

も比較的よい結果が得られているが、症例によってはいくつかのエクソンでは症例によって不安定なものがある。今後、さらに多数例について検討する。

結 論

Charcot-Marie-Tooth病の診断DNA Chipチップは完成し、本システムで遺伝子異常の検出を効果的に行いうる。

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Electrophysiological features of late-onset transthyretin Met30 familial amyloid polyneuropathy unrelated to endemic foci

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Abstract *Background* Through the development of gene diagnostic techniques, late-onset transthyretin Met30-associated familial amyloid polyneuropathy (FAP TTR Met30) has been shown to be more prevalent than is generally believed. *Objective* To examine the electrophysiological features of late-onset FAP TTR Met30 unrelated to endemic foci. *Methods* Nerve conduction findings in 44 cases with an onset of more than 50 years of age in a non-endemic area were assessed and compared with findings from 21 earlier-onset cases related to endemic foci. *Results* The extent of the reduction of the compound muscle action potential and, especially, the sensory nerve action potential was more profound in the late-onset group even when the decline of these indices with aging in normal control subjects was taken into account. The feature of predominant lower-limb involvement seemed to be more conspicuous in the late-onset group. Electrophysiological indices tended to

be aggravated as the duration of neuropathic symptoms increased in the early-onset group, while most of these indices in the late-onset group did not show this correlation. A slowing of conduction velocity and a prolongation of distal latency, which suggests demyelination, were conspicuous in some patients. Pathologically, a predominant loss of small-fibers was not conspicuous in sural nerve biopsy specimens from late-onset patients. Large myelinated fiber density showed a negative correlation with the disease duration in early-onset cases, but not in late-onset cases. *Conclusions* Electrophysiological differences between late- and early-onset cases were present, probably reflecting the different underlying pathogenic mechanisms of neuropathy. The demyelinating feature does not exclude the possibility of this disease.

Key words familial amyloid polyneuropathy · amyloid · transthyretin

Introduction

Transthyretin Met30-associated familial amyloid polyneuropathy (FAP TTR Met30), in which methionine is substituted for valine at position 30 of transthyretin, is the most common type of familial amyloid polyneuropathy in Japan, as well as in Western countries [3, 10–12,

26, 28, 29, 35]. In Japan, these patients have been known to concentrate in two geographic areas (Arao and Ogawa) [11, 12, 26]. Although there are exceptions, typical cases of FAP TTR Met30 in these endemic foci show characteristic features including the age at onset from late 20s to early 40s, a high penetrance rate, marked autonomic dysfunction, loss of superficial sensation including nociception and thermal sensation (i.e., sensory dissociation

tion), atrioventricular conduction block requiring pacemaker implantation, and the presence of anticipation of age at onset [2, 11, 16, 24, 26].

In contrast to conventional FAP TTR Met30 with relatively early age at onset in the endemic foci, we have reported the presence of a late-onset type of FAP TTR Met30 unrelated to the endemic foci that is widely distributed throughout Japan [16, 18, 23]. The typical features of these cases were distinct from those related to the endemic foci. These differences include an age at onset of over 50 years, a low penetrance rate, relatively mild autonomic dysfunction, loss of all sensory modalities rather than sensory dissociation, the frequent presence of cardiomegaly, extreme male preponderance, and the absence of anticipation of age at onset [16, 18, 23, 24, 34]. Differences in geographic distribution and clinical features between early- and late-onset types of FAP TTR Met30 have also been reported in Portugal [5, 6, 35]. Previously, we reported pathologic differences between these two types that corresponded well to clinical differences [18]. The causal mechanism of the different clinical and pathologic features of the early- and late-onset types of FAP with the same mutation in the transthyretin gene has not yet been determined. The late-onset type of FAP TTR Met30 in a non-endemic area may be a prototype of FAP TTR Met30 and some environmental or genetic factors in the endemic foci induced anticipation and created the early-onset type of FAP TTR Met30. Thus, late-onset FAP TTR Met30 is considered to be more prevalent in Japan and elsewhere than is generally believed, but it is still not widely recognized. Wide geographic distribution, sporadic occurrence, and atypical clinical features still may result in missed diagnoses. Before the availability of molecular diagnosis, late-onset FAP TTR Met30 patients residing outside of endemic foci frequently were misdiagnosed with polyneuropathy of undetermined cause or CIDP [1, 28].

In the present study, we investigated electrophysiological features in patients with late-onset FAP TTR Met30 unrelated to endemic foci to provide more recognition for this type of FAP with apparently nonspecific clinicopathological features.

Patients and methods

We examined the nerve conduction findings in 44 patients (36 men, 8 women) with late-onset FAP TTR Met30 from non-endemic areas of Japan who were referred to the hospitals of Nagoya University Graduate School of Medicine, Kumamoto University School of Medicine, or Shinshu University School of Medicine. Twenty-one patients (10 men, 11 women) with early-onset cases from endemic foci were also assessed for comparison. Inclusion criteria for late-onset cases were FAP TTR Met 30 with an onset age over 50 years and no relationship to the endemic foci within the two most recent prior generations [16, 18, 23]. For early-onset cases, inclusion criteria were an onset age under 50 years and a relationship to one of the two Japanese endemic foci within the two most recent prior generations [16, 18, 23]. No pa-

tient in the study belonged to the same kindred as another. In order to confirm the diagnosis of FAP TTR Met30, DNA analyses for mutation of the transthyretin gene were performed in all patients as described previously [26, 31]. Informed consent was obtained, and all aspects of the study were approved by the ethics committees of Nagoya University Graduate School of Medicine, Kumamoto University School of Medicine, and Shinshu University School of Medicine.

Rough backgrounds and clinical characteristics of the cases are described in Table 1. The age at onset of neuropathic symptoms in the late-onset group was 64.0 ± 6.4 (mean \pm SD) years, and that of the early-onset group was 34.4 ± 6.4 years. The duration from onset of neuropathy to the examination was shorter in the late-onset group (3.1 ± 2.8 years) than in the early-onset group (6.0 ± 4.6 years), while the activity of daily living assessed by the modified Rankin Scale [41] was not very much different between the groups (2.0 ± 0.8 in the late-onset group and 2.2 ± 1.2 in the early-onset group). Clinical features of the two groups of patients included in the present study were well within accordance with previous descriptions [12, 16, 23]. Because a previous large-scale comparative study divided patients solely by their age at onset, but not their relationship to endemic foci [16], the characteristics of the two groups in the present study are more distinct.

As a routine practice for detection of peripheral neuropathy, motor and sensory conduction was measured in the median, tibial, and sural nerves, using a standard method with surface electrodes for stimulation and recording [20]. Motor conduction was investigated in the median and tibial nerves, recording from the abductor pollicis brevis and abductor hallucis brevis, respectively. Wrist to elbow for the median nerve and ankle to popliteal fossa for the tibial nerve were used for calculating motor conduction velocity (MCV). Sensory con-

Table 1 Background and clinical features of FAP TTR Met30

Features	Late-onset group in non-endemic area n=44	Early-onset group in endemic foci n=21
Men/women	36/8	10/11
Age of onset (years)	64.0 ± 6.4	34.4 ± 6.4
Age of examination (years)	67.0 ± 6.0	40.5 ± 7.6
Presence of family history	17 (39)	21 (100)
Initial complaint		
Neuropathic symptoms*	39 (89)	10 (48)
Autonomic symptoms**	4 (9)	11 (52)
Cardiac symptoms***	1 (2)	0 (0)
Sensory deficit		
Superficial sensation-dominant	8 (18)	19 (90)
All modality	36 (82)	2 (10)
Deep sensation-dominant	0 (0)	0 (0)
Modified Rankin Scale****	2.0 ± 0.8	2.2 ± 1.2

Values are expressed as numbers of patients with percentages in parentheses, or as the mean \pm SD

* Numbness or weakness in distal portion of the extremities

** Orthostatic hypotension, syncope, nausea/vomiting, diarrhea/constipation, urination, or impotence

*** Dyspnea or edema in the lower extremities

**** Modified Rankin Scale [41]: 0, asymptomatic; 1, nondisabling symptoms not interfering with lifestyle; 2, minor disability from symptoms leading to some restriction of lifestyle but not interfering with patients' capacity to look after themselves; 3, moderate disability from symptoms significantly interfering with lifestyle or preventing totally independent existence; 4, moderately severe disability from symptoms clearly precluding independent existence, though not requiring 24-hour attention from a caregiver; and 5, severe disability and total dependence, requiring constant attention day and night

duction was investigated in the median and sural nerves. These were recorded antidromically using ring electrodes at the second digit for the median nerve and bar electrodes at the ankle for the sural nerve. Sensory nerve conduction velocity (SCV) was calculated for the distal segment. Amplitudes of compound muscle action potential (CMAP) and sensory nerve action potential (SNAP) were measured from the baseline to the first negative peak. Age-matched control values for the late- and early-onset groups were obtained separately because age-associated change of electrophysiological indices may be present in normal subjects [20]. Control values were obtained from 60 normal volunteers (66.9 ± 6.9 years of age; male:female, 28:32) for the late-onset group and 50 normal volunteers (40.3 ± 7.3 years of age; male:female, 31:19) for the early-onset group.

A sural nerve biopsy was performed in 21 patients of the late-onset group and results were compared to that of 9 subjects of the early-onset group as described previously [9, 17, 32]. The duration from onset of neuropathy to biopsy was 2.8 ± 1.6 years in the late-onset group and 3.2 ± 2.0 years in the early-onset group. Pathologic findings in 11 patients of the late- and 9 patients of the early-onset groups were reported previously [18]. Specimens were fixed in 2.5% glutaraldehyde in 0.125 M cacodylate buffer (pH 7.4) and embedded in epoxy resin for light and electron microscopy. Semithin sections were stained with toluidine blue for morphometric assessment with light microscopy. The density of myelinated fibers was assessed in toluidine blue-stained semithin sections using a computer-assisted image analyzer (Luzex FS; Nikon, Tokyo, Japan), and the densities of small and large myelinated fibers were calculated as described previously [25, 27, 32]. For electron microscopy, epoxy resin-embedded specimens were cut into ultrathin transverse sections and stained with uranyl acetate and lead citrate. In order to assess the density of unmyelinated fibers, electron microscopic photographs were taken at a magnification of 4000x in a random fashion to cover the area of ultrathin sections as described previously [15, 17, 22]. The remainder

of the glutaraldehyde-fixed sample was processed for the teased-fiber study as described previously [17, 32].

