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Clinical, electroneuromyographic and morphological studies of pure neural leprosy in a Brazilian referral centre

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Summary Nineteen patients with pure neural leprosy were analysed with clinical examination, electroneuromyography and histopathology of nerve biopsies. Clinical examination showed sensory loss (78·9%), paresis (78·9%), nerve enlargement (68·4%) and nerve pain (42·1%). Electroneuromyographic study revealed an axonal pattern in 18 patients (94·7%) and a demyelinating pattern in one (0·5%). Mononeuropathy multiplex was the most frequent presentation (78·9%), followed by mononeuropathy simplex (10·5%) and polyneuropathy (10·5%). The histopathological study showed the presence of inflammatory infiltrate composed of epithelioid granuloma (42·1%), mononuclear infiltrate (36·8%) or macrophages positive for bacilli (21%). Fibrosis was present in 78·9% of the biopsies. Examination of semithin sections revealed, besides inflammatory infiltrate, myelinated fibre loss (94·7%), remyelination (42%), axonal degeneration (10%) as well as regeneration (31·5%). Based on these results, the pathogenesis of leprosy neuropathy in this group of patients is briefly discussed.

Introduction

Neuropathy is the hallmark of the leprosy disease. There are no leprosy patients without involvement of the peripheral nervous system[9] and the mechanism of nerve damage in

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leprosy remains a controversial issue. Large and small myelinated fibre loss may be observed. However, morphological signs of active axonal degeneration or demyelination, although present, were not prominent in leprosy neuropathy.[1,10] The axonal loss and demyelination have been ascribed to the endoneