

# Inflammatory vasculopathy in multifocal diabetic neuropathy

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## Summary

Besides the common distal symmetrical sensory–motor polyneuropathy (DSP) that is often associated with autonomic dysfunction, diabetic patients may develop multifocal sensory–motor deficits (MDN) secondary to roots, plexus and nerve trunk involvement. Nerve ischaemia has been suggested as a common mechanism for the different patterns of diabetic neuropathies, yet the important clinical differences that exist between DSP and MDN suggest concurrent factors. In order to learn more on the subject, we prospectively studied 22 consecutive diabetic patients with MDN, for which other causes of neuropathy were excluded by appropriate investigations, including biopsy of a recently affected sensory nerve. Three patients had a relapsing course, and the others an unremitting subacute-progressive course. Painful MDN progressed over 2–12 months. The neurological deficit predominated in distal lower limbs which were involved in all patients, unilaterally in seven, bilaterally in the others, with an asynchronous onset in most cases. In addition, a proximal deficit of the lower limbs was present on one side in seven patients, and on both sides in six. Thoracic radiculoneuropathy was present bilaterally in two patients, and unilaterally in one. The ulnar nerve was involved in one patient, and the radial nerve in two. The CSF protein ranged from 0.40 to 3.55 g/l; mean: 0.87 g/l. Electrophysiological testing showed severe, multifocal,

axonal nerve lesions in all cases. Asymmetrical axonal lesions were found in all nerve specimens. The mean density of myelinated axons was reduced to  $1340 \pm 1070$  per  $\text{mm}^2$  of endoneurial area versus  $8370 \pm 706$  myelinated fibres/ $\text{mm}^2$  in controls. The mean density of unmyelinated fibres was reduced to  $5095 \pm 6875$  per  $\text{mm}^2$  (extremes: 0–26 600). On teased fibre preparations,  $34 \pm 31\%$  of the fibres were at different stages of axonal degeneration (extremes 0–99%);  $7 \pm 6\%$  of the fibres showed segmental demyelination or remyelination. Necrotizing vasculitis of perineurial and endoneurial blood vessels were found in six patients. Endoneurial seepage of red cells was present in 11 specimens, and endoneurial haemorrhage in five. Ferric iron deposits that characterize previous bleeding were found in seven patients, including two who had no red cells in the endoneurium. Perivascular mononuclear cell infiltrates were present in the nerve specimens of 21 out of 22 patients, prominently in four patients. In comparison, nerve biopsy specimens of 30 patients with severe distal symmetrical diabetic polyneuropathy showed mild epineurial mononuclear cell infiltrate in one patient and endoneurial seepage of red cells in another. We conclude that MDN is related to pre-capillary blood vessel involvement in elderly diabetic patients with a secondary inflammatory response.

**Keywords:** diabetic neuropathy; nerve biopsy; multifocal diabetic neuropathy; vasculitic neuropathy

**Abbreviations:** DSP = distal symmetrical polyneuropathy; MDN = multifocal diabetic neuropathy; PDN = proximal diabetic neuropathy

## Introduction

A wide range of neuropathic syndromes are observed in diabetic patients, the most common of which is the distal symmetrical predominantly sensory–motor, polyneuropathy (DSP) that progresses following a fibre length-dependent

pattern, often associated with autonomic disturbances (Thomas and Tomlinson, 1993; Said, 1996). Some diabetic patients develop proximal neuropathy of the lower limbs (Raff and Asbury, 1968; Raff *et al.*, 1968; Asbury 1987; Said

*et al.*, 1994; Said and Thomas, 1999) characterized by weakness and pain of proximal lower limbs, in which setting inflammatory nerve lesions and vasculitis have been documented (Said *et al.*, 1994; Krendel *et al.*, 1995; Llewelyn *et al.*, 1998; Dyck *et al.*, 1999; Kelkar *et al.*, 2000). Besides these two well characterized patterns of neuropathy, some diabetic patients are disabled by a severe, subacute, multifocal sensory-motor neuropathy (MDN) that massively predominates in distal lower limbs, and variously affects other territories including proximal lower limbs, thoracic nerves and the upper extremities. Although a common ischaemic mechanism has been suggested for the different patterns of diabetic neuropathy, the self-limited, yet protracted course of MDN and its clinical distribution are quite different from those of DSP.

In an attempt to understand better the pathophysiology of MDN and the relationship between the different patterns of diabetic neuropathy, we performed a prospective clinico-pathological study of 22 consecutive diabetic patients with multifocal neuropathy and subsequently compared the lesions of nerve blood vessels found in nerve biopsy specimens with those of a series of patients with severe distal symmetrical sensory polyneuropathy.

## Patients and methods

### Patient inclusion criteria

Insulin- or non-insulin-dependent (NIDDM) diabetic patients with motor and sensory deficit affecting simultaneously or successively more than one nerve or root territory with asymmetric or asynchronous motor and sensory deficit in a radicular or nerve trunk distribution in distal lower limbs were included in the study. Patients gave informed consent to be included in the study, which was approved by the Bicêtre Hospital Ethics Committee.

### Patient exclusion criteria

Any detectable cause of neuropathy other than diabetes was excluded after complete workout, including nerve and muscle biopsy, confirmed by long-term follow-up. Diabetic patients with generalized demyelinating polyneuropathy fulfilling electrophysiological or neuropathological criteria for chronic inflammatory demyelinating polyneuropathy were excluded, as well as those with clinical or biological markers of a polysystemic disorder, abnormal blood cell count, presence of monoclonal gammopathy or abnormal chest radiogram. Patients with isolated sensory manifestations or with isolated proximal neuropathy were also excluded.