Quantitative data of the late-onset group were presented as the mean \pm SD, and were compared with those of the early-onset group. Electrophysiological indices of the late- and early-onset groups were also compared to respective age-matched control values. Statistical analyses were performed using the Mann-Whitney *U* test or Pearson's correlation coefficient analysis as appropriate. *p* values less than 0.05 were considered to indicate significance.

Results

Nerve conduction studies are summarized in Table 2. The results indicate mainly axonal neuropathy with some slowing of conduction velocities and prolongation of distal latency (DL) in both the late- and early-onset groups. Although the duration of neuropathic symptoms was shorter in the late-onset group, electrophysiological indices were rather worse in this group. In the median nerve, a variable reduction of CMAP was observed in both groups, but were more profound in the late-onset group. The average values of respective groups were compared to age-matched control values to assess whether there is an affect from a normal decline of CMAP with aging in normal subjects. However, the extent of reduction remained more conspicuous in the late-onset group (42% and 61% of control values for the late- and early-onset groups, respectively). In the tibial

Table 2 Nerve conduction studies

	1. Late-onset FAP TTR Met30 n=37-44	2. Control for late-onset cases n=60	3. Early-onset FAP TTR Met30 n=14-21	4. Control for early-onset cases n=50	p values		
					1 vs 2	3 vs 4	1 vs 3
Median nerve							
MCV (m/s)	43.7 \pm 9.6	56.5 \pm 3.3	47.8 \pm 7.3	58.2 \pm 4.0	< 0.0001	< 0.0001	NS
DL (ms)	5.5 \pm 2.0	3.5 \pm 0.3	4.6 \pm 1.7	3.3 \pm 0.5	< 0.0001	< 0.0001	NS
CMAP (mV)	3.2 \pm 2.7	7.6 \pm 2.6	5.1 \pm 3.9	8.3 \pm 2.8	< 0.0001	< 0.01	NS
Not elicited	2/44 cases (5%)		1/21 cases (5%)				
SCV (m/s)	47.8 \pm 7.7	55.0 \pm 5.3	48.3 \pm 8.4	57.3 \pm 5.2	< 0.001	< 0.0001	NS
SNAP (μ V)	2.1 \pm 3.5	22.7 \pm 8.8	8.8 \pm 9.3	30.9 \pm 12.6	< 0.0001	< 0.0001	< 0.01
Not elicited	26/41 cases (63%)		5/20 cases (25%)				
Tibial nerve							
MCV (m/s)	37.6 \pm 6.9	44.8 \pm 3.2	42.4 \pm 7.8	47.0 \pm 3.7	< 0.0001	< 0.05	NS
DL (ms)	5.8 \pm 1.5	4.0 \pm 0.7	5.6 \pm 1.8	4.0 \pm 0.6	< 0.0001	< 0.001	NS
CMAPs (mV)	0.7 \pm 1.2	10.7 \pm 3.3	2.6 \pm 5.1	12.7 \pm 3.6	< 0.0001	< 0.0001	NS
Not elicited	17/37 cases (46%)		8/18 cases (44%)				
Sural nerve							
SCV (m/s)	39.8 \pm 7.1	49.1 \pm 5.0	42.4 \pm 5.3	49.1 \pm 4.1	< 0.01	< 0.05	NS
SNAP (μ V)	0.4 \pm 1.4	14.9 \pm 9.3	2.8 \pm 5.0	18.6 \pm 9.7	< 0.0001	< 0.0001	< 0.05
Not elicited	36/41 cases (88%)		9/14 cases (64%)				

MCV motor nerve conduction velocity; DL distal latency; CMAP compound muscle action potential; SCV sensory nerve conduction velocity; SNAP sensory nerve action potential; NS not significant. Values are expressed as the mean \pm SD.

Control values were obtained in age-matched 60 normal volunteers (66.9 ± 6.9 years of age; male:female, 28:32) for the late-onset group and 50 normal volunteers (40.3 ± 7.3 years of age; male:female, 31:19) for the early-onset group.

Statistical analyses were performed using the Mann-Whitney *U* test

nerve, the reduction of CMAP was severe in both groups, except for some of the early-onset cases with a short duration of neuropathic symptoms. Similar to the median nerve results, the extent of the reduction in the tibial nerve was more profound in the late-onset group (7% and 20% of average values of the respective control groups). The reduction of SNAP was more profound compared to CMAP. As in the CMAP, the degree of the reduction was more conspicuous in the late-onset group compared to the early-onset group ($p < 0.01$ for the median nerve, $p < 0.05$ for the sural nerve). The average values of SNAP in the median nerve were 9% and 28% of the age-matched controls for the late- and early-onset groups, respectively. Those of the sural nerve were 3% and 15%, respectively. The degree of reduction of CMAP and SNAP was greater in the lower limbs than the upper limbs in both groups, but the feature of predominant lower-limb involvement seemed to be more conspicuous in the late-onset group judging from the respective mean values. Slowing of MCV and SCV, and a prolongation of DL were observed more or less in both groups. As for the late-onset group, MCV ranged from 14.2 to 57.4 m/s in the median nerve and 20.0 to 48.7 m/s in the tibial nerve, while SCV ranged from 34.6 to 64.0 m/s in the median nerve and 33.0 to 48.0 m/s in the sural nerve when the potentials were recorded. DL ranged from 3.3 to 10.0 ms in the median nerve and 3.2 to 9.9 ms in the tibial nerve. CMAP was correlated to MCV positively ($r = 0.583$, $p < 0.0001$ in the median nerve and $r = 0.460$, $p < 0.05$ in the tibial nerve) and to DL negatively ($r = -0.414$, $p < 0.01$ in the median nerve and $r = -0.532$, $p < 0.05$ in the tibial nerve) in the late-onset group.

As for the correlation between electrophysiological indices and the duration of neuropathic symptoms, the late-onset group did not show any correlation except for MCV in the tibial nerve ($r = -0.598$, $p < 0.01$). In the early-onset group, however, correlations were observed for MCV ($r = -0.612$, $p < 0.01$), DL ($r = 0.715$, $p < 0.001$), CMAP ($r = -0.553$, $p < 0.01$), SCV ($r = -0.537$, $p < 0.05$), and SNAP ($r = -0.621$, $p < 0.01$) in the median nerve, MCV ($r = -0.685$, $p < 0.05$) and CMAP ($r = -0.502$, $p < 0.05$) in the tibial nerve, and SCV ($r = -0.988$, $p < 0.001$) and SNAP ($r = -0.670$, $p < 0.01$) in the sural nerve. The relationship between electrophysiological indices and the duration of neuropathic symptoms in the individual cases for the median nerve only are illustrated in Fig. 1, because the potentials in most cases could be recorded in the median nerve, while many potentials were not elicited in the tibial and sural nerves. These findings indicate that electrophysiological indices normally tend to be preserved in the early phase of neuropathic symptoms in the early-onset group. However, they are aggravated as the duration of neuropathic symptoms becomes longer in this group. On the other hand, they tend to be severely affected even in cases with short duration of neuropathic symptoms in the late-onset group (Fig. 1).

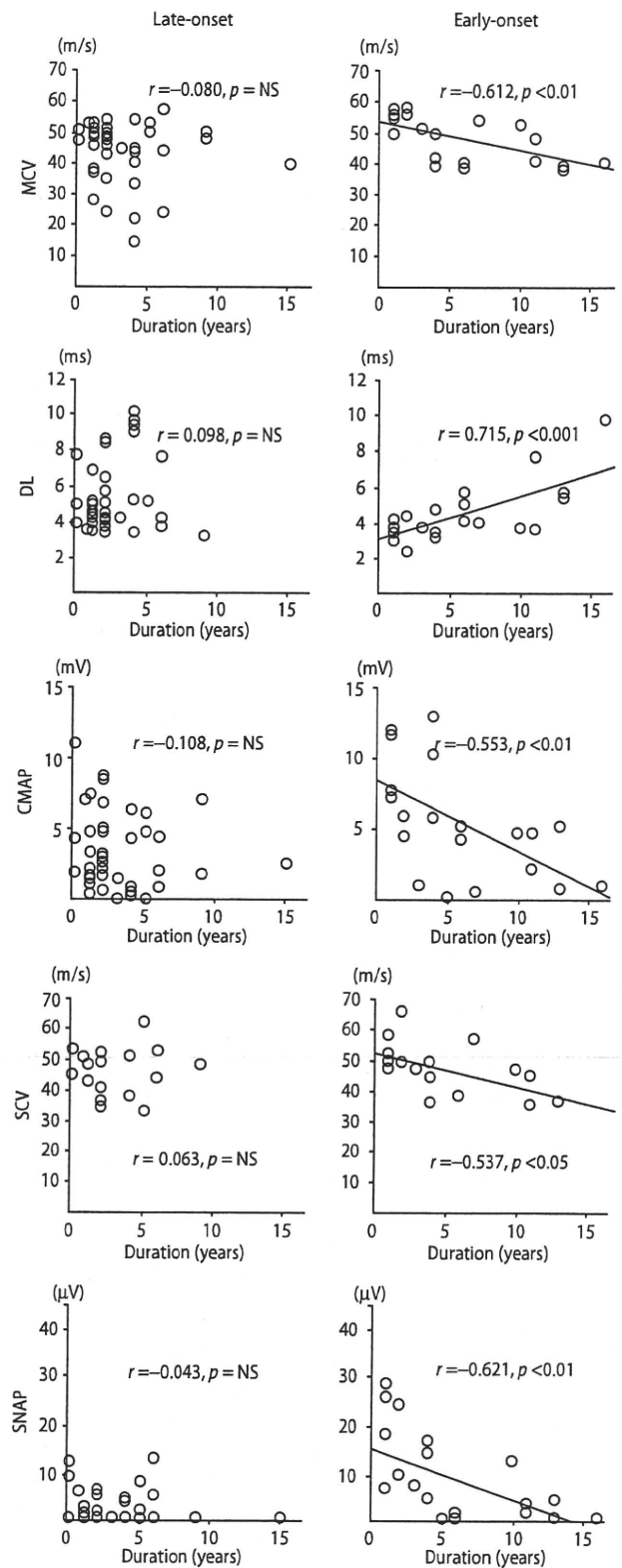
Sural nerve biopsy specimens showed a variable degree of myelinated fiber loss in both groups. Large myelinated fibers tended to be preserved and small-fiber predominant axon loss was obvious in early-onset cases with relatively short duration of neuropathic symptoms, as previously described [7, 18, 30]. As a whole, the densities of large myelinated fibers for late- and early-onset cases were 259 ± 311 and 914 ± 1018 fibers/mm², respectively, while those of small myelinated fibers were 713 ± 801 and 451 ± 608 fibers/mm², respectively. The densities of unmyelinated fibers were 5536 ± 4593 and 993 ± 1305 fibers/mm² for late- and early-onset cases, respectively, which is significantly reduced in early-onset cases ($p < 0.01$). Little or no axonal sprouting of myelinated fibers was observed in early-onset cases, while it was abundant in some late-onset cases, consistent with a previous report [18]. However, small-fiber predominant loss as that observed in early-onset cases was not obvious when the presence of regenerating small myelinated fibers was taken into consideration. The density of large myelinated fibers negatively correlated with the duration of neuropathic symptoms ($r = -0.769$, $p < 0.05$) in early-onset cases, while such a correlation was not found in late-onset cases (Fig. 2). In the teased-fiber study, the frequencies of axonal degeneration for late- and early-onset cases were 22.2 ± 8.1 and $19.5 \pm 6.9\%$, respectively, while those of segmental demyelination and remyelination were 7.5 ± 6.1 and $6.5 \pm 2.9\%$, respectively.

Discussion

In this study, we examined the electrophysiological features of late-onset FAP TTR Met30 cases unrelated to endemic foci and compared the findings to conventional early-onset cases in endemic foci. Although the activity of daily living, as assessed by the modified Rankin scale, was not very much different between the two groups, the following differences were present: (1) the extent of reduction of the CMAP and SNAP, especially the latter, was more profound in the late-onset group even when the decline of these indices with aging in normal control subjects were taken into consideration; (2) the feature of predominant lower-limb involvement tended to be more conspicuous in the late-onset group; and (3) electrophysiological indices tended to be aggravated as the duration of neuropathic symptoms become longer in the early-onset group, while most of these indices in the late-onset group did not show this correlation.

In order to verify the association between the aggravation of electrophysiological indices and disease duration, we evaluated pathologic findings in the sural nerve biopsy specimens and their correlation with the duration of neuropathic symptoms. The results were similar to those of the electrophysiological findings because the

Fig. 1 Relationship between the electrophysiological indices of the median nerve and the duration of neuropathic symptoms in 44 late-onset FAP TTR Met30 cases from non-endemic areas (left panels) and 21 early-onset FAP TTR Met30 cases from endemic foci (right panels). Bold lines represent regression lines. In the early-onset cases, motor nerve conduction velocity (MCV), compound muscle action potential (CMAP), sensory nerve conduction velocity (SCV), and sensory nerve action potential (SNAP) showed a negative correlation and distal latency (DL) showed a positive correlation with the duration of neuropathic symptoms. In contrast, such correlations were not found in late-onset cases. *NS* not significant



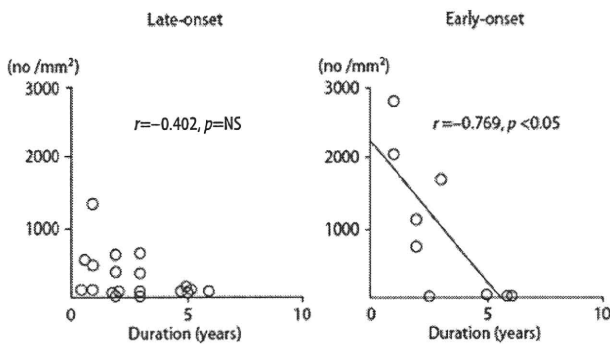


Fig. 2 Relationship between the density of large myelinated fibers in the sural nerve biopsy specimens and the duration of neuropathic symptoms in 21 late-onset FAP TTR Met30 cases from non-endemic areas (left panel) and 9 early-onset FAP TTR Met30 cases from endemic foci (right panel). Bold lines represent a regression line. In the early-onset cases, large myelinated fiber density showed a negative correlation with the duration of neuropathic symptoms. In contrast, such a correlation was not found in late-onset cases. *NS* not significant

large myelinated fiber density showed a negative correlation to the disease duration in early-onset cases, but not in late-onset cases. These findings support the view that these two forms of FAP TTR Met30 possess different underlying mechanisms that result in neuropathy.

Through the development of the gene diagnostic techniques, transthyretin-related FAP has been shown to exist in many nations worldwide [13, 28, 29]. For example, sporadic late-onset FAP TTR Met30 is apparently not as rare in Japan as previously thought [16]. However, the classic concept of FAP is strong in the minds of many neurologists; therefore, sporadic cases are often late to receive a correct diagnosis [1, 13]. Thus, recognizing the variability in clinical, electrophysiological, and histopathological features of FAP, especially, sporadic late-onset FAP TTR Met30 in non-endemic areas, is becoming more important. Previous studies suggested that the characteristic finding of sural nerve biopsy specimens in FAP TTR Met30 in endemic foci was small-fiber predominant loss including unmyelinated fibers in association with the presence of sensory dissociation and marked autonomic dysfunction [18, 30, 33, 38]. However, this feature is not common in late-onset cases in non-endemic areas [18]. Therefore, clinicopathological features in these cases tend to be nonspecific.

Indeed, physicians did not consider FAP until amyloid became evident in sural nerve biopsy specimens in many of our late-onset cases. However, amyloid deposition may appear negative by rough examination in these cases [23], so that careful histopathological examination is needed. Therefore, recognition of the possibility for sporadic late-onset FAP TTR Met30 is needed at the time of the initial clinical and electrophysiological evaluation to avoid an incorrect diagnosis. Late-onset FAP TTR Met30 cases may be too old to undergo liver transplantation, but recent advances in therapeutic strategies, such

as the prevention of amyloid fibril formation by stabilizing transthyretin tetramers or the reduction of transthyretin deposition by immunization [39, 40], render the value of early diagnosis of these patients more important. The demyelinating feature in FAP TTR Met30 is another impediment to correct diagnosis. The slowing of conduction velocity and prolongation of DL, which suggest the presence of demyelinating changes, were conspicuous in some of our patients. These demyelinating features were indicated by the presence of segmental demyelination in teased-fiber preparations [28, 30]. If a biopsy was not performed, physicians may diagnose these patients as CIDP [28]. Electrophysiological features indicating demyelination are not as conspicuous in typical axonal neuropathies, including alcoholic and beriberi neuropathies [14, 15, 17, 19], although the presence of secondary segmental demyelination due to axonal atrophy has been reported in association with these neuropathies [17, 21]. On the other hand, the electrophysiological features indicating demyelination should be taken into consideration for FAP TTR Met30. The demyelinating feature, at first glance, does not exclude the possibility of this disease.

The finding that CMAP and SNAP were reduced in the late-onset group compared to the early-onset group in spite of a similar activity of daily living may reflect the tendency of small-fiber predominant loss in early-onset cases and the loss of the entire range of nerve fibers in late-onset cases. The nerve conduction studies performed here reflect the abnormalities in large myelinated fibers, and these findings support the view that the differential modality of nerve fiber involvement is not confined to the sural nerve. However, this does not explain the difference in the mechanisms of axonal loss between early- and late-onset cases.

Although the pathogenesis of peripheral neuropathy in amyloidosis has not been clarified, the obliteration or dysfunction of small vessels [4, 8], compression or infiltration of amyloid deposition [7, 30], or toxic effect of amyloid precursors [36, 37] may be involved. According to our previous study of biopsy and autopsy cases, nerve fibers tended to be more severely reduced as a whole, while the amount of amyloid deposition tended to be milder in late-onset FAP TTR Met30 cases in non-endemic areas than in early-onset cases in endemic foci [18].

In addition, axonal sprouting was abundant in late-onset cases, even when the age at examination was taken into account [18]. This observation suggests a long history of nerve damage and repair in late-onset cases. Amorphous material, which was transthyretin-positive, but Congo-red-negative, suggesting the presence of nonfibrillar amyloid precursor, was shown to be abundant in a late-onset case [18]. Thus, amyloid may not start to deposit until the patients become elderly, but the toxicity of amyloid precursors may have a subclinical

affect during the presymptomatic stage in late-onset cases.

In this study, most of the electrophysiological indices in the early-onset group tended to remain normal during the initial phase of neuropathic symptoms and seemed to become aggravated with increased duration of neuropathic symptoms. However, most of these indices in the late-onset group were aggravated in the initial phase. The relationship between large myelinated fiber density in the sural nerve biopsy specimens and the duration of neuropathic symptoms also supports this view. Taken together, our results suggest that toxicity of non-fibrillar form of transthyretin may affect the peripheral nervous system for a long period prior to deposition of amyloid fibrils in late-onset cases. This long-standing amyloid precursor toxicity may induce the whole range of axonal damage in late-onset cases, although this issue should be further assessed. The tendency of more predominant lower limb involvement, which may be interpreted as a length-dependency of axonal damage similar

to that caused by the toxicity of ethanol or its metabolites [21], is more conspicuous in late-onset cases. Therefore, toxic factors are more likely to participate in these cases. In early-onset cases, the deposition of amyloid fibrils may be severe even in the early phase of neuropathic symptoms judging from the amount of amyloid deposition compared to late-onset cases [18]. Amyloid fibrils, therefore, may cause small-fiber predominant axonal loss as previously suggested [30]. Thus, differential duration of exposure to toxic amyloid precursor may create a different mode of axonal loss.

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Rapidly developing weakness mimicking Guillain-Barré syndrome in beriberi neuropathy: Two case reports

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Abstract

Objective: We examined the diagnostic difficulty in thiamine deficiency.

Methods: We report on two patients with polyneuropathy associated with thiamine deficiency (i.e., beriberi neuropathy) that presented with acute motor symptoms mimicking Guillain-Barré syndrome.

