) score for the neck flexors was the most significant prognostic factor for the primary endpoint (HR 0.74, p<0.001), *loss of speech* (HR 0.66, p<0.001), and *loss of swallowing function* (HR 0.73, p<0.001), and was one of the significant prognostic factors for *loss of upper limb function*, *difficulty turning in bed*, and *loss of walking ability* (p=0.001, 0.002, and 0.008, respectively). The MRC score for the neck flexors was also a significant prognostic factor for covariates of the previously reported prognostic factors.

Conclusions Neck weakness is an independent prognostic factor for survival and deterioration in ADL in Patients with ALS.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterised by progressive upper and lower motor neuron loss, which leads to limb and bulbar paralysis and respiratory failure.[1] Symptoms develop at a progressive rate, and the median survival time from disease onset is 2–4 years.[2–4] However, patients with ALS show extensive variability in clinical courses, with durations ranging from a few months to more than 10 years. Furthermore, major symptoms that differentially affect activities of daily living (ADL) and prognosis also show variability among patients with different disease forms.[5] A better understanding of the factors influencing deterioration in ADL and prognosis would help physicians and patients determine whether and when to introduce non-invasive positive pressure ventilation, tube feeding, tracheostomy and artificial ventilation, and would lead to effective stratification strategies in clinical trials. Several studies have shown that age,[6–10] bulbar symptom onset,[6 7] respiratory function,[3 8 11 12] time from symptom onset to diagnosis,[2 6 10 13 14] functional score[2 14] and rate of disease progression[2 15–17] are predictors of survival. Muscle weakness in particular regions of the body affect the prognosis of ALS, although it has not been sufficiently determined which regions are most predictive.[18] To investigate the longitudinal course of patients with ALS and clarify the emergence of muscle weakness, which affects deterioration in ADL and ALS prognosis, we conducted a prospective, multicentre study.

METHODS

Patient registry and follow-up system

We constructed a multicentre registration and follow-up system called the Japanese Consortium for Amyotrophic Lateral Sclerosis research (JaCALS), which consists of 21 neurology facilities in Japan. Patients with ALS diagnosed in these facilities were consecutively registered with written informed consent. The ethics committees of all the participating institutions approved the study. Full clinical examinations were conducted at registration by neurologists in each of the respective institutions. Muscle strength was manually tested and scored with the scale of the Medical Research Council (six points, range: 0–5)[19] in nine muscle groups as follows: neck flexors; bilateral abductors of shoulders as representatives of proximal upper extremity muscles; wrist extensors muscles as representatives of distal upper extremity muscles; bilateral flexors of hips as representatives of proximal lower extremity muscles; and ankle dorsiflexion muscles as representatives of distal lower extremity muscles. All manual muscle testing was performed with standard positioning and procedures by certified neurologists.[20] The MRC score of the neck

flexors was determined with the patient in the supine position. We confirmed the inter-rater reliability of the manual muscle testing method employed in this study using 23 patients with neuromuscular disease. The values of the kappa statistics of each muscle ranged from 0.65 to 0.93. To standardise the procedures and the examinations, the three organising doctors (NA, RN, HaW) visited each participating facility and ascertained the evaluation methods for this study.

Disease onset was defined as when the patients became initially aware of muscle weakness or impairment of swallowing, speech, or respiration. We enrolled patients who fulfilled the revised El Escorial criteria.[21] The diagnostic accuracy of the enrolled patients was then assessed by members of the steering committee of the JaCALS. Included patients were then followed-up with telephone surveys conducted by clinical research coordinators (CRC) or with examinations by neurologists every 3 months, and the degree of deterioration in ADL was determined at each time point. We employed the Japanese version of the ALSFRS-R as a scale for ADL, which was validated by Ohashi *et al*, using a telephone survey system.[22] We confirmed the reliability of the telephone survey system for the Japanese version of the ALSFRS-R previously,[23] and the English version of the telephone survey system has been confirmed in several previous studies.[24–26] Prior to the study, we informed and trained the CRCs of the study plan, procedures for the telephone survey, ethical issues relevant to the study, and requisite considerations for patients with ALS and caregivers, and then provided them with a general knowledge of ALS.

We defined a primary endpoint and ADL milestones in the disease course of the patients with ALS and determined their occurrence by telephone survey or examinations by neurologists. The introduction of tracheostomy positive pressure ventilation (TPPV) or death of the patient was defined as the primary endpoint, and TPPV-free survival was defined as survival. *Loss of speech function*, *loss of swallowing function*, *loss of upper limb function*, *difficulty turning in bed*, and *loss of walking ability* were set as ADL milestones. The time at which each ADL milestone occurred was defined as follows: *loss of speech function* was determined to have occurred when non-vocal communication became needed; *loss of swallowing function* was determined to have occurred when parenteral or enteral feeding became needed exclusively; *loss of upper limb function* occurred when the patient needed to be fed and became unable to grip a pen; *difficulty turning in bed* occurred when the patient became unable to turn in bed alone; *loss of walking ability* occurred when the patient became unable to walk without assistance.