### Population

Twenty-two diabetic patients, 13 females, nine males; mean age:  $65 \pm 9$  years, range 41–81 years were included in the study from May 1992 to February 2000.

### Clinical assessment

Neurological examination was carried out in all patients. Muscle testing was performed giving a five-grade scale motor score according to the Medical Research Council for global motor scores. Light touch, pinprick, temperature sensation at  $+4^{\circ}\text{C}$  and at  $+40^{\circ}\text{C}$ , vibratory sensation and position sense were tested to explore the functions of different subpopulations of axons.

### Laboratory investigations

These included routine blood tests, CSF examination and other appropriate investigations to exclude causes of neuropathy other than diabetes, including familial, toxic, drug abuse, nutritional, metabolic, dysglobulinaemic, amyloid, inflammatory and malignancy. All patients had routine glycaemic control, measurements of glycosylated haemoglobin, assessment of renal function and ophthalmological examination.

### Neurophysiological investigations

Electrophysiological tests included evaluation of motor and sensory conduction velocities in all four limbs; nerve and muscle compound action potentials and nerve conduction velocity, and distal motor and F-wave latencies measured by standard methods. Sensory nerve conduction studies were performed on sural, superficial peroneal, median, radial and ulnar nerves. Motor studies included peroneal, posterior tibial, radial, median and ulnar nerves. Both sides were studied. The sensory action potentials (SAPs) were recorded antidromically. When SAPs were not detectable by surface electrodes, the near-nerve needle method was used. EMG examination of muscles in clinically affected territories was performed using a monopolar needle. A summary of relevant results is given in Table 1.

### Morphological studies

After written informed consent, a nerve biopsy was performed in a territory recently affected clinically. The superficial peroneal nerve and the neighbouring peroneus brevis muscle were sampled during the same procedure in 20 patients. The superficial radial nerve was biopsied in one patient (patient 4) and the intermediate cutaneous nerve of the thigh and the adjacent quadriceps muscle in one (patient 22).

The nerve samples were fixed at  $4^{\circ}\text{C}$  in 3.6% glutaraldehyde, buffered at pH 7.4. One nerve fragment was embedded in paraffin, cut at  $6\text{ }\mu\text{m}$  thickness and examined after haematoxylin and eosin staining. Serial sections of all paraffin-embedded specimens were examined.

Another fragment was post-fixed in 1% osmium tetroxide in buffer for 3 h at  $4^{\circ}\text{C}$  and embedded in epon. Thionin-stained  $1\text{ }\mu\text{m}$  thick transverse sections were used for morphometry and  $0.1\text{ }\mu\text{m}$  thick sections stained with uranyl

**Table 1** Summary of electrophysiological and morphometrical data

Patient	Summary of electrophysiological data	Nerve fibre density/ mm <sup>2</sup>	Teased fibre preparations	Blood vessels and inflammation	Immunolabelling	Endoneurial red cells and Perls staining				
		MF	UF	Normal	WD	SA	Total			
1	Severe axonal lesion of the right peroneal nerve and distal axonal polyneuropathy.	1340	8400	26	61	13	100	Occlusion of perineurial vessel and inflammatory infiltrate.	ND	RC++, P+ muscle and nerve
2	Severe axonal neuropathy; no detectable sural nerve SAP	570	2030	4	3	0	7	Small inflammatory infiltrates around endoneurial capillaries	CD68, CD4 and CD8	RC+, P-
3	Multifocal axonal neuropathy	860	350	48	30	20	100	Marked inflammatory infiltrate	CD4 and CD8	RC-, P-
4	Multifocal axonal neuropathy. Mild reduction of the SAPs. Sural nerve SAPs	220	170	4	93	3	100	Epi-, peri and endoneurial lymphocytic vasculitis of the radial nerve	ND	RC-, P-
5	Severe multifocal sensory-motor axonal neuropathy	580	2600	78	21	1	100	Small endoneurial inflammatory infiltrate	ND	RC-, P-
6	Severe axonal neuropathy of the LL. Unexcitable sural nerves	980	7600	55	43	2	100	Epineurial artery and pericapillary infiltrates	CD4 and CD8	RC-, P-
7	Severe axonal neuropathy-unexcitable sural, peroneal and posterior tibial nerves	550	1200	16	59	25	100	Necrotizing arteritis of an epineurial artery	CD4 and CD8	RC-, nerve+
8	Decreased CMAPs in both peroneal nerves, normal CV	4840	26200	87	10	3	100	Small inflammatory infiltrates	CD4 and CD8	RC-, P-
9	Axonal neuropathy predominating on the left peroneal nerve	1240	3600	20	75	5	100	Necrotizing vasculitis	Predominantly CD68	RC+++; P+ muscle and nerve
10	Severe sensorimotor axonal neuropathy	1450	2500	93	5	2	100	Vasculitis of endo- and epineurial blood vessels	ND	RC+++; P+ nerve
11	Undetectable SAPs in all four limbs.	120	1200	1	99	0	100	Inflammatory infiltrate of some blood vessels	ND	RC+, P-
12	Unexcitable peroneal nerves Severe axonal neuropathy. Unexcitable peroneal and sural nerves	1440	6200	5	78	17	100	Marked perineurial infiltrate by mononuclear cells. Fibrinoid necrosis of endoneurial blood vessels	CD4, CD8 and CD68	RC+, P-
13	Severe multifocal axonal pattern	890	1400	3	28	8	39	Vasculitis of endo- and epineurial blood vessels; marked inflammatory infiltrate	CD4, CD8 and CD68	RC-, P-
14	Severe multifocal axonal pattern	1310	250	54	39	7	100	Epineurial necrotizing vasculitis and endoneurial capillaries	ND	RC-, P-
15	Severe multifocal axonal neuropathy of the lower extremities	2530	18500	81	13	6	100	Epineurial and endoneurial necrotizing vasculitis	CD4 and CD8	RC+++; P+ nerve
16	Multifocal axonal neuropathy	1050	3250	87	8	5	100	Epineurial lymphocytic vasculitis and increased endoneurial cellularity	CD68 only	RC+++; P-
17	Decreased CMAP and MCV in the LL	3360	17300	82	6	14	100	Mild endoneurial lymphocytic infiltration	CD4 and CD8	RC+, P-
18	Severe axonal neuropathy of the LL.	2740*	0	91	2	8	100	Epi- and endoneurial vasculitis with haemorrhage	CD68+	RC+++; P-
19	Unexcitable sural nerves. Normal CV in the UL; decreased in the LL in keeping with axon loss	1785	3230	86	9	5	100	Epineurial mononuclear cell infiltrates	Predominantly CD68;	RC-, P--
20	Multifocal axonal pattern	1190	2520	37	53	10	100	Severe depletion of axons. No vasculitis or inflammation	Occasional CD4 and CD8 cells	RC-, P-