Results: The cause of the thiamine deficiency was associated with gastrectomy to treat cancer in a 46-y-old man and with dietary imbalance in a 33-y-old man. The thiamine deficiency was not related to alcohol intake in either patient. In both patients, the upper and lower extremities showed a rapidly progressive weakness over the course of 1 mo. Muscle weakness in the first patient progressed even after admission to the hospital, and urinary retention, Wernicke's encephalopathy, lactic acidosis, paralytic ileus, and heart failure appeared subsequently. Clinical symptoms in both patients showed improvement after initiation of thiamine administration, although some residual deficit remained.

Conclusion: Thiamine deficiency must be actively considered as a possible cause of polyneuropathy, and variability in its clinical features should be taken into consideration. © 2008 Elsevier Inc. All rights reserved.

Keywords:

Thiamine; Beriberi; Neuropathy; Guillain-Barré syndrome

Introduction

Thiamine (vitamin B1) deficiency may occur in persons with a decreased intake of thiamine caused by consumption of high-carbohydrate, low-thiamine diets [1–3], chronic alcoholism [4,5], anorexia nervosa [6], or hyperemesis gravidarum [7]. In addition, it may occur in persons with increased thiamine requirements such as laborers performing heavy outdoor work [1] and pregnant or lactating women [8]. Thiamine deficiency can result in peripheral neuropathy, heart failure, and Wernicke-Korsakoff syndrome. The disorder has been largely forgotten by many physicians, especially those in developed countries, because prophylac-

tic thiamine administration in high-risk cases and nutritional education have considerably decreased the occurrence of thiamine deficiency. However, some investigators have suggested that neuropathy associated with thiamine deficiency is not uncommon in patients with a variety of background factors including chronic alcoholism [5,9,10], prolonged parenteral nutrition [11], a postgastrectomy state to treat ulcers or neoplasms [3,5,12], and after operations to treat morbid obesity (i.e., bariatric surgery) [13]. Therefore, thiamine deficiency should still be considered in various clinical settings.

In this report, we present two cases of polyneuropathy associated with thiamine deficiency (i.e., beriberi neuropathy), one associated with gastrectomy to treat cancer and one with dietary imbalance, that presented with acute motor symptoms and posed a diagnostic difficulty by mimicking Guillain-Barré syndrome. A rough clinical profile of the

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Table 1
Nerve conduction study in patients 1 and 2

	Median nerve					Tibial nerve			Sural nerve	
	Motor		Sensory			Motor			Sensory	
	MCV (m/s)	DL (ms)	CMAP (mV)	SCV (m/s)	SNAP (μ V)	MCV (m/s)	DL (ms)	CMAP (mV)	SCV (m/s)	SNAP (μ V)
Patient 1	55.0	4.68	4.66	40.3	10.40	45.2	4.83	8.22	49.2	1.00
Patient 2	57.4	3.94	3.78		NE	40.0	5.58	0.13		NE
Controls (mean \pm SD)*	57.8 \pm 3.7	3.4 \pm 0.4	10.7 \pm 3.5	57.8 \pm 4.7	23.5 \pm 8.4	46.9 \pm 3.5	4.5 \pm 0.8	10.9 \pm 3.8	51.0 \pm 5.1	11.5 \pm 4.7

CMAP, compound muscle action potential; DL, distal latency; MCV, motor nerve conduction velocity; NE, not elicited; SCV, sensory nerve conduction velocity; SNAP, sensory nerve action potential

* Control values were obtained in 191 normal volunteers (mean age \pm SD 48.7 \pm 16.5 y, male:female 97:94) for the median nerve, 121 (mean age \pm SD 49.9 \pm 15.0 y, male:female 64:57) for the tibial nerve, and 133 (mean age \pm SD 50.6 \pm 15.6 y, male:female 74:59) for the sural nerve [9,12].

former case was reported previously as 1 of 17 cases with postgastrectomy polyneuropathy in thiamine deficiency [12], and the latter is a new case. We believe that these case studies provide further insights into the current knowledge of this classic but occasionally forgotten disease.

Case reports

Patient 1

A 46-y-old man noted weakness in his lower extremities at 10 d before admission to the hospital. He reported no preceding episode of upper respiratory or gastrointestinal infection. Numbness in the distal portions of the lower extremities followed the initial weakness. The weakness gradually progressed over 10 d until the patient had great difficulty in walking by himself. The patient had undergone a total gastrectomy for adenocarcinoma of the stomach 3 y previously, with reconstruction using a jejunal pouch interposition. His condition had been good without complications. The patient and his wife took particular care with nutritional balance since the operation, and he completely abstained from alcohol. Neurologic examination revealed distal-dominant weakness in the lower extremities. A mild sensory deficit involving all modalities was present in the legs. Patellar and Achilles tendon reflexes were absent. Examination of the upper extremities was normal except for hypoactive deep tendon reflexes. Plantar responses were flexor on both sides. Cerebrospinal fluid examination revealed no abnormalities in protein content or cell count. A nerve conduction study, performed on the day of admission, revealed a reduction in sensory nerve action potential in the sural nerve, indicative of axonal neuropathy (Table 1). After admission to the hospital, the weakness rapidly worsened, ascending from the lower to the upper extremities to render the patient bedridden after 3 d. Urinary retention developed at 17 d after the first symptom, and, at 21 d from onset, ocular movements had become affected. A sural nerve biopsy specimen, obtained at 22 d from onset, revealed axonal

degeneration, with a predominant loss of large myelinated fibers (Fig. 1). Severe edema in the subperineurial space also was observed. Decreases in the level of consciousness and the occurrence of lactic acidosis appeared on day 27. Retention of gas in the gastrointestinal tract with a decrease of bowel sounds also was noted. On day 28, severe heart failure that was unresponsive to diuretic agents appeared. A 100-mg intravenous dose of fursultiamine was given to initiate therapy on that day. The total thiamine concentration in the whole blood at this time was 15 ng/mL (normal 20–50 ng/mL, representing a mean \pm 2 SD for 100 normal control subjects [5,9,12]). The patient recovered from heart failure and from the disturbance of consciousness in several days. His neurologic symptoms also showed gradual improvement beginning from that day. Six months later, he was just able to walk by himself.

Patient 2

A 33-y-old man noted numbness in his distal lower limbs at 2 wk before admission. Weakness in the lower limbs

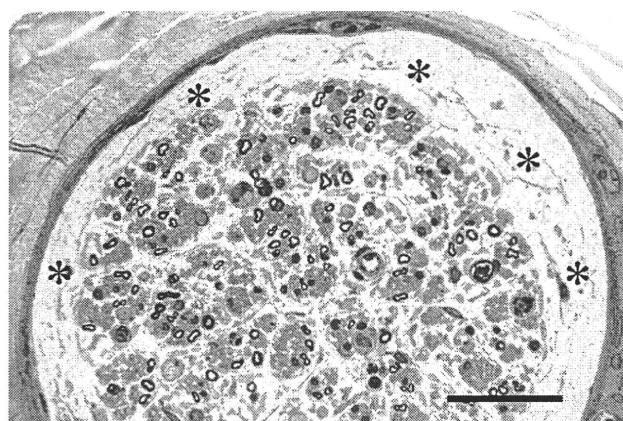


Fig. 1. Transverse section of a sural nerve biopsy specimen in patient 1. Loss of predominantly large myelinated fibers is observed. Extensive endoneurial edema with enlargement of the subperineurial space is indicated by asterisks. Scale bar = 50 μ m.

appeared at 5 d before admission and subsequently progressed rapidly in the lower limbs and the upper limbs. The patient was bedridden at the time of admission to the hospital. He did not have any preceding infectious episode. He did not like to eat meat or vegetables, preferring milled white rice and noodles with no side dishes. He took these meals at very irregular intervals. The patient reported only occasional intake of alcohol, which did not exceed 20 g/d of ethanol consumption. He had maintained these dietary patterns for 6 y. Once he was engaged in heavy outdoor work, but he then lost his job. He had no history of surgical procedures including gastrectomy. Neurologic examination revealed severe distal-dominant weakness in the lower and upper extremities. The grasping power of both hands was 0 kg. Plantarflexion and dorsiflexion of the foot could not be performed bilaterally. Severe sensory deficits involving all modalities were present in the legs, whereas sensory loss was mild in the hands. Deep tendon reflexes were moderately decreased in the upper limbs and were absent in the lower limbs. Plantar responses were flexor on both sides. Consciousness was intact, and cranial nerve function was normal. Cramping pain was present in the muscles of the lower limbs. Edema was not noted in the limbs. Chest radiographs and echocardiogram revealed no signs of heart failure. Cerebrospinal fluid examination revealed no abnormalities. Nerve conduction study showed reductions in compound muscle action potentials that were mild in the upper limbs and severe in the lower limbs (Table 1). Sensory nerve action potentials could not be evoked in the upper and lower limbs. A diagnosis of Guillain-Barré syndrome was considered initially, but predominant axonal features and a history of dietary imbalance led us to consider beriberi neuropathy. A 100-mg intravenous dose of fursultiamine was administered to begin treatment. Total thiamine concentration in the whole blood at that time was 7 ng/mL. Symptoms showed gradual improvement from that day. He was able to walk with a cane 1 mo later.

Discussion

Beriberi neuropathy, caused by dietary imbalance without associated alcohol intake, is currently considered rare in developed countries. Therefore, clinicians are relatively unaware of thiamine deficiency as a possible cause of polyneuropathy, especially in patients without Wernicke's encephalopathy or heart failure at the initial phase, as in the present cases. Moreover, thiamine deficiency is not widely recognized to be related to various background factors. Previous studies of neuropathy associated with thiamine deficiency have suggested that the major causes of thiamine deficiency in Japan are heavy alcohol intake, gastrectomy to treat cancers or ulcers, and dietary imbalance [3,5,12]. In Western countries, where morbid obesity is prevalent, bariatric surgery has become a risk factor [13]. Gastrectomy to

treat cancers or ulcers, and not associated with the treatment of obesity, is not widely appreciated as a possible cause of thiamine deficiency, especially when a patient's clinical condition is favorable without complications for a long period after surgery. The varied symptoms of polyneuropathy are another impediment to correct diagnosis. Previous studies have indicated the presence of variable clinical features of beriberi neuropathy, including the progression and relative predominance of motor and sensory deficits [3,5,10,12]. Some patients, especially those with rapid progression mimicking Guillain-Barré syndrome, as in the present cases, may become worse when intravenous thiamine is not administered soon after admission. Because the pathologic characteristic of beriberi neuropathy is considered to be a length-dependent dying-back axonal neuropathy, patients with thiamine deficiency, without exception, manifest symmetric polyneuropathy with more involvement in the lower than upper limbs, showing a centripetal pattern of progression [3,5,12]. In contrast, weakness that is predominant in the proximal portions of the limbs, which may be caused by simultaneous involvement of skeletal muscles due to thiamine deficiency [14], may amplify the variability of clinical features of beriberi neuropathy in some patients.

Similar to variable neuropathic features, the combination of other symptoms associated with thiamine deficiency such as Wernicke's encephalopathy and heart failure varies among individual patients [3,5,12]. If thiamine deficiency is exacerbated without treatment due to delayed reference or diagnosis, as occurred in patient 1 in the present report, this combination of symptoms may tend to worsen. However, the presence of interindividual differences in susceptibility to the development of thiamine deficiency-related disorders and differential vulnerabilities to thiamine deficiency of certain tissues and cell types has been suggested [12,15]. The cause of these variations has not been determined. Genetic factors may cause susceptibility to thiamine deficiency for individual organs [15]. For example, genetic variability of transketolase may relate to susceptibility to Wernicke's encephalopathy [16,17]. Thiamine-responsive megaloblastic anemia associated with diabetes mellitus and deafness results from a mutation of the gene encoding the high-affinity thiamine transporter protein [18–20]. This genetic defect preferentially involves hematopoietic cells, pancreatic islet cells, and auditory apparatus cells. Seasonal ataxic syndrome in western Nigeria, which is caused by eating the pupae of an African silkworm that possesses a heat-resistant thiaminase, is known to manifest ataxia as the main clinical feature of thiamine deficiency [21,22]. Ethnic variation of genetic background may explain the clustering of clinical symptoms in thiamine deficiency on ataxia in this region.

Since the introduction of high-performance liquid chromatography in the 1980s [23,24], direct thiamine evaluation has become widely used in routine practice. Before this method became available, thiamine status mostly had been evaluated indirectly through a functional determination of

activity of erythrocyte transketolase, a thiamine-dependent enzyme [25]. In addition, pyruvate and lactate may accumulate in thiamine deficiency because pyruvate, the final glycolytic product, cannot be utilized in the citric acid cycle for adenosine triphosphate generation; instead, it is converted to lactate [26]. Therefore, elevation of serum pyruvate or lactate concentration may support the diagnosis of thiamine deficiency. Because genetic factors have been suggested to determine individual susceptibility to thiamine deficiency [3,12,15,16], laboratory and clinical data should be assessed in a comprehensive manner. In our patients total thiamine concentrations in whole blood evaluated by high-performance liquid chromatography were abnormally low and neuropathic symptoms improved by thiamine supplementation. However, one may argue that deficiencies of other vitamins, including nicotinic acid [27], vitamin B2 [28], vitamin B6 [29], vitamin B12 [30,31], or folate [32], might have contributed the pathogenesis of neuropathy in our patients. However, characteristic symptoms associated with these individual vitamin deficiencies were not present. These clinical pictures include anorexia, diarrhea, erythematous and hyperkeratotic dermatides, and mental changes in pellagra (nicotinic acid deficiency); cheilosis, glossitis, keratoconjunctivitis, and dermatitis involving nasolabial folds, scrotum, and labia in vitamin B2 deficiency; and myelopathy in vitamin B12 and folate deficiency. Thus, we believe that these vitamins were not major causal factors of neuropathy in our patients.

As for the prognosis of beriberi neuropathy, some residual deficits persist in many patients, although the functional status improved after thiamine administration [12]. According to a previous study, substantial functional recovery, particularly in motor involvement, was achieved by thiamine supplementation within 6 mo, but sensory symptoms and Korsakoff's psychosis were likely to show residual deficits [12]. Indeed, patient 1, whose muscle weakness progressed even after admission to the hospital and various symptoms associated with thiamine deficiency other than neuropathy had appeared, took longer to recover ambulation than patient 2, whose clinical manifestations are those related to neuropathy alone. Thus, treatment should be initiated as early as possible.

In conclusion, thiamine deficiency should be actively considered as a possible cause of polyneuropathy, and variability of its clinical features also should be taken into consideration.

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Neuropathic pain correlates with myelinated fibre loss and cytokine profile in POEMS syndrome

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ABSTRACT

Objective: To reveal characteristic clinicopathological correlates of polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes (POEMS) syndrome.

Methods: The clinical features of 22 patients with POEMS syndrome were investigated and correlated with the histopathological features of sural nerves and serum cytokine profiles.

Results: More than half of the patients complained of pain in the lower extremities, which is closely related to hyperalgesia. Assessment of the total nerve fibre population using complete transverse sural nerve cross-sections, excluding the marked enlargement of endoneurial areas due to intrafascicular oedema, showed that myelinated fibres, especially small myelinated fibres, were reduced, whereas unmyelinated fibres were preserved. Uncompacted myelin lamellae and segmental demyelination were seen more frequently in the small, rather than the large, myelinated fibres. The presence of hyperalgesia was electrophysiologically associated with a reduction of sensory nerve action potentials in the sural nerve ($p < 0.05$) and histopathologically associated with myelinated fibre loss ($p < 0.01$). Serum levels of proinflammatory cytokines (interleukin-1 β , interleukin-6 and tumour necrosis factor- α), but not their soluble receptors, were significantly elevated in patients with hyperalgesia ($p < 0.05$ – 0.01).

Conclusions: Hyperalgesia seen in patients with POEMS syndrome is closely related with a reduction in the myelinated, but not unmyelinated, fibre population. Elevation of proinflammatory cytokines is also correlated with hyperalgesia. The painful symptoms in POEMS syndrome may be generated by well-preserved unmyelinated C-fibres due to the lack of inhibitory myelinated A-fibres, along with cytokine sensitisation.

POEMS syndrome—an acronym for polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes—is a unique multisystem disorder that is also known as Crow–Fukase syndrome.¹ Because it is strongly associated with plasma-cell dyscrasia, especially osteosclerotic myeloma, monoclonal proliferation of plasma cells has been thought to play an important role in the development of various POEMS symptoms.^{1,2} In addition, serum concentrations of cytokines, such as interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumour necrosis factor- α (TNF- α), are elevated.³ Recent studies implicate vascular endothelial growth factor (VEGF) as another pathogenetic factor.^{4,5} As this syndrome manifests a variety of symptoms, it has been typically examined from the viewpoint of

wide-ranging organ involvement;^{1,2,6,7} reports exclusively examining its neuropathic features are relatively rare. Furthermore, because electrophysiological and histopathological features are those of demyelinating neuropathy,^{7,8} neuropathy in POEMS syndrome has often been diagnosed as chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) when the associated symptoms other than neuropathy are not conspicuous.^{9,10} New therapeutic approaches to POEMS syndrome have been described that differ from CIDP approaches.^{6,10–13} Therefore, clarification of the clinical and pathological features and their correlations unique to POEMS syndrome is needed to improve the diagnostic accuracy and subsequent therapeutic consequences.

The present study describes the clinical, pathological and cytokine profile features that are unique to POEMS syndrome.

PATIENTS AND METHODS

Patients

Twenty-two consecutive patients with POEMS syndrome who were referred to the Department of Neurology of Nagoya University Hospital from 1987 to 2007 were investigated. Patients included 13 men and 9 women aged 54.8 ± 13.6 (mean \pm SD) years. Clinical features and laboratory data assessed before the initiation of treatment are summarised in table 1. As for the assessment of neuropathic pain, patients were asked whether they have spontaneous pain. Mechanical stimuli, both normally painful and normally non-painful, were applied on the distal portion of the lower limbs (dorsum of foot and lateral surface of the lower leg) by examiners to evaluate hyperalgesia. The patient's subjective responses to these stimuli were qualified and the amount of pain was described in categorical scale (none, mild, moderate and severe). Because patients with POEMS syndrome lack a contralateral homologous normal side due to their symmetrical polyneuropathy pattern, responses to normally painful stimuli such as pinprick and pinwheel were compared with those on a more proximal portion of the limbs (anterior thigh). Abnormal sensations without pain were not considered to be hyperalgesia. Patients' functional status was assessed at the peak phase according to the modified Rankin Scale.¹⁴ Motor and sensory conduction was measured in the median, tibial and sural nerves in all patients during their initial clinical assessment by neurologists, using a standard method with surface electrodes for stimulation and recording.

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Table 1 Clinical features of POEMS syndrome

Case	Age/Sex	Organomegaly	Endocrinopathy	M-protein	Skin change	Bone lesion	VEGF level* (pg/ml)	Cerebrospinal fluid protein (mg/dl)
1	43/W	CM, HM, SM, LA	—	IgA λ	HP, HT	Sclerotic	1310	93
2	68/W	CM, HM, SM	—	IgA λ	HP, HT	Sclerotic	2290	63
3	47/W	HM, SM	—	IgG λ	HP	—	1670	38
4	47/W	CM, HM, SM, LA	Hy	IgA λ	HP, HT	—	>2000	95
5	31/M	HM, SM	Gy, Im	IgG λ	HP, HT	Sclerotic, lytic	5500	130
6	54/W	HM, LA	—	IgG κ	HP, HT	Sclerotic	756†	107
7	48/M	LA	Gy	IgG λ	HP	Sclerotic	1830	63
8	46/M	HM, LA	Gy	IgG λ	HP	Sclerotic	>2000	108
9	62/M	SM	Gy	IgA λ	HP, HT	Sclerotic	>2000	165
10	58/M	—	Im	IgA λ	HP, HT	Sclerotic	>2000	53
11	57/W	CM, HM, SM	Hy	IgA λ	HP, HT	Sclerotic	>2000	179
12	28/W	HM, SM	—	IgG λ	HP, HT	Sclerotic, lytic	1120	238
13	72/M	HM, SM	Gu	IgG λ	HP	—	ND	124
14	67/W	CM, SM, LA	Hy	IgG λ	HP	—	>2000	97
15	63/M	HM, SM, LA	Hy, Gy, Im	IgG λ	HP, HT	—	ND	82
16	45/M	LA	Gu, Hy, Im	IgA λ	HP, HT	—	ND	196
17	69/W	ND	—	IgA λ	HP	—	ND	108
18	60/M	LA	Hy	IgA λ	HP, HT	—	ND	62
19	63/M	SM	Im	—	HP	Sclerotic	814†	78
20	45/M	HM	Gu, Gy	—	HP, HT	—	1280	360
21	85/M	CM, HM, SM, LA	Gy	IgA λ	HP	—	500†	57
22	47/M	HM, SM	Im	IgG λ	HP, HT	Lytic	1280	81

VEGF, vascular endothelial growth factor (normal <707 pg/ml for serum, <115 pg/ml for plasma); —, absent; CM, cardiomegaly; Gu, glucose intolerance; Gy, gynecomastia; HM, hepatomegaly; HP, hyperpigmentation; HT, hypertrichosis; Hy, hypothyroidism; Im, impotence; LA, lymphadenopathy; ND, not determined; POEMS, polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes; SM, splenomegaly.