Patients

A total of 520 patients with ALS were initially registered in the JaCALS from January 2006 to June 2011. We excluded 26 patients with known gene mutations: 17 patients with SOD-1 mutations, two patients with TDP-43 mutations, two patients with FUS/TLS mutations, three patients with angiogenin mutations, and two patients with C9ORF72 repeat expansions. We also excluded 13 patients with family histories of ALS and 40 patients who were categorised as clinically possible or suspected according to the revised El Escorial criteria. An additional 20 patients for whom we could not obtain follow-up information to their refusal to participate in the telephone survey were also excluded. Twenty patients were excluded due to invalid data. The study finally included 401 sporadic patients with ALS diagnosed as clinically definite, probable, or probable laboratory-supported. Of these, 382 patients were followed for more than a year or died within a year of registration, and 19 patients were censored within a year from registration. Eleven patients declined the telephone survey during the course of the study, and we lost contact with eight patients during the survey.

Statistical analysis

The clinical data of the registered patients were anonymised in each participating facility of the JaCALS and assigned unique patient numbers. The data were then sent to the clinical data centre located at the Nagoya University Graduate School of Medicine and inputted into the JaCALS database.

We performed Cox proportional hazards regression analyses for the time of registration to the primary endpoint or onset of each ADL milestone to evaluate the impact of muscle weakness on the time to the primary endpoint and each decline in ADL. Specifically, for the primary endpoint and each ADL, we evaluated the HR for the MRC scores in nine muscle groups (ie, neck flexors, left and right abductors of shoulders, wrist extensor muscles, flexors of hips and ankle dorsiflexion muscles) at registration, identifying the muscles groups associated with the primary endpoint and five common ADL milestones. Additionally, we examined the HR for each muscle group after adjusting for known prognostic factors as follows: age at registration,[6–10] gender (male vs female),[6 27] disease duration,[2 6 10 13 14] percent vital capacity (%VC),[3 8 11 12] ALSFRS-R score,[14] riluzole use (yes vs no),[28] bulbar symptom,[6 7] and classification according to the revised El Escorial criteria (definite vs probable or probable laboratory-supported).[7 8 10 14] We compared the time from registration to the primary endpoint or each of the previously defined ADL milestones in the patients divided by their degree of muscle weakness using the Kaplan–Meier method. The log-rank test was used to test the null hypothesis that all the Kaplan–Meier curves were equal. A two-sided $p<0.05$ was considered statistically significant. All statistical analyses were conducted using the PASW V.18.0 program (SPSS Inc, Chicago, Illinois, USA).

RESULTS

Demographic characteristics of the registered patients

The patient sample comprised 244 men and 157 women. The median age at disease onset was 62.2 years (IQR: 53.5–68.5 years), and the mean follow-up period was 2.1±1.5 years. The follow-up rate at 1 year after registration was 95.3%. As initial symptoms, 47.4% of the patients showed upper limb weakness, 31.4% lower limb weakness, 22.9% dysarthria, 5.5% dysphagia and 2.0% cervical weakness. At registration, the median score on the ALSFRS-R was 38 (IQR: 32–42). (see online supplementary table S1).

Identification of weakened muscle groups that affect survival and progression to the ADL milestone

Cox proportional hazard regression analyses for the primary endpoint and the ADL milestones

Table 1 shows the results of Cox proportional hazard regression analyses for the primary endpoint and the five ADL milestones, including the MRC scores of the nine muscle groups. The MRC score for the neck flexors was the most significant negative prognostic factor for the primary endpoint, *loss of speech*, and *loss of swallowing function* (HR 0.74, $p<0.001$, HR 0.66, $p<0.001$, HR 0.73, $p<0.001$, respectively). For the *loss of upper limb function*, *difficulty turning in bed* and *loss of walking ability*, the MRC score for the neck flexors was a significant negative prognostic factor (HR 0.77, $p=0.001$, HR 0.77, $p=0.002$, and HR 0.80, $p=0.008$, respectively). Whereas, the MRC score for the left wrist extensors was a significant positive prognostic factor for the primary endpoint and each ADL milestone except for *difficulty turning in bed*.

Table 1 Multivariate Cox regression analyses for the primary endpoint and each activity of daily living milestone using the MRC score of each muscle group at registration

	Primary endpoint		Loss of speech		Loss of swallowing function		Loss of upper limb function		Difficulty turning in bed		Loss of walking ability	
Muscle group	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
Neck flexors	0.74 (0.65to 0.86)	<0.001	0.66 (0.56 to 0.76)	<0.001	0.73 (0.63 to 0.85)	<0.001	0.77 (0.66 to 0.89)	0.001	0.77 (0.66 to 0.91)	0.002	0.80 (0.67 to 0.94)	0.008
Left shoulder abductors	0.87 (0.69 to 1.11)	0.266	0.89 (0.69 to 1.14)	0.363	0.89 (0.71 to 1.12)	0.309	0.62 (0.49 to 0.79)	<0.001	0.72 (0.56 to 0.93)	0.012	0.75 (0.57 to 1.00)	0.049
Right shoulder abductors	0.98 (0.77 to 1.25)	0.890	1.11 (0.87 to 1.43)	0.399	1.02 (0.81 to 1.29)	0.867	1.19 (0.94 to 1.50)	0.159	1.08 (0.85 to 1.39)	0.529	0.99 (0.75 to 1.30)	0.917
Left wrist extensors	1.29 (1.04 to 1.59)	0.018	1.28 (1.03 to 1.59)	0.026	1.24 (1.02 to 1.51)	0.034	1.42 (1.14 to 1.77)	0.002	1.24 (1.00 to 1.55)	0.054	1.39 (1.08 to 1.79)	0.010
Right wrist extensors	0.90 (0.74 to 1.08)	0.254	0.88 (0.73 to 1.07)	0.193	0.92 (0.77 to 1.11)	0.380	0.73 (0.60 to 0.88)	0.001	0.80 (0.66 to 0.98)	0.029	0.98 (0.79 to 1.22)	0.884
Left hip flexors	0.99 (0.72 to 1.36)	0.964	0.96 (0.73 to 1.28)	0.791	0.85 (0.62 to 1.15)	0.284	0.74 (0.55 to 0.99)	0.040	0.77 (0.56 to 1.06)	0.115	0.90 (0.61 to 1.32)	0.585
Right hip flexors	0.96 (0.69 to 1.34)	0.830	0.95 (0.70 to 1.28)	0.719	1.09 (0.79 to 1.50)	0.613	1.18 (0.87 to 1.62)	0.290	1.18 (0.84 to 1.66)	0.331	1.06 (0.69 to 1.64)	0.788
Left ankle extensors	1.14 (0.93 to 1.40)	0.214	1.13 (0.94 to 1.34)	0.185	1.14 (0.95 to 1.37)	0.166	1.26 (1.04 to 1.52)	0.021	1.09 (0.91 to 1.30)	0.367	0.93 (0.71 to 1.21)	0.583
Right ankle extensors	0.94 (0.76 to 1.15)	0.530	0.95 (0.79 to 1.14)	0.564	0.94 (0.77 to 1.14)	0.539	0.85 (0.70 to 1.04)	0.125	0.81 (0.68 to 0.97)	0.023	0.72 (0.57 to 0.91)	0.007

According to table 1, the MRC score for the neck flexors was commonly identified as a possible prognostic factor for the primary endpoint and the five ADL milestones. We further examined its impact after adjusting for the other established or potential risk factors, that is, age at registration, gender, disease duration from onset to registration, percent vital capacity (% VC) at registration, ALSFRS-R score at registration, classification according to revised El Escorial criteria, riluzole use and bulbar symptom at registration (table 2). As seen in table 2, the MRC score for the neck flexors was an independent and significant prognostic factor for the primary endpoint, *loss of speech*, *loss of swallowing*, *loss of upper-limb function* and *difficulty turning in bed* in patients with ALS except for *loss of walking ability*. ($p<0.001$, $p=0.001$, $p=0.003$, $p<0.001$, $p=0.027$, respectively). At registration, there were moderate and significant correlations between the MRC score for the neck flexors and the % VC or the ALSFRS-R score. Pearson's correlation coefficients were 0.367 ($p<0.001$) and 0.496 ($p<0.001$), respectively.

Differences in survival time and time to ADL milestones in patients in terms of the MRC score grade for the neck flexors

We divided the registered patients into four categories according to their MRC score for the neck flexors (ie, 5, 4, 3 and ≤2). Figure 1 shows the Kaplan–Meier curves for the four categories for the primary endpoint and each ADL milestone. All the differences between the curves were significant according to a log-rank test ($p<0.001$).

DISCUSSION

In a prospective and multicentre cohort study, we identified that weakness of the neck flexors is a potent factor for the prediction of survival and for the deterioration of ADL, such as speech, swallowing, upper limb function, turning in bed, and walking, in sporadic patients with ALS.

The neck flexors consist of the sternocleidomastoid muscle (SCM), the platysma muscle, hyoid muscle, longus capitis muscle, longus colli and scalenus. These muscles are innervated by motor neurons in the cervical cord (C1–8) and accessory nerve nuclei,[29 30] primarily the C2–4 segments. By contrast, respiratory muscles consist of the diaphragm and the internal and external intercostals muscles, which are innervated by motor neurons of the upper cervical cord (C3–5) and thoracic cord (Th1–Th12), respectively.[30] Thus, the muscles for neck flexion and those for respiration partially share spinal segments of the motor neuron column for their motor innervations. Furthermore, significant correlations are present between compound muscle action potentials of the SCM and those of the diaphragm in patients with ALS,[31] suggesting that neck muscle weakness is correlated with weakness of the diaphragm to some extent in ALS. Because the main cause of death in patients with ALS is respiratory insufficiency, it is reasonable that neck flexor weakness was associated with respiratory impairments and, eventually, survival time. The motor response amplitude of the phrenic nerve motor neurons which are located in the C3–5 segments has been shown to be a significant prognostic factor for survival in patients with ALS.[32] This supports our findings.

Why then is weakness of the neck flexors a determinant factor for the deterioration of ADL for speech, swallowing, upper limb function, truncal turning and walking ability? Recently, some studies have suggested that the degeneration of motor neurons is initially a focal process in ALS that later spreads contiguously throughout the three-dimensional anatomy of connected or neighbouring neurons.[33–36] Dysfunction of speech and swallowing involves the impairment of motor

Table 2 Multivariate Cox regression analyses with the adjustments of the covariates that we selected for the primary endpoint and each activity of daily living milestone using known predictive factors

	Primary endpoint		Loss of speech		Loss of swallowing function		Loss of upper limb function		Difficulty turning in bed		Loss of walking ability	
	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
MRC score of neck flexors at registration	0.72 (0.62 to 0.83)	<0.001	0.78 (0.67 to 0.90)	0.001	0.80 (0.69 to 0.93)	0.003	0.76 (0.65 to 0.88)	<0.001	0.83 (0.70 to 0.98)	0.027	0.95 (0.79 to 1.15)	0.601
Age at registration (years)	1.03 (1.02 to 1.04)	<0.001	1.02 (1.01 to 1.03)	0.002	1.03 (1.01 to 1.04)	<0.001	1.01 (0.99 to 1.02)	0.264	1.01 (0.99 to 1.02)	0.265	1.00 (0.98 to 1.01)	0.890
Gender (male vs female)	1.14 (0.85 to 1.52)	0.381	0.85 (0.65 to 1.12)	0.247	1.13 (0.85 to 1.49)	0.398	1.27 (0.97 to 1.68)	0.088	1.01 (0.76 to 1.33)	0.947	0.85 (0.61 to 1.17)	0.309
Duration from onset to registration (years)	0.64 (0.57 to 0.72)	<0.001	0.72 (0.64 to 0.80)	<0.001	0.69 (0.62 to 0.77)	<0.001	0.82 (0.75 to 0.9)	<0.001	0.74 (0.65 to 0.85)	<0.001	0.75 (0.66 to 0.87)	<0.001
%VC at registration	0.98 (0.98 to 0.99)	<0.001	0.98 (0.97 to 0.99)	<0.001	0.98 (0.98 to 0.99)	<0.001	0.99 (0.98 to 1.00)	0.001	0.99 (0.99 to 1.00)	0.007	1.00 (0.99 to 1.00)	0.491
ALSFRS-R at registration	0.97 (0.94 to 0.99)	0.008	0.99 (0.97 to 1.02)	0.483	0.96 (0.93 to 0.98)	0.001	0.96 (0.94 to 0.98)	0.001	0.89 (0.86 to 0.92)	<0.001	0.91 (0.88 to 0.94)	<0.001
El Escorial criteria (probable or probable laboratory-supported)	0.72 (0.53 to 0.99)	0.043	0.61 (0.45 to 0.82)	0.001	0.76 (0.56 to 1.04)	0.087	0.67 (0.50 to 0.90)	0.007	0.71 (0.52 to 0.97)	0.031	0.63 (0.44 to 0.88)	0.008
Riluzole administration	1.02 (0.75 to 1.37)	0.916	1.09 (0.82 to 1.44)	0.551	0.97 (0.73 to 1.29)	0.843	0.95 (0.72 to 1.25)	0.694	0.84 (0.63 to 1.13)	0.258	0.94 (0.68 to 1.31)	0.721
Bulbar symptom at registration	0.91 (0.67 to 1.22)	0.524	2.04 (1.52 to 2.73)	<0.001	1.41 (1.06 to 1.86)	0.018	0.68 (0.50 to 0.93)	0.015	0.63 (0.47 to 0.84)	0.002	0.68 (0.49 to 0.96)	0.028

%VC, percent vital capacity; ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale.

neurons relayed via the glossopharyngeal, vagus, accessory and hypoglossal nerves to the medulla oblongata.[30] The medulla oblongata and cervical cord motor neurons innervating the neck flexion muscles are anatomically different in their three-dimensional layering, while these two groups of neurons are rather contiguously located. Thus, it may be speculated that if the contiguous spreading of motor neuron degeneration occurs according to the local spreading hypothesis, neck flexion impairment may eventually affect speech and swallowing functions. Furthermore, motor neurons for the neck flexion muscles, which are located in the C1–8 segments,[29 30] are also contiguous or overlapping with those for the upper limb muscles in the C5–Th1 segments, particularly the proximal upper limb muscles.[29 30] Neck flexion and upper limb function may be correlated with disease progression through the local spreading view of motor neuron degeneration. Truncal turning and walking require not only lower limb muscle activities but also power in a broad area of the chest, abdominal and back muscles, which are innervated by the cervical to lumbar cord.[37–39] Therefore, propagation of weakness from the cervical and lumbar areas may affect truncal turning or walking. We need, however, further investigations to demonstrate the underlying mechanisms of the correlation between the neck muscles and other muscles of the body that together determine ADL.

In this study, the MRC score for the left wrist extensors shows a positive prognostic factor for the primary endpoint and some ADL milestones, the reason for which might be that the weakness of the distal muscle in the non-dominant arm was least relevant to survival, or ADL declines so that it was shown to be a positive factor in the multivariate analyses.

A number of studies have demonstrated survival curves for patients with ALS and some factors that influence these survival curves.[18] The majority of these studies have found that older age is a strong risk factor for shorter survival in patients with ALS,[6–10] and the onset of bulbar symptoms is associated with a worse prognosis than the onset of spinal symptoms.[6 7] Several studies have found that a longer diagnostic delay correlates with a better prognosis,[2 6 10 13 14] and that a lower %VC or a percent forced vital capacity (%FVC) is correlated with shorter survival for patients with ALS.[3 8 11 12] The progression rate of the ALSFRS-R at the time of diagnosis was also related to ALS prognosis.[17] Neck flexor weakness has not been listed as a prognostic factor for patients with ALS, and most of these studies evaluated survival alone as an endpoint, and did not determine the onset of loss of speech, swallowing, limb and truncal function. In this study, we showed that neck flexor weakness was not only a novel prognostic factor for survival but also a significant prognostic marker for non-survival events related to ADL decline for patients with ALS.

In the course of ALS, patients must make some difficult decisions, including the use of gastrostomy for tube feeding, the use of assisted ventilation, and end-of-life planning, which require the support of the attending physician and a multidisciplinary team. All patients with ALS should be provided with sufficient information concerning these interventions and given sufficient opportunity for the careful consideration of their decision. In the medical, nursing and social care of patients with ALS, simple and robust indicators for predicting the status of each patient for several months or a year after diagnosis are necessary for patient management. Medical staff and caregivers need to have a predictor of the patient's status in the near future, including survival prognosis and also estimates for the loss of speech, swallowing, limb and truncal function. Our findings may contribute to such prediction.

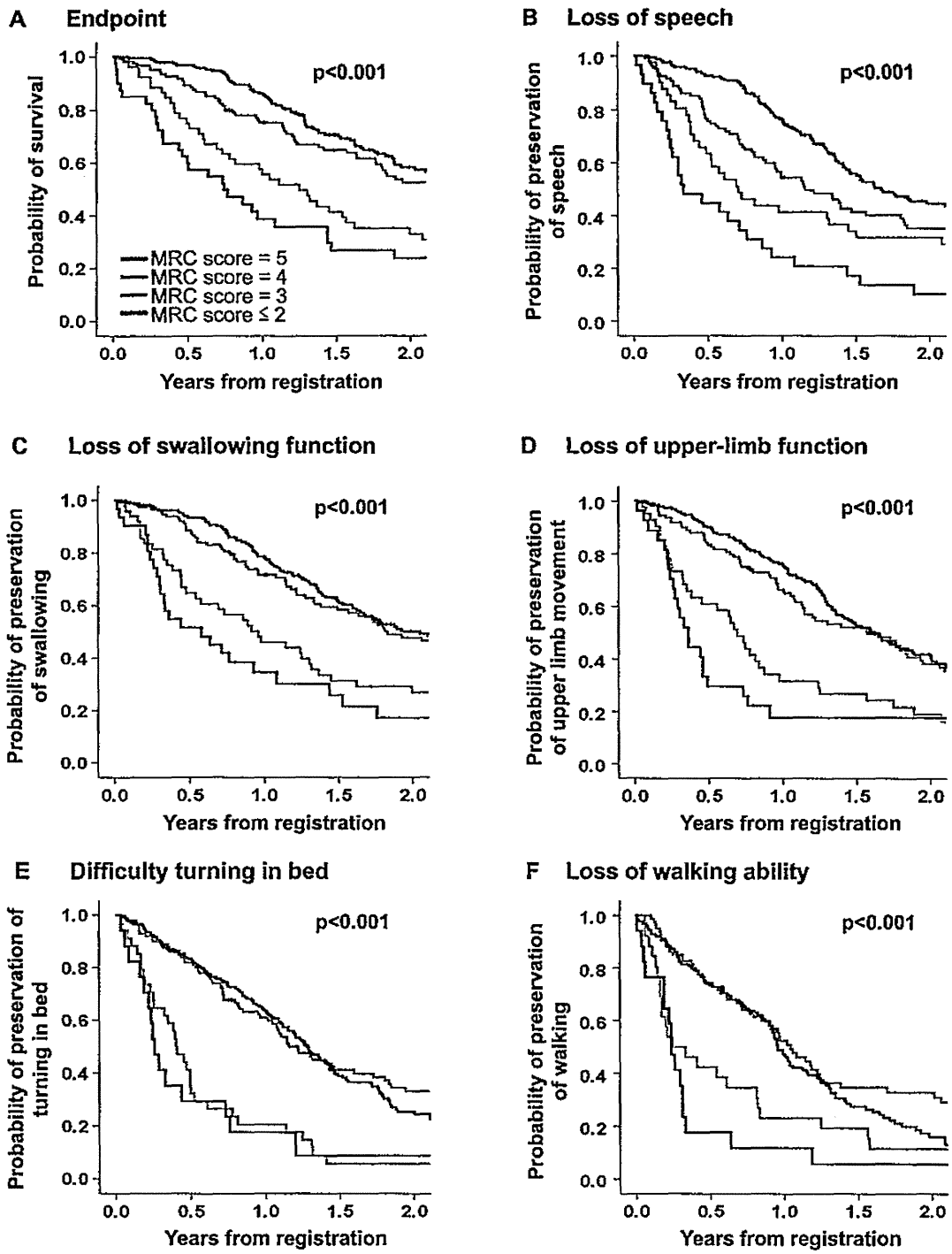

Figure 1 Kaplan–Meier curves according to the MRC score for the neck flexors. The Kaplan–Meier curves for the primary endpoint and each activity of daily living milestone among four categories divided according to the MRC score for the neck flexors were compared by the log-rank test. Curves are shown for the MRC score 5 (blue), the MRC score 4 (green), the MRC score 3 (orange), and the MRC score ≤2 (purple) groups. All the differences of the curves were significant (p<0.001).

The course of ALS is highly variable between patients,[5] which is one of the major factors limiting the power of ALS clinical trials.[40 41] Therefore, robust stratification factors that could divide ALS patient groups depending upon prognosis are needed for designing trials. Compared with known prognostic factors for patients with ALS, such as age, duration from onset to registration, ALSFRS-R at registration, and presence of bulbar symptom, weakness of the neck flexors was a potent and independent prognostic factor. Thus, the MRC score for the neck flexors might be used for stratification factor in a future clinical trial.

Neck extensor muscle weakness with head drop as an early symptom has been reported in a few patients with ALS.[42 43] However, Katz *et al*[44] wrote that neck flexor weakness is typically observed. We assert that neck flexor weakness is commonly observed in patients with ALS, and is useful for the prediction of prognosis.

The limitations of this study are as follows: registered patients were followed-up by telephone survey, and we did not examine longitudinal changes in the strength of multiple muscles. As we demonstrated, the relationship between the involved muscle groups and survival prognosis and estimates of ADL deterioration would offer insights into the modalities of progression in patients with ALS. However, to examine the pattern of spread more precisely, a cohort study that observes longitudinal changes in the strength of muscle groups and extensions of muscle weakness will be required.

This study analysed a multicentre cohort of patients with ALS in a single nation, Japan. Although the clinical profiles of ALS are broadly similar among countries in previous reports, the outcome of our study would be better confirmed in cohorts of patients with ALS in multiple countries.

In conclusion, we showed that neck weakness is an independent prognostic factor for survival and deterioration in ADL in patients with ALS. We hope that our report will be helpful for clinicians who want to provide medical, social and nursing care to patients with ALS with proper timing, and to researchers as they plan clinical trials for ALS.

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Neck weakness is a potent prognostic factor in sporadic amyotrophic lateral sclerosis patients

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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.*, 2011a). 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.*, 2011a). 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.

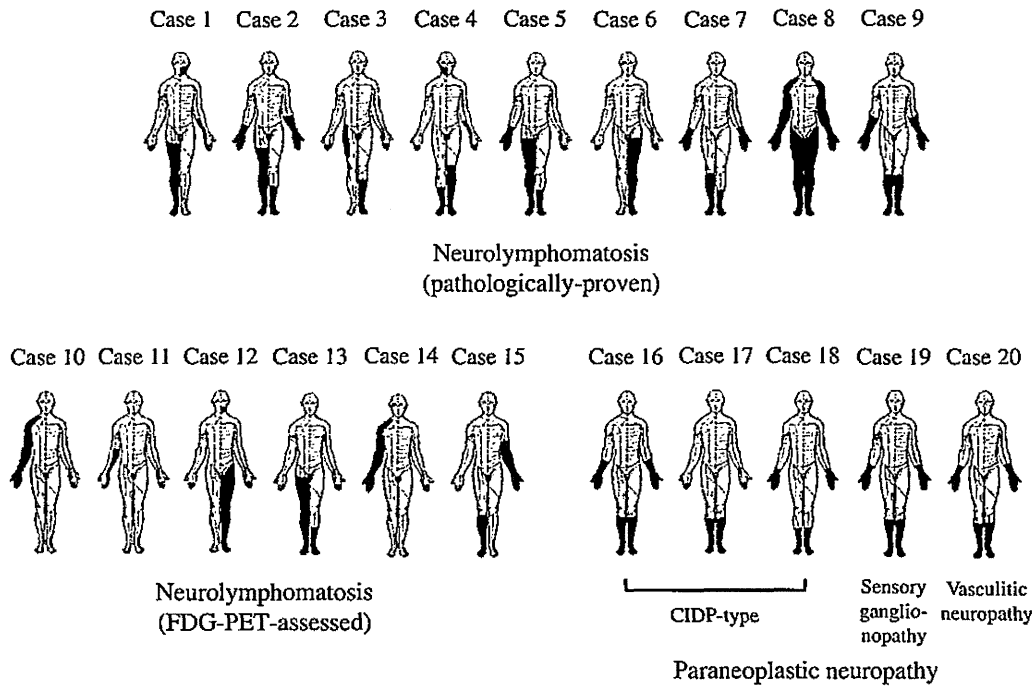

Figure 1 Distribution of sensory deficits in representative cases. Cases 1 to 9 and 10 to 15 represent pathologically-proven and FDG-PET-assessed neurolymphomatosis, respectively. All cases in this category, except for Cases 8 and 9, showed multiple mononeuropathy patterns, whereas Cases 8 and 9 manifested 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.*, 2011a). 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.*, 2011a), 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).

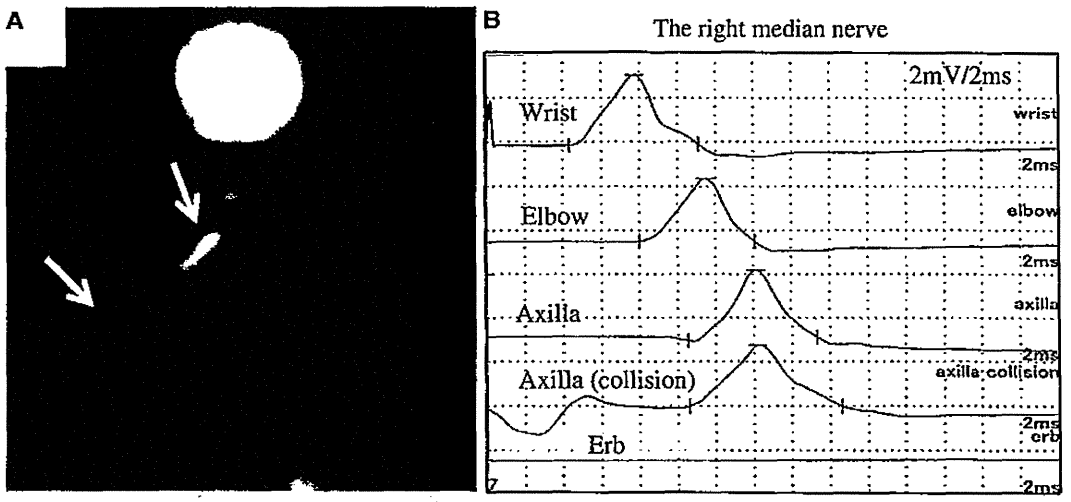

Figure 2 Findings of whole-body FDG-PET and nerve conduction studies in Patient 10, who had diffuse large B cell lymphoma. (A) Accumulation of FDG along the right brachial plexus (arrow) was observed. (B) A conduction block was present between the erb and the axilla, which was located between the proximal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

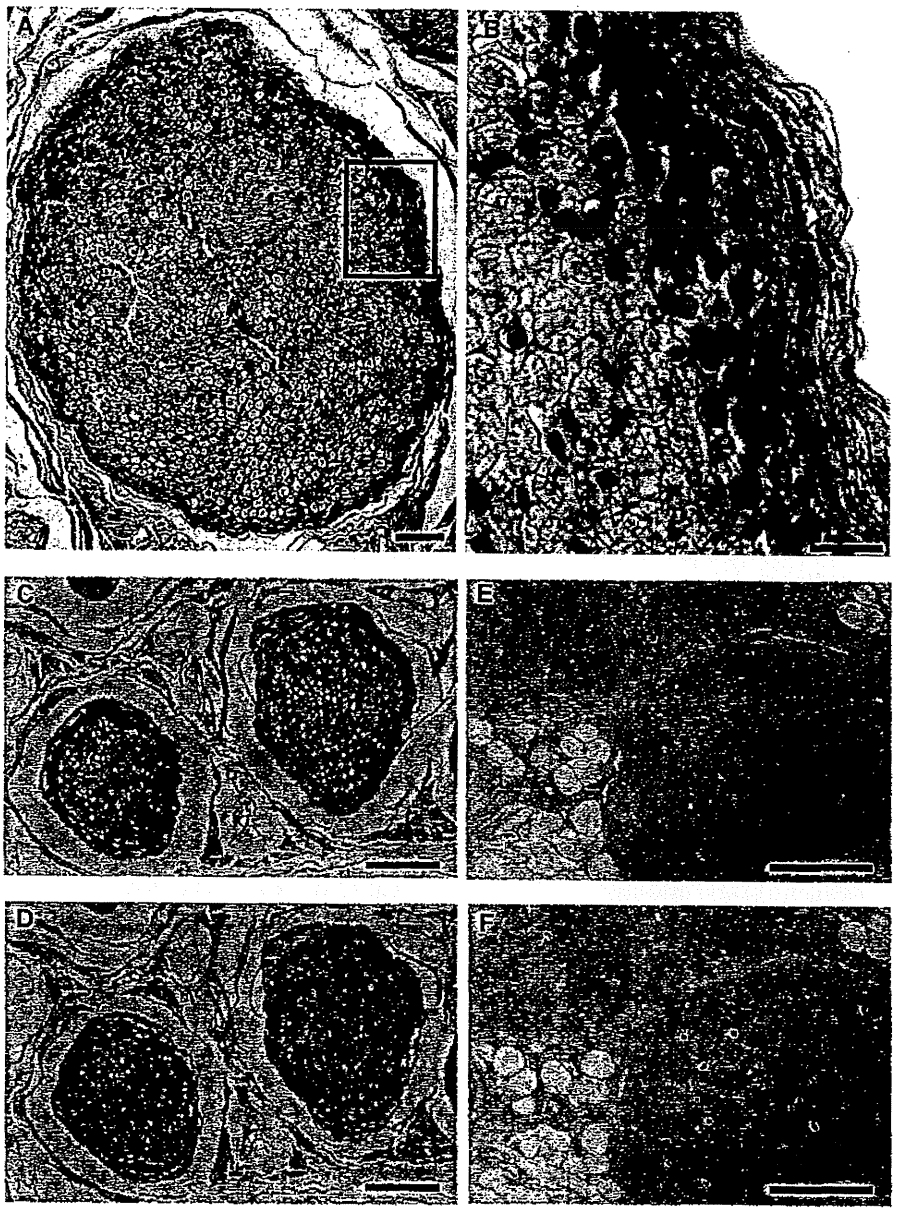

Figure 3 Sciatic nerve from Patient 1 with diffuse large B cell lymphoma. **(A)** Invasion of lymphomatous cells was primarily observed in the subperineurium through haematoxylin-eosin staining. Cross section. **(B)** In a high power view, invading cells exhibited atypical cellular appearance. **(C)** 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.

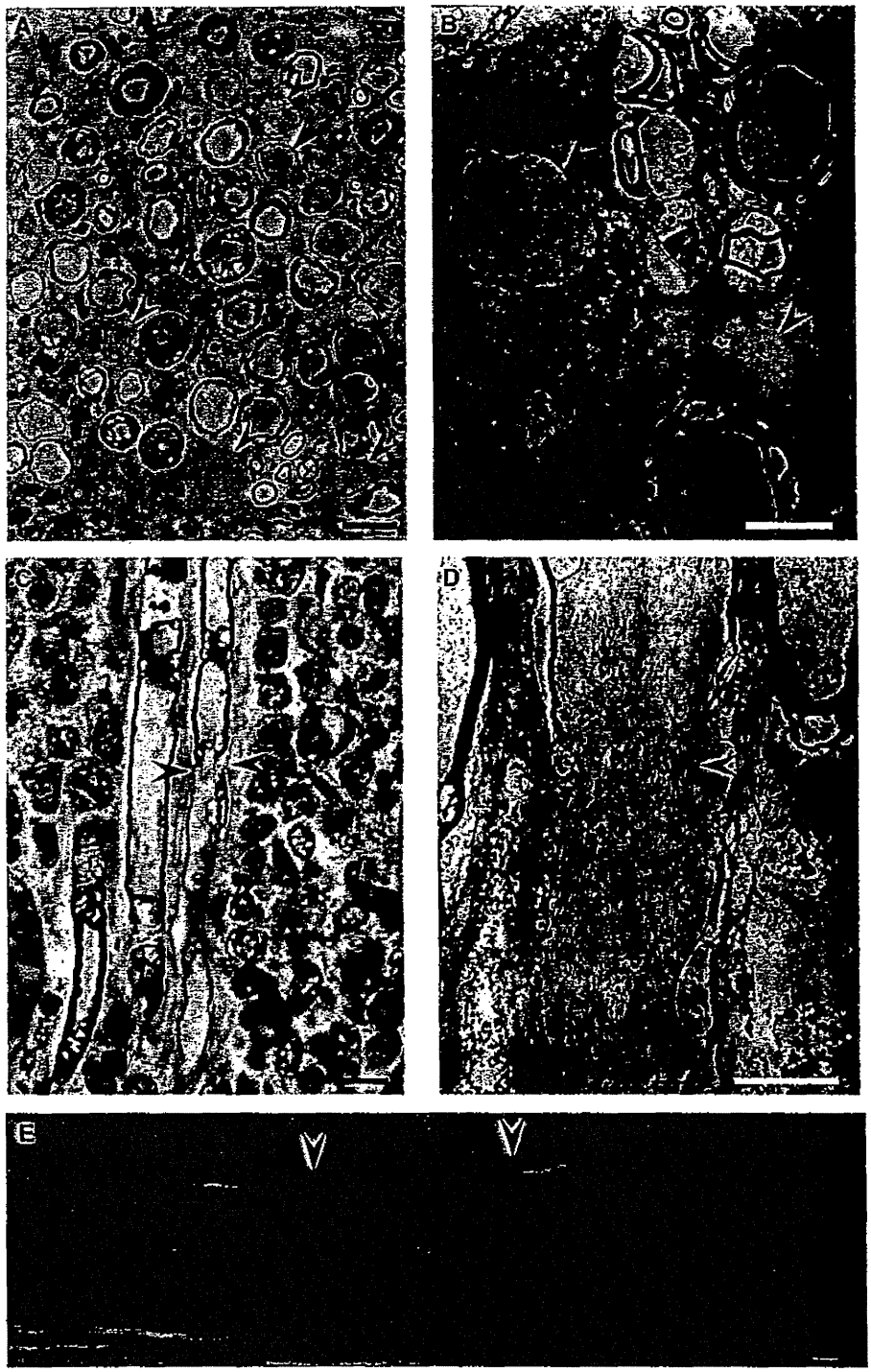

Figure 4 L4 anterior root from Patient 8 with Burkitt's lymphoma. (A) Demyelination (arrowhead) was observed near the infiltration of lymphomatous cells (asterisk) in toluidine blue-stained semi-thin cross sections. (B) Demyelination (arrowhead) near the infiltration of lymphomatous cells (asterisk) 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