Table 1 Continued

Patient	Summary of electrophysiological data	Nerve fibre density/mm <sup>2</sup>	Teased fibre preparations	Blood vessels and inflammation	Immunolabelling	Endoneurial red cells and Perls staining			
21	Unexcitable left peroneal nerve, axonal pattern on the right. No detectable SAPs in the LL	1120	77	15	8	100	Asymmetrical axon depletion within fascicles. Necrotizing vasculitis of endoneurial blood vessels	Predominantly CD68; occasional CD4 and CD8	RC+, P+ nerve
22	Severe axonal neuropathy of the femoral, peroneal and radial nerves	476	84	8	8	100	Severe axon loss with mild inflammation	CD68 only	RC-, P+ nerve
	Mean fibre density	1340 ± 1070	5095 ± 6875	34 ± 31%	7 ± 6%				

WD = Wallerian degeneration; SA = segmental abnormalities of the myelin sheath, including demyelination and/or remyelination; R = right; SAP = sensory action potential; CV = conduction velocity; CMAP = compound muscle action potential; LL = lower limbs; RC = upper limbs; RC = endoneurial red cells; P = Perls staining; MCV = motor conduction velocity; \* = fibre density in the less affected fascicle.

acetate and lead citrate for electron microscopic examination. The density of myelinated fibres was calculated on enlarged photographic montages of 1 µm thick sections and compared with control values of the laboratory.

### Teased fibre study

After post-fixation in osmium tetroxide, another nerve fragment was macerated in 66% glycerin for 48 h before dissection in pure glycerin. One hundred consecutive nerve fibres were isolated over >10 internodes, and classified according to their morphology into normal fibres; fibres showing segmental abnormalities of myelination; and fibres showing Wallerian degeneration as early and late stages of Wallerian degeneration following the size of row of ovoids and balls of myelin debris that decrease in size with time.

### Immunopathology

Immunolabelling of cellular infiltrates was performed on frozen and/or paraffin-embedded specimens of patients whose nerve biopsy specimens showed inflammatory infiltrates, namely in specimens of patients 3, 4, 7–11, 14, 16, 17 and 19–22. Monoclonal antibodies to common leukocyte antigens, CD68, CD3, CD4, CD8 and CD20 were thus used (Dako, Denmark).

## Results

### Diabetic status

Patients 2 and 4 had insulin-dependent diabetes mellitus for >35 years, with glycosylated haemoglobin at 7 and 8.8%, respectively, without renal or retinal complication. Twenty patients had NIDDM, which had been discovered on average 11.7 ± 7.8 years prior to the onset of neuropathic symptoms; extremes: 6 months to 30 years. Seven patients with NIDDM required treatment with insulin. In the NIDDM patients, the glycosylated haemoglobin level was between 6 and 8% in nine (patients 1, 3, 5, 6, 9, 12, 16, 17 and 22); between 8 and 10% in nine (patients 2, 4, 8, 10, 11, 14, 15, 18 and 20); and >10% in four (patients 7, 13, 19 and 21) (N < 6 IU). Blood urea and creatinine levels were normal in all patients; microalbuminuria was above normal values in three patients (patients 4, 12 and 13). Patients 16 and 22 had mild proteinuria. Background retinopathy was present in four patients (patients 1, 8, 10 and 15); proliferative retinopathy was present in patient 12 only. Patients 1, 5 and 15 had symptomatic macroangiopathy, affecting the lower limbs in patients 1 and 5, and the coronary arteries in patient 15.

### Neurological data at referral

The onset period before referral extended over 2–12 months (see Table 1). Patients 1, 20 and 19 had two neuropathic episodes within 1, 2 and 3 years, respectively, with spontan-

eous remission after the first episode. Pain and motor deficit of subacute onset and progression were the most constant complaints. Only one patient (patient 17) did not complain spontaneously of pain. Spontaneous pains, often burning, more disturbing at night, and contact dysaesthesia had been present for several weeks in the others. Pains were graded as mild to moderate in eight patients (patients 2, 4–7, 11, 12 and 19) and severe in the other 13 patients, who all required administration of opiates.

Neuropathic manifestations started in the distal lower limbs in 16 patients (patients 1, 2, 4–10, 12, 14, 16 and 18–21). At referral, all patients had a motor and sensory deficit of the distal lower limbs. The motor deficit was severe in most patients, with distal strength at 0–2 on a scale of 5. In seven patients, the neurological deficit was unilateral and predominated in the peroneal nerve territory (patients 1, 6, 8, 9, 14, 19 and 22). In the other 15 patients, the distal deficit was bilateral, with an asynchronous onset in most cases. Proximal deficit of the lower limbs was the first manifestation in three patients (patients 3, 11 and 22). At referral, the proximal deficit was present in 13 patients, unilaterally in seven patients (patients 4, 6, 8, 15, 19, 21 and 22) and bilaterally in six (patients 2, 7, 11, 13, 14 and 16). Bilateral thoracic radiculoneuropathy with spontaneous burning pains, hypoaesthesia and allodynia in the corresponding territory was the first manifestation in two patients (patients 13 and 15). One additional patient had a unilateral thoracic neuropathy (patient 17). The ulnar nerve was involved in patient 1. The median nerve was affected on both sides, in patient 2, without electrophysiological signs of carpal tunnel entrapment. A motor and sensory deficit in the radial nerve territory was present in two patients (patients 1 and 4).

Symptomatic autonomic disturbances included some degree of impotence in all male patients, and sphincter disturbances in patients 6 and 16. Patients 2 and 16 had asymptomatic light-near dissociation of pupillary reflexes. Postural hypotension and gastrointestinal autonomic neuropathy were not found. The CSF was acellular in all patients; the CSF protein ranged from 0.40 to 3.55 g/l; mean: 0.87 g/l.

### ***Electrophysiological data***

All patients had severe, multifocal, axonal neuropathy, with unexcitable nerves or a major decrease of nerve action potentials in affected territories in all cases. Sural nerve sensory action potentials were abolished in 18 patients. They were within the normal range in patients 4, 8, 17 and 22. Conduction blocks were not found.

### ***Morphological data***

#### ***Alteration of nerve fibres observed by nerve cross-section***

Asymmetrical axonal lesions, within and between nerve fascicles, were a consistent finding. The mean density of

myelinated axons was reduced to  $1340 \pm 1070$  per  $\text{mm}^2$  of endoneurial area (extremes ranged from 4840 fibres/ $\text{mm}^2$  in patient 14 to 15 myelinated fibres/ $\text{mm}^2$  in patient 8), versus  $8370 \pm 706$  myelinated fibres/ $\text{mm}^2$  in the controls. On electron microscopic examination, the nerve fibre abnormalities included demyelination and remyelination, axonal degeneration and clusters of regenerating myelinated and unmyelinated axons. Major fibre loss was noted in nerve biopsy specimens of patients 4, 8 and 9, with only fibroblasts and Schwann cell processes forming pockets of collagen surviving. The mean density of unmyelinated fibres was reduced to  $5095 \pm 6875$  per  $\text{mm}^2$  (extremes: 0–26 600), versus an average  $32\,600 \pm 3500$  per  $\text{mm}^2$  in non-diabetic control patients. Clusters of regenerating myelinated and unmyelinated axons were common in nerve specimens of patients 1–3, 5, 15, 16 and 19. Rigid residual basal lamina often persisted around clusters of regenerating axons, single myelinated fibres and bundles of collagen fibres.

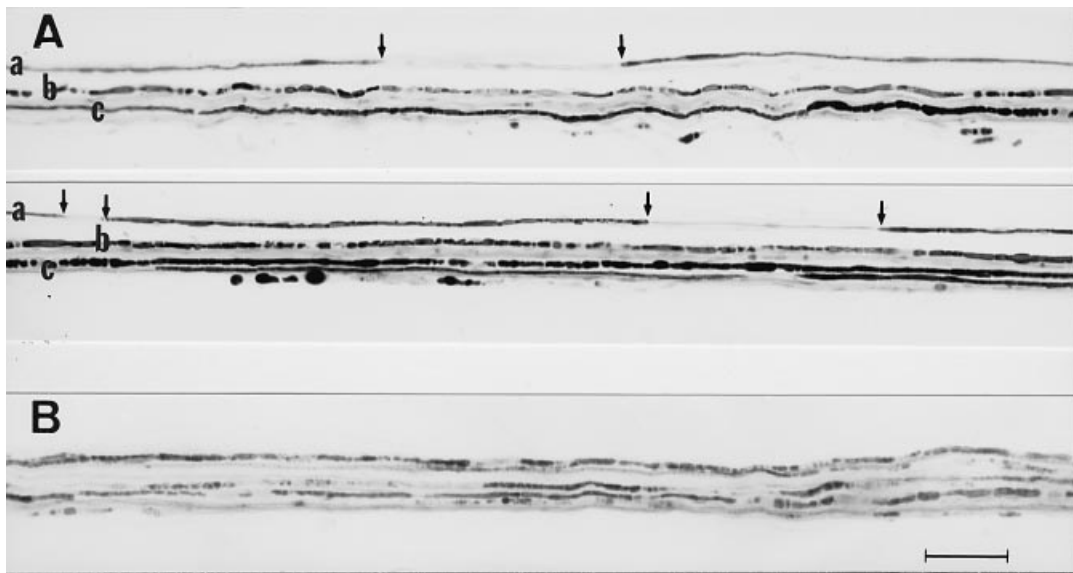
#### ***Nerve teased fibre preparations***

On teased fibre preparations,  $34 \pm 31\%$  of the fibres were at different stages of axonal degeneration (extremes 0–99%);  $7 \pm 6\%$  of the fibres showed segmental abnormalities of the myelin sheath including demyelinated and short, thinly remyelinated internodes (extremes: 0–25%). Demyelination/remyelination was noted in 10% or more of the surviving fibres in six patients (patients 1, 3, 7, 12, 17 and 20) (see Fig. 1).

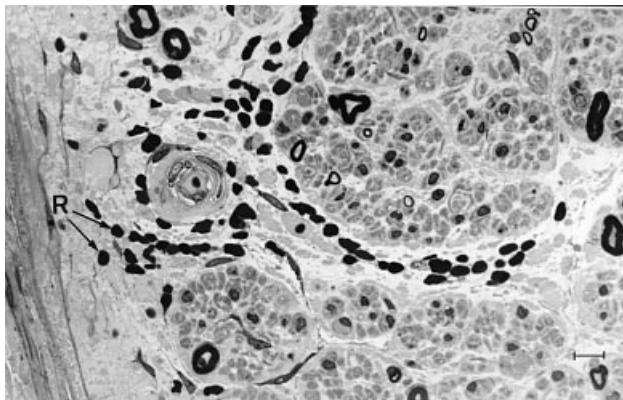
#### ***Nerve blood vessels and endoneurial red cell seepage***

Diabetic microangiopathy, characterized by increased capillary wall and basement membrane thickness on 1  $\mu\text{m}$  thick sections and on electron microscopic examination were observed in 20 specimens, although with a variable intensity (Fig. 2). Necrotizing vasculitis of perineurial and endoneurial blood vessels was found in six patients (patients 7, 9, 12, 14, 15 and 21) in association with mononuclear cell infiltrates (Fig. 3). In some sections, however, fibrinoid necrosis affected only part of the vessel, without inflammatory infiltration.

Red cell seepage in the endoneurium was observed in 11 patients (patients 1, 2, 9–12, 15–18 and 21), with frank haemorrhage in five of them (patients 2, 3, 11, 12 and 18). Ferric iron deposits were identified by Perls staining in seven patients (patients 1, 7, 9, 10, 15, 17 and 21). Thus, a total of 12 patients had signs of previous and/or current endoneurial bleeding. On 1  $\mu\text{m}$  thick preparations and on electron microscopic examination of thin sections, many of the red cells were engulfed by phagocytic cells (Figs 1 and 4). None of the patients was taking anti-platelet aggregants or anticoagulants during the weeks that preceded performance of the biopsy. In one patient (patient 21), a longitudinal paraffin



**Fig. 1** Multifocal diabetic neuropathy. Teased fibre preparations of the endoneurial content after osmication. (A) Patient 14: the first two rows show fibres at different stages of Wallerian degeneration and one fibre (fibre a) showing three demyelinated segments (between arrows). Osmium tetroxide staining. (B) Patient 9: all the fibres of this group are undergoing axonal degeneration. Bar: 50 µm.

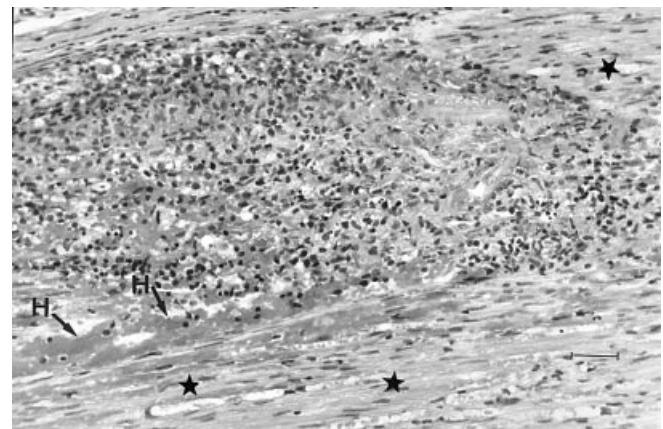


**Fig. 2** Blood vessel lesions and red cell seepage in the endoneurium of patient 10. A 1 µm thick cross-section showing an endoneurial capillary with thickened wall and red cell (R) seepage in the endoneurium. Note the marked reduction of the density of myelinated nerve fibres. Thionin blue staining. Bar: 10 µm.

section of the serial sections clearly showed a damaged blood vessel bleeding in the endoneurium (Fig. 4). The end stage of the process is characterized by complete occlusion of the perineurial blood vessel associated with major axon loss, but still with endoneurial red cells (Fig. 6)

### Muscle biopsy findings

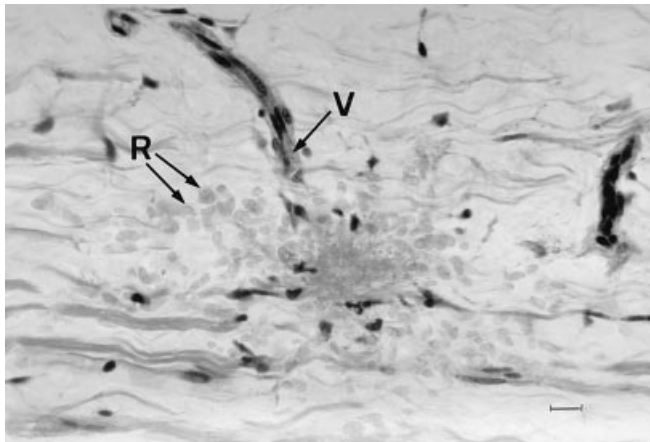
Examination of serial sections of muscle biopsy specimens showed neurogenic atrophy in 11 patients; Perls staining was positive in patients 1 and 9; inflammatory infiltrates were present in patients 2, 5 and 10; necrotizing vasculitis in patient 7; and non-necrotizing vasculitis in patient 9.