*Serum VEGF levels were measured except in patients 6, 19 and 21.

†Plasma VEGF levels were measured.

Pathological assessment of sural nerve biopsy specimens

Sural nerve biopsy was performed in 21 patients before the initiation of treatment as described previously.^{15–17} Specimens were divided into two portions. The first was fixed in 2.5% glutaraldehyde in 0.125 M cacodylate buffer (pH 7.4) and embedded in epoxy resin for morphometric and ultrastructural study. Densities of small and large myelinated fibres were assessed in toluidine-blue-stained semithin sections using a computer-assisted image analyser (Luzex FS; Nikon, Tokyo, Japan), as described previously.^{15–16–18} To determine the extent of nerve fibre loss, the total number of nerve fibres in complete transverse sections of sural nerves was estimated because the density might be reduced without actual nerve fibre loss when the endoneurium was enlarged by a pathological condition such as endoneurial oedema. Therefore, the total endoneurial area and subperineurial space devoid of nerve fibres were assessed using the image analyser (Luzex FS) in cases in which complete transverse sections of the sural nerve could be obtained; we estimated the total number of nerve fibres in these cases. Two cases (patients 9 and 14) were excluded because their sural nerve was only partially obtained. To determine the total numbers per complete cross-section of the sural nerve, the density of each morphometric index was multiplied by the endoneurial area from which the subperineurial space devoid of nerve fibres was subtracted.¹⁷ The remainder of the glutaraldehyde-fixed sample was processed for the teased-fibre study, in which at least 200 single fibres were isolated and their pathological condition was assessed microscopically according to criteria described previously.^{15–19} Each teased fibre was divided into two categories based on the diameter of the middle portion of the largest segment. Fibres of 6.73 μ m or more in diameter were designated as large fibres and those of less than 6.73 μ m as small fibres.¹⁵ Fibres showing axonal degeneration were not included when the diameter of the fibres was evaluated. The second portion of

each specimen was fixed in 10% formalin solution and embedded in paraffin. Sections were cut by routine methods and stained with hematoxylin and eosin, Congo red, the Klüver–Barrera method and the Masson trichrome method.

For the electron microscopic study, epoxy resin-embedded specimens were cut into ultrathin transverse sections and stained with uranyl acetate and lead citrate. To assess the density of unmyelinated fibres, electron microscopic photographs were taken at a magnification of 4000 \times in a random fashion to cover the area of ultrathin sections, as described previously.^{15–16–20} These electron micrographs were enlarged to about 6500 \times . The total area analysed in electron micrographs was at least 0.03 mm², obtained from at least three fascicles. Unmyelinated fibres were distinguished from Schwann cell cytoplasmic profiles by their round or oval shape, a lighter appearance than Schwann cell cytoplasm, and often a higher incidence of microtubules.²¹ The presence of mesaxon-like structures and a greater density of axolemma than Schwann cell membranes were also criteria for identifying unmyelinated fibres.²¹ Disproportionately large unmyelinated fibres over 3 μ m in diameter were not counted because these fibres were considered to be originally myelinated fibres and formed as a consequence of demyelination.^{17–22} A conglomerate of Schwann cell processes with or without unmyelinated axons, and enclosed by a continuous loop of basal lamina, was designated as a “Schwann cell subunit”, as described previously.^{17–22} Only Schwann cell subunits related to unmyelinated fibres were counted. Unmyelinated fibres found in Schwann cell subunits that previously contained myelinated axons (ie, bands of Büngner) were not counted because they could be sprouts from regenerating myelinated fibres.^{21–23} In addition, unmyelinated fibres and Schwann cell subunits that took part in the formation of regenerating clusters of myelinated axons were not counted for the same reason.²² The bands of Büngner were

Table 2 Neuropathic features of POEMS syndrome

Case	Initial symptom	Duration until biopsy (m)	Motor signs			Sensory signs*				Modified Rankin Scale‡
			Weakness		Muscle atrophy	Spontaneous pain	Pain†	Vibration	Joint sense	
UE (P/D)	LE (P/D)									
1	N	12	+1/+2	+2/+1	+1	0	+1	-3	-2	2
2	N	5	+1/+2	+2/+3	0	0	-2	-3	-2	4
3	N	3	+1/0	+2/+1	+1	+2	+2	-1	0	3
4	N	24	+2/+1	+2/+1	+1	+3	+3	-3	-2	2
5	W	12	0/0	+2/+3	0	+2	+2	-2	-2	3
6	N	7	0/+1	+1/+3	0	0	-2	-3	-1	3
7	N	10	0/0	+2/+1	0	+3	-3§	-3	-3	2
8	W	1	0/+1	+2/+3	0	0	0	-2	-1	3
9	N	6	+1/+1	+2/+3	+1	+3	+3	-2	-2	3
10	N	4	0/+2	+1/+3	+1	+2	+2	-3	-2	3
11	N	4	0/+2	+3/+3	+2	0	-2	-3	-3	3
12	W	9	+2/+3	+3/+3	+2	0	0	-2	0	4
13	W	5	+1/+1	+2/+3	0	0	+1	-1	-1	3
14	W	7	0/0	+2/+3	0	0	0	-2	0	2
15	N	6	+1/+2	+1/+1	0	+3	+3	-3	-3	2
16	N	1	+1/+1	+1/+2	0	0	0	-3	-1	2
17	N	12	+1/+1	+2/+3	+1	+1	+2	-3	-1	3
18	N	3	+1/+1	+1/+1	+1	0	+2	-3	-2	2
19	N	7	0/0	+2/+3	0	+3	+2	-3	-2	3
20	W	2	0/0	+2/+3	0	+1	+3	-2	-2	3
21	N	12	0/0	0/0	0	+1	+1	-2	-1	2
22	N	3¶	0/0	+1/+3	+1	+2	+2	-1	0	2

+, positive findings; -, negative findings; 0, none.

+1, +2, and +3 represent mild, moderate and severe degree of muscle weakness, atrophy and sensory signs.

-1, -2, and -3 represent mild, moderate and severe reduction for sensory signs.

D, distal portion; LE, lower extremities; N, numbness of the lower extremities; P, proximal portion; POEMS, polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes; UE, upper extremities; W, weakness of the lower extremities.

*Sensory signs were assessed in the distal portion of the lower limbs.

†Pain is divided into two categories; hyperalgesia (+1, +2 or +3) and hypoalgesia (-1, -2 or -3).

‡Modified Rankin Scale:¹⁴ 0, asymptomatic; 1, non-disabling symptoms not interfering with lifestyle; 2, minor disability from symptoms leading to some restriction of lifestyle but not interfering with patients' capacity to look after themselves; 3, moderate disability from symptoms significantly interfering with lifestyle or preventing totally independent existence; 4, moderately severe disability from symptoms clearly precluding independent existence, although not requiring 24-hour attention from a caregiver; and 5, severe disability and total dependence, requiring constant attention day and night.

§Painful sensation in patient 7 was so severe that the response to mechanical stimuli was not obvious.

¶Duration until first referral to the hospital.

distinguished from subunits of non-myelinating Schwann cells, as described previously.^{17 21 22}

The widening of major dense lines of myelin lamellae was designated as uncompacted of myelin. Myelinated fibres were considered as presenting uncompacted myelin lamellae (UML) when at least three lamellae were not compacted along a semi-circumference of the myelin sheath or in an additional loop.²⁴ The percentage of fibres with UML in the large myelinated fibres and small myelinated fibres were separately assessed. The area of ultrathin section was overviewed by photographs at a magnification of 2000 \times . Fibres with UML confirmed by greater magnification were marked on the photographs and the diameter of each myelinated fibre was assessed using a computer-assisted image analyser (WinROOF; Mitani, Fukui, Japan). At least 300 fibres were assessed to determine the frequency of UML.

Control values were obtained from five autopsy cases in which the patients died of non-neurological diseases (male:female, 1: 4; age range: 35-71 years, mean \pm SD: 55.8 \pm 14.5 years). Specimens were processed in the same manner as for POEMS syndrome patients.

Assessment of serum cytokines and cytokine receptors levels

All patients were examined before initiation of treatment. Measurements of IL-1 β , IL-6 and TNF- α were performed in 15 patients and values were compared to 20 control patients with other neurological diseases, including spinocerebellar ataxia,

multiple system atrophy and spinobulbar muscular atrophy. Measurements of IL-1 receptor antagonist (IL-1ra), soluble IL-6 receptor (sIL-6r), soluble TNF receptor I (sTNFrI) and soluble TNF receptor II (sTNFrII) were performed in 12 patients and values were compared to those of 7 patients with other neurological diseases. Peripheral blood was taken in dry tubes and centrifuged within 3 hours. The sera were kept frozen at -80°C until analysed. Blood samples were obtained in the absence of overt fever, infection and shock. An enzyme-linked immunosorbent assay kit was used for the quantification of IL-1 β (BioSource, Nivelles, Belgium), TNF- α (JIMRO, Takasaki, Japan), IL-1ra (R&D Systems, Minneapolis, USA), sIL-6r (R&D Systems), sTNFrI (R&D Systems) and sTNFrII (R&D Systems), and a chemiluminescent enzyme immunoassay kit was used for the quantification of IL-6 (Fujirebio, Tokyo, Japan).

Statistical analyses

Quantitative data, presented as the mean \pm SD, were compared with control values. Statistical analyses were performed using χ^2 test or the Mann-Whitney *U* test as appropriate. Values of *p* less than 0.05 were considered to indicate significance.