**Fig. 3** Vasculitis and endoneurial haemorrhage in patient 18. Longitudinal section of a paraffin-embedded nerve showing an important inflammatory infiltrate with mononuclear cells around and within the wall of an epineurial blood vessel. Massive endoneurial haemorrhage was present in this patient (H). Note that the nerve fibres are undergoing axonal degeneration. Endoneurial space (star). Haematoxylin and eosin staining. Bar: 50 µm.

### Inflammatory infiltrates

Inflammatory infiltrates were present in the nerve specimens of 21 out of 22 patients, prominently in patients 3, 12, 13 and 18 (Fig. 2). They were made of mononuclear cells infiltrating the perineurium and epineurial blood vessels in all, and endoneurial capillaries in eight of them (patients 2, 3, 4, 6, 13, 14, 15 and 18). Perivascular infiltrates were composed of a mixture of CD4/CD8 T cells and macrophages. Endoneurial macrophages labelled with anti-CD68 monoclonal antibodies were closely



**Fig. 4** Acute lesion of an endoneurial blood vessel on a paraffin section from patient 21. Endoneurial bleeding from a capillary (V). A number of red cells (R) are spread in the endoneurium. Haematoxylin and eosin staining. Bar: 10 µm

associated with myelin debris that resulted from axonal degeneration of myelinated fibres. Polymorphonuclear cells were seldom seen in the perivascular infiltrates.

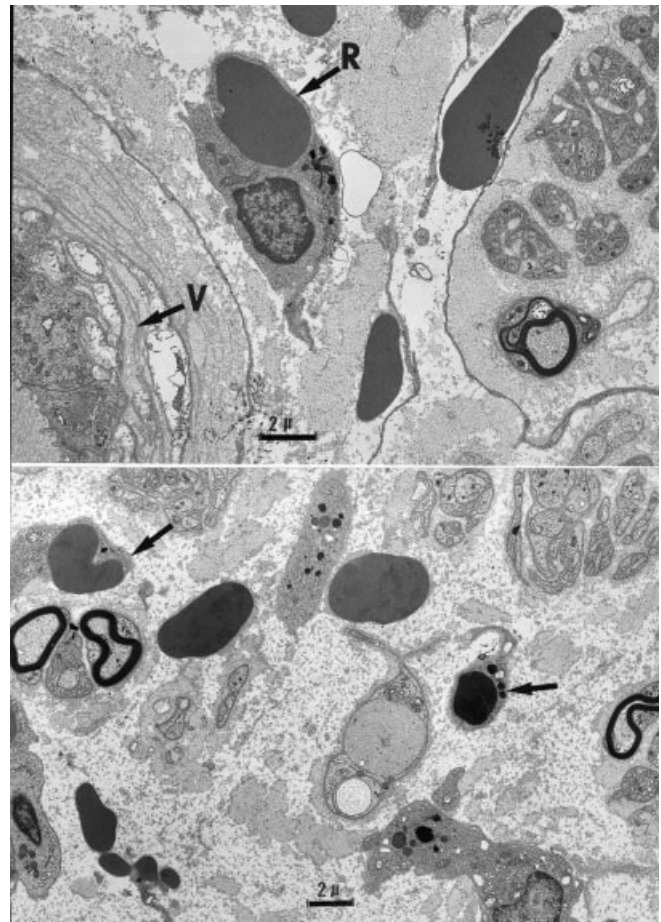
After these findings, we re-examined cross and longitudinal plastic and paraffin sections of nerve biopsy specimens of 30 patients with different patterns of severe distal symmetrical diabetic polyneuropathy, including some patients reported in previous series (Said *et al.*, 1983, 1992, 1998). Although severe diabetic microangiopathy was observed in all those with longstanding diabetes, only one of these nerve specimens showed mild epineurial mononuclear cell infiltrate and one had minimal endoneurial seepage of red cells.

### Follow-up evaluation

Eighteen patients were followed-up in our service for 2–10 years. Hyperglycaemia was well controlled in all patients. Pain subsided spontaneously within a few weeks in four patients (patients 1, 5, 11 and 19). Of the four patients who received high doses of immunoglobulins, pain subsided in one (patient 3). Prednisone 0.75 mg/kg/day was prescribed in 10 patients. After 3–4 weeks, prednisone was tapered gradually to zero over the following 6 months on average. Treatment of diabetes required adjustment with insulin in all the patients who received corticosteroids. Pain subsided within a few days in all patients treated with corticosteroids (patients 4, 6, 7, 9, 13–15, 21 and 22). In patient 22, pain recurred in the radial nerve territory after withdrawal of corticosteroids, and then decreased spontaneously over 1 year. Gradual improvement of motor deficit took several months. Minor to moderate residual deficit persisted in half of the patients.

### Discussion

All patients included in this series manifested a multifocal motor and sensory neuropathy that markedly predominated in

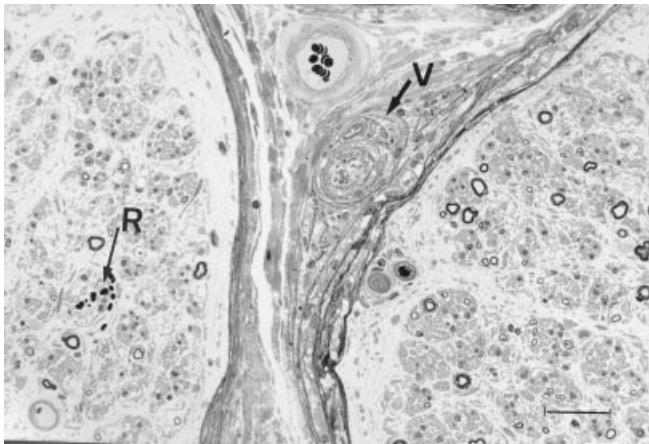


**Fig. 5** Electron photomicrographs. Patient 10 (top): red cells engulfed in macrophages (R) or free in the endoneurium. Note the reduplication of basal lamina of the endoneurial capillary (V). Patient 1 (bottom): note the presence of several red cells free or inside phagocytic cells (arrow). Uranyl acetate and lead citrate staining.

distal lower limbs. Unilateral or asymmetrical distal motor and sensory deficit was associated with involvement of proximal lower limbs in half of the patients, with thoracic neuropathy in three, and with upper limbs sensory–motor deficit in six patients. In all patients, pain and sensory–motor deficit were prominent, with few or no symptoms of autonomic disturbances.

### Multifocal neuropathy and the other patterns of neuropathy in diabetic patients

Multifocal diabetic neuropathy differs markedly from the common distal symmetrical, predominantly sensory, polyneuropathy that progresses following a fibre length-dependent pattern. In the latter, autonomic disturbances are prominent and sometimes life threatening, and motor deficit is very uncommon. Although reported in early descriptions of severe diabetic polyneuropathy, at a time when no treatment of diabetes was available (Charcot, 1890), symptomatic



**Fig. 6** End stage of the inflammatory vasculopathy in MDN in patient 1. A 1  $\mu$ m thick section of the nerve specimen showing an occluded epineurial blood vessel (V) and marked axonal loss. Some red cells (R) are still present in the endoneurium. Thionin blue staining.

motor deficit is seldom observed in patients with DSP in industrialized countries. Motor deficit is a late event in the natural history of DSP. When observed in this setting, motor deficit is always bilateral, distal and roughly symmetrical. Its onset is also more gradual and indolent, with an unrelenting course. In contrast, in patients with MDN, the onset of the neurological deficit is acute or subacute, sometimes with spontaneous remissions and relapses, as in three patients in this series. In this respect, MDN can be paralleled by proximal diabetic neuropathy (PDN) in which patients with documented inflammatory nerve lesions also have a spontaneously favourable outcome (Said *et al.*, 1997).

Patients of this series differ from the usual pattern of PDN, in which condition proximal involvement is a constant feature and distal neurological deficit is only occasional. However, it is our opinion that PDN, as well as the cases of ischaemic mononeuropathy multiplex (Raff and Asbury, 1968; Raff *et al.*, 1968) and the recently termed diabetic lumbosacral radiculoplexus neuropathies (Dyck *et al.*, 1999) are variants of MDN. Some patients with the so-called lumbosacral radiculoplexus neuropathies also had upper limb involvement. We think thus that the term multifocal neuropathy is more appropriate to this clinical pattern.

The occurrence of lumbosacral radiculoplexus neuropathy in non-diabetic patients has been underlined recently (Bradley *et al.*, 1990; Dyck *et al.*, 2000). In this respect, it is important to remember that none of the neuropathic patterns observed in diabetic patients is specific to this population. For example, proximal neuropathy was observed in diabetic patients in only 14 out of the 19 cases reported by Calverley and Mulder (1960). As a rule, when a diabetic patient manifests a peripheral neuropathy, causes other than diabetes must be excluded before concluding that the neuropathy is of diabetic origin (Mulder *et al.*, 1961;

Lozeron *et al.*, 2002). The conjunction of a neuropathic pattern common in diabetic patients with the absence of another detectable cause of neuropathy is necessary to incriminate diabetes as the sole cause of the neuropathy.

Overlapping and association between the different patterns of diabetic neuropathy is common. Silent or symptomatic DSP, the most frequent type of diabetic neuropathy, is often associated with PDN (Bastron and Thomas, 1981). In a previous report, all 10 patients with PDN had signs of DSP, which was symptomatic in one patient only (Said *et al.*, 1994). The same proportion of patients had MDN and signs of DSP in this series.

Acute or subacute, asymmetrical sensory-motor deficit can also be symptomatic of an acquired demyelinating polyneuropathy in a diabetic patient. Subacute or chronic inflammatory demyelinating polyneuropathy (CIDP) seems more common in diabetic patients than in the general population (Cornblath *et al.*, 1987; Stewart *et al.*, 1996). However, the higher frequency of CIDP in diabetic than in non-diabetic patients should be confirmed by epidemiological studies to determine whether or not diabetes is a risk factor for CIDP. The diagnosis of CIDP must be considered when a diabetic patient manifests a subacute neurological deficit with prominent loss of function of large myelinated fibres, namely motor deficit and loss of proprioception, which is not common in diabetic DSP. The results of electrophysiological testing point to a demyelinating process in diabetic CIDP, while it has an axonal pattern in MDN. In some cases, however, only the nerve biopsy permits identification of CIDP in this setting, due to the association with severe axonal loss related to pre-existing diabetic neuropathy.