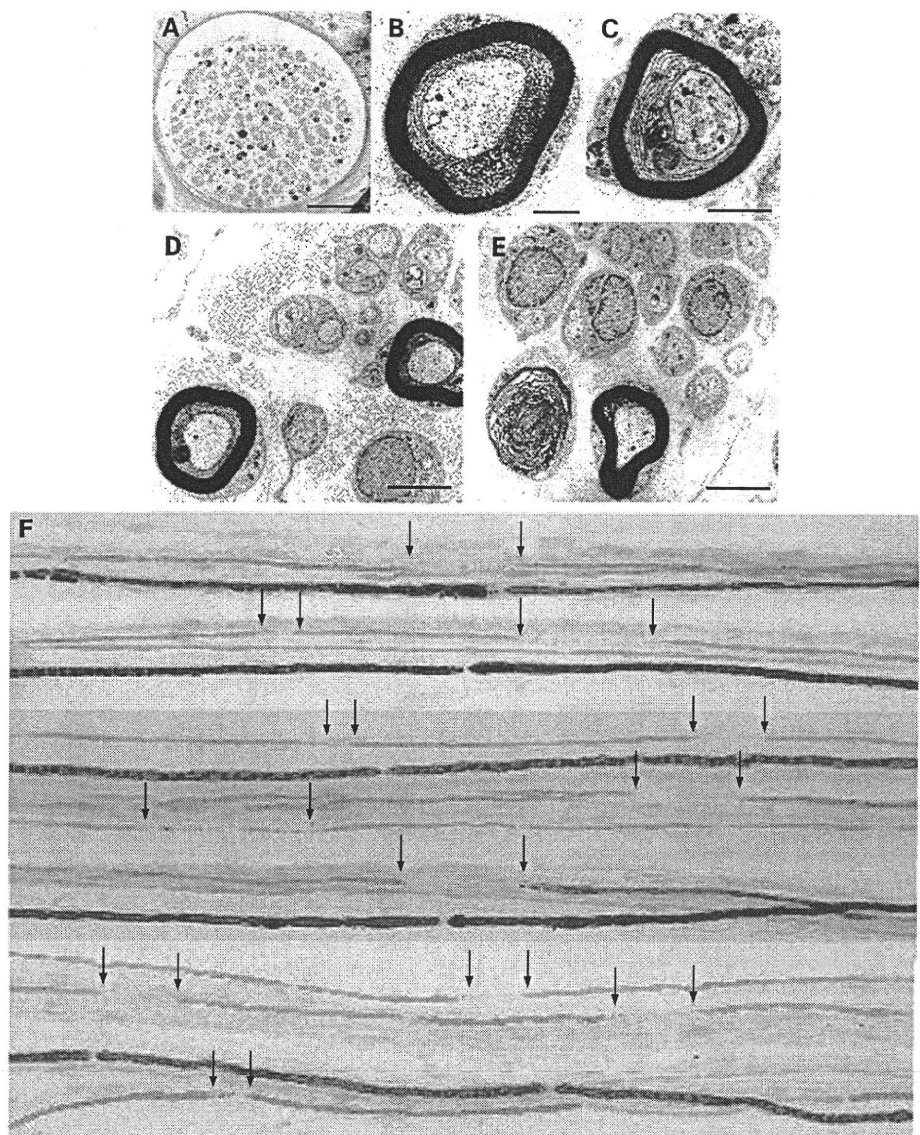
RESULTS

Neuropathical features

Neuropathic features are summarised in table 2. All patients showed a symmetrical polyneuropathy pattern with greater

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Figure 1 Sural nerve biopsy specimen from a patient with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes (POEMS) syndrome. (A) In light microscopy, subperineurial oedema is conspicuous. (B, C and D) In electron microscopy, uncompact myelin lamellae are more frequent in small myelinated fibres. (E) Unmyelinated fibres are preserved, whereas a remnant of myelin sheath, suggestive of axonal degeneration of myelinated fibres, is seen. (F) Teased fibres from a patient with POEMS syndrome. Segmental demyelination resulted from widening of nodes of Ranvier (arrows) is conspicuous in small myelinated fibres. Scale bars = 50 μ m (A), 1 μ m (B and C) and 3 μ m (D and E).



involvement of the lower rather than the upper limbs. Sensory symptoms were moderate to severe in most patients in the distal portion of the lower extremities as a whole. Nineteen patients (86%) complained of numbness in the lower extremities, most of which were uncomfortable sensations, such as aching, tingling or prickling. Twelve patients (55%) reported spontaneous pain. Painful symptoms generally were brought on, or made worse, by gentle manual pressure or pinching. Many of the patients complained of pain as they walked because pressure was applied on the soles of their feet. Patients also reported pain when a blanket was put on their feet. Distally accentuated hyperalgesia to pin-prick or pinwheel was also observed. As a whole, 14 patients (64%) reported hyperalgesia, as described above. In some patients, pain was the most characteristic feature, significantly compromising activities of daily living. Seven patients (32%) reported having difficulty walking due to pain (patients 1, 4, 9, 10, 15, 18 and 21). On the other hand, three patients (14%) did not complain of any positive sensory symptoms despite the presence of subjective sensory deficit (patients 8, 12 and 14). Autonomic symptoms were reported in 4 patients (18%): these consisted of impairment of sweating in the extremities in patients 8, 13 and 15; urinary retention in patient 13; and constipation and

orthostatic hypotension defined as a fall of 20 mm Hg in systolic blood pressure following arising from the supine position in patient 18. In addition, 6 of 13 male patients (patients 5, 10, 15, 16, 19 and 22) complained of impotence, although this may have been related to endocrine disorder.

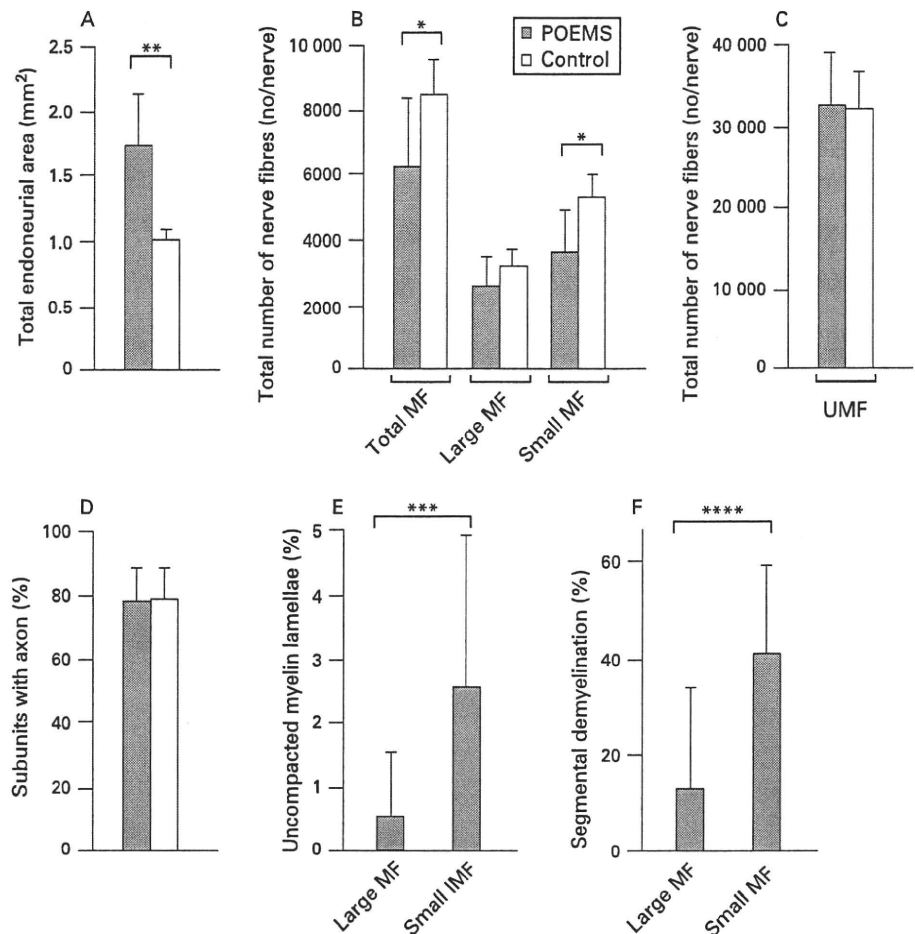
Nerve conduction studies revealed slowing of motor and sensory conduction velocities and prolongation of distal latencies in all patients, as shown in Supplementary Material 1.

No significant difference in the type of M-protein, duration of neuropathy, or relative predominance of weakness and sensory deficit was present between the group with mechanical hyperalgesia and that without it. Functional status assessed by the modified Rankin Scale was not significantly different between the two groups. Electrophysiological features were different only in the amplitude of sensory nerve action potentials of the sural nerve, which was more profoundly reduced in the group with hyperalgesia ($p < 0.05$).

Pathological findings of sural nerve biopsy specimens and their correlation to neuropathic pain

Quantitative data, including those of nerve fibre density of individual cases, are listed online in Supplementary Material 2.

Figure 2 Morphometric assessments of sural nerve biopsy specimens. Black columns indicate polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes (POEMS) syndrome and white columns indicate controls. Each error bar represents the standard deviation. (A) Total endoneurial area. Total endoneurial area was markedly enlarged compared with controls ($p < 0.01$). (B) Estimated number of myelinated fibres per complete cross-section of the sural nerve. The estimated number of total myelinated fibres per sural nerve cross-section was significantly reduced compared with controls ($p < 0.05$). There was no significant difference in the number of large myelinated fibres between POEMS syndrome and controls, but there was a significant difference in small myelinated fibres ($p < 0.05$). (C) The estimated number of unmyelinated fibres per complete cross-section of the sural nerve. In contrast to the reduction of myelinated fibres, there was no difference in the number of unmyelinated fibres. (D) Percentage of subunits with unmyelinated fibres in the total population of Schwann cell subunits associated with unmyelinated fibres. The percentage was not different from that of the control group, suggesting that unmyelinated fibres were preserved. (E) The percentage of fibres with uncompacted myelin lamellae in myelinated fibres. The percentage was significantly more frequent in small myelinated fibres than in large myelinated fibres ($p < 0.001$). (F) Teased-fibre studies. The frequency of fibres with segmental demyelination due to widened nodes of Ranvier was significantly more frequent in small fibres than in large fibres ($p < 0.0001$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ with Mann-Whitney *U* test. MF, myelinated fibres; UMF, unmyelinated fibres.