### Pathological findings

On a pathological basis, asymmetrical axonal loss within and between nerve fascicles and axonal degeneration were common in this series, which fits well with an ischaemic process (Said *et al.*, 1988; Fujimura *et al.*, 1991). The incidence of fibres showing segmental abnormalities, including recently demyelinated and segmentally remyelinated fibres, was relatively high in a few patients, which suggests that several mechanisms including demyelination secondary to axonal injury, a metabolic process due to hyperglycaemia and the consequences of endoneurial inflammatory infiltrates could play a role. Mononuclear cell infiltrates, consisting of CD8<sup>+</sup>, CD4<sup>+</sup> and macrophages, were present in nearly all patients. In some of them, however, fibrinoid necrosis of the vessel wall was not associated with inflammatory infiltrates, which suggests that damage of the vessel wall was the first pathological event. Inflammatory infiltrates predominated around epineurial, perineurial and, less often, endoneurial blood vessels. In addition to the perivascular infiltrates, many endoneurial CD68-positive macrophages had been attracted by axonal and myelin debris during massive axonal degeneration.



### ***Lesions of nerve blood vessels and endoneurial haemorrhage***

Occlusion of epineurial and perineurial blood vessels was present in most cases. Extension of fibrinoid necrosis to endoneurial capillaries was also observed in some cases. Endoneurial haemorrhage and iron deposits were present in half of the nerve biopsy specimens. The presence of iron deposits in nerve biopsies from patients with lumbosacral radiculoplexus neuropathy was also emphasized recently by Dyck *et al.* (1999), but the presence of endoneurial bleeding had not been documented in this setting. Fresh blood in a nerve biopsy specimen suggests surgical trauma, in which case red blood cells are usually free in the epineurium and less often in the endoneurium. In the nerve specimens studied, phagocytosis of debris of red cells by endoneurial macrophages and the presence of iron deposits in nerve specimens cannot result from the surgical procedure, since both phenomena require time to develop. Endoneurial bleeding thus is definitely related to the pathological process.

### ***Conclusion***

Endoneurial bleeding is common in necrotizing vasculitis, and red cell seepage is observed in different patterns of inflammatory-infectious polyneuropathies. Demonstration of ongoing endoneurial bleeding with numerous Perls-positive iron deposits points to a sustained haemorrhagic process. Both inflammation, which makes the blood-nerve barrier more permeable to cells, and focal destruction of the vessel wall by fibrinoid necrosis can lead to endoneurial bleeding. However, haemorrhage of the type observed in some patients of this series occurs only in patients with necrotizing vasculitis of the polyarteritis nodosa type. Besides loss of weight, our patients had no general manifestation, organ involvement or biological markers of a general inflammatory illness. When present, necrotizing vasculitis affected epineurial, perineurial and often endoneurial blood vessels, but leukocytoclasia and polymorphonuclear cell infiltrates, which are classical features of polyarteritis nodosa, were uncommon in diabetic patients.

In spite of these differences, the focal and multifocal neuropathy observed in middle-aged diabetic patients fits well with our current knowledge of ischaemic neuropathy complicating occlusion of small and middle sized nerve arteries. In addition to the much higher frequency of endoneurial bleeding and of inflammatory infiltrates than in diabetic symmetrical sensory polyneuropathy, occlusion or necrosis of the small and middle sized epineurial and perineurial nerve arteries sharply differentiates the multifocal pattern from the distal symmetrical pattern of diabetic neuropathy. These findings suggest the presence of an inflammatory vasculopathy, which is different from diabetic microangiopathy, and probably results from damage of pre-capillary and small nerve artery walls by diabetes in elderly patients, at an age when atherosclerotic lesions are common.

Association of inflammation with atherosclerosis is increasingly recognized (Libby *et al.*, 2002). It has been shown that after initiation of an atherogenic diet, patches of arterial endothelial cells begin to express on their surface selective adhesion molecules that bind to various classes of leukocytes. In particular, the role of vascular cell adhesion molecule-1 that binds the type of cells found in early human and experimental atheroma, the monocyte and T lymphocytes, has been shown recently in genetically engineered mice (Cybulsky *et al.*, 2001). Hyperglycaemia associated with diabetes can also lead to modification of macromolecules by forming advanced glycation end products and can augment the production of proinflammatory cytokines (Libby *et al.*, 2002). Inflammatory responses have also been observed in atherosclerotic lesions of the carotid artery (Carr *et al.*, 1997; Jander *et al.*, 1998). These findings may account for the presence of inflammatory arterial lesions in elderly diabetic patients. A superimposed immune mechanism triggered by altered vessel wall may also be considered.

The good response of these patients with refractory pain to short courses of corticosteroids is probably related to the control of the inflammatory reaction. The overall favourable course of neuropathic manifestations in patients with MDN contrasts with the gradual worsening, or at best an apparent steady state, of diabetic patients with a length-dependent polyneuropathy. In MDN, improvement is comparable with that observed in long-term follow-up of patients with necrotizing arteritis and ischaemic neuropathy. The discrepancy in the outcome of the two patterns of neuropathy strongly suggests that metabolic control plays a greater role in DSP than in MDN, and that nerve ischaemia secondary to lesions of pre-capillary blood vessels is far more important in MDN than in DSP.

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