As for myelinated fibres, the density varied from 1396 to 6558 fibres/mm². Density of large myelinated fibres was 1897 ± 652 fibres/mm² (61% of control), whereas that of small myelinated fibres was 2564 ± 832 fibres/mm² (50% of control), indicating a relatively predominant reduction of small myelinated fibres in most cases. Remarkable oedema in the endoneurium was present in most cases (fig 1A). The total endoneurial area was significantly increased compared with controls (1.725 ± 0.434 mm² vs 1.089 ± 0.061 mm², $p < 0.01$; fig 2A). The estimated total number of myelinated fibres in the complete cross-section of the sural nerve was significantly reduced compared with controls (6367 ± 2123 fibres vs 8684 ± 959 fibres, $p < 0.05$; fig 2B). The number of large myelinated fibres was reduced, but not to a significant extent (2663 ± 920 fibres vs 3289 ± 494 fibres; fig 2B), whereas the number of small myelinated fibres was significantly reduced (3704 ± 1340 fibres vs 5396 ± 628 fibres, $p < 0.05$; fig 2B). Axonal sprouting of myelinated fibres and onion-bulb formation were not conspicuous in any case compared with controls.

In contrast to myelinated fibres, a reduction of unmyelinated fibres was not apparent. Although the densities of unmyelinated fibres ranged from 15736 to 32323 fibres/mm² and significantly decreased compared with controls (22947 ± 5032 fibres/mm² vs 30655 ± 2731 fibres/mm², $p < 0.01$), the estimated total number of unmyelinated fibres in the complete cross-section of the sural nerve in POEMS syndrome was almost the same as that seen in controls (32932 ± 6570 fibres vs 32348 ± 4174 fibres; fig 2C). The population of unmyelinated fibres was preserved, even in cases with a marked loss of myelinated fibres. In two patients who had impaired glucose tolerance (patients 13 and 16), ballooning of unmyelinated fibres, suggestive of degenerating fibres, and clusters of small unmyelinated fibres, suggestive of regenerating fibres, were observed. One of the patients (patient 13) had increased numbers of unmyelinated fibres due to the regenerating fibres. Findings suggestive of unmyelinated fibre degeneration or regeneration were not obvious in other cases. The percentage of Schwann cell subunits with unmyelinated fibres

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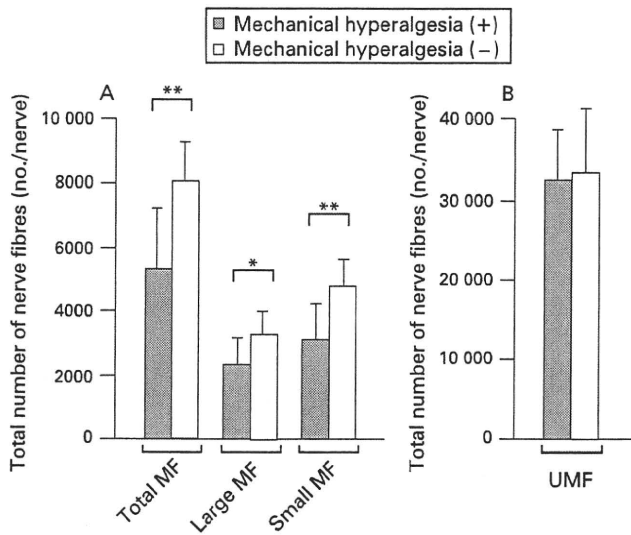


Figure 3 The relationship between hyperalgesia and total number of nerve fibres per complete cross-section of the sural nerve. Black columns indicate the group with hyperalgesia and white columns indicate the group without hyperalgesia. Each error bar represents the standard deviation. (A) The reduction of total myelinated fibres was significant in the group with hyperalgesia compared to those without it ($p < 0.01$). Both large and small myelinated fibres were significantly reduced ($p < 0.05$ for large myelinated fibres, $p < 0.01$ for small myelinated fibres). (B) No difference in the number of unmyelinated fibres was observed between the groups with and without hyperalgesia. * $p < 0.05$ and ** $p < 0.01$ with Mann-Whitney *U* test. MF, myelinated fibres; UMF, unmyelinated fibres.

was not different from that in controls ($78.3 \pm 9.5\%$ vs $79.6 \pm 8.3\%$; fig 2D). This finding suggests that there was no increase of empty subunits and further supports the view that unmyelinated fibres were preserved.²¹

The percentage of fibres with UML was $1.7 \pm 1.7\%$. They were more frequent in small myelinated fibres than in large myelinated fibres ($2.5 \pm 2.4\%$ vs $0.5 \pm 1.0\%$, $p < 0.001$; fig 1B, C, D, and 2E).

In teased-fibre studies, irregularity of myelin was conspicuous and both segmental demyelination and axonal degeneration were frequently found. Segmental demyelination resulted from widening of the nodes of Ranvier, and was more frequently found in small fibres ($41.8 \pm 17.6\%$ of small fibres vs $14.2 \pm 19.3\%$ of large fibres, $p < 0.0001$; fig 1C, 2F).

The total myelinated fibres per complete cross-section of the sural nerve was significantly less in the group with hyperalgesia than those without it (5385 ± 1895 fibres vs 8051 ± 1304 fibres, $p < 0.01$; fig 3A). Both large and small myelinated fibres were significantly reduced in the group with hyperalgesia compared with that without hyperalgesia (2290 ± 820 fibres vs 3304 ± 741 fibres, $p < 0.05$ for large myelinated fibres; 3095 ± 1144 fibres vs 4747 ± 988 fibres, $p < 0.01$ for small myelinated fibres; fig 3A). Compared with controls, the number of both large and small myelinated fibres was significantly reduced in the group with hyperalgesia ($p < 0.05$ for large myelinated fibres, $p < 0.01$ for small myelinated fibres). On the other hand, in the group without hyperalgesia, the number of large myelinated fibres was not reduced compared with controls, and the number of small myelinated fibres was only slightly reduced. There was no difference in the number of unmyelinated fibres in the complete cross-section of the sural nerve between the groups with and

without hyperalgesia (32308 ± 6010 fibres vs 33912 ± 7764 fibres; fig 3B). As for the frequency of fibres with UML and the frequency of segmental demyelination in teased-fibre studies, these were not significantly different between the groups with and without hyperalgesia except for a higher frequency of segmental demyelination in small fibres in the group with hyperalgesia than that without it ($p < 0.01$).

Cytokine profiles and their correlation to neuropathic pain

Individual cases showed extensive variation of the concentration for proinflammatory cytokines (IL-1 β , IL-6 and TNF- α) in the group with POEMS syndrome (fig 4A, B and C). As a whole, a significant increase was found only for IL-6 ($p < 0.01$). Levels of these proinflammatory cytokines for the POEMS syndrome and control groups were 17.3 ± 25.9 and 5.6 ± 5.4 pg/ml for IL-1 β , 45.4 ± 96.2 and 2.3 ± 2.5 pg/ml for IL-6, and 138.7 ± 349.5 and 2.8 ± 6.7 pg/ml for TNF- α . Levels of the anti-inflammatory cytokine (IL-1ra) were significantly lower in the POEMS syndrome group than in the control group (175.3 ± 71.7 and 469.9 ± 146.2 pg/ml, $p < 0.001$; fig 4D). Levels of cytokine receptors (sIL-6r, sTNFrI and sTNFrII) were significantly higher in the group with POEMS syndrome than the control group (fig 4E, F and G). These levels for the POEMS syndrome and control groups were 28.9 ± 6.6 and 22.6 ± 4.1 ng/ml ($p < 0.05$) for sIL-6r, 2241.7 ± 671.2 and 1241.7 ± 175.3 pg/ml ($p < 0.001$) for sTNFrI, and 3178.3 ± 738.1 and 2223.3 ± 401.7 pg/ml ($p < 0.01$) for sTNFrII.

All patients who showed extensive elevation of proinflammatory cytokines (IL-1 β , IL-6 and TNF- α) had hyperalgesia. All of these proinflammatory cytokines were significantly elevated in the group with hyperalgesia compared to the group without it ($p < 0.05$ for IL-1 β and IL-6, $p < 0.01$ for TNF- α ; fig 4A, B and C). No significant difference between the two groups was found for IL-1ra, sIL-6r, sTNFrI and sTNFrII (fig 4D, E, F and G). Compared with controls, the group with hyperalgesia showed significant elevation of IL-1 β ($p < 0.05$; fig 4A), IL-6 ($p < 0.01$; fig 4B), TNF- α ($p < 0.05$; fig 4C), sTNFrI ($p < 0.01$; fig 4F) and sTNFrII ($p < 0.05$; fig 4G), and reduction of IL-1ra ($p < 0.01$; fig 4D). The group without hyperalgesia showed significant elevation for sTNFrI ($p < 0.01$; fig 4F) and sTNFrII ($p < 0.05$; fig 4G) and reduction for IL-1ra ($p < 0.01$; fig 4D) compared with controls. In patient 7, who complained of severe spontaneous pain without hyperalgesia, none of the proinflammatory cytokines was elevated compared with controls. On the other hand, proinflammatory cytokines were examined in two patients (patients 1 and 13) who had hyperalgesia without spontaneous pain and all cytokines were elevated in both patients.

DISCUSSION

Because POEMS syndrome affects a wide range of organs, its detailed neuropathic features have not yet been fully described. Evaluating the extent of nerve fibre loss in sural nerve biopsy specimens from POEMS syndrome patients is difficult due to marked enlargement of the endoneurial area due to extensive oedema. When nerve fibre loss is estimated by density, the extent may be overestimated when the cross-sectional area is enlarged. To exclude this possibility, we estimated the total number of nerve fibres in the complete cross-section of the sural nerve and determined the precise extent of nerve fibre loss. Thus, we were able to clearly demonstrate the clinical and pathological correlates of POEMS syndrome. Although sensory symptoms were variable, ranging from the positive to the negative, the most characteristic feature in our series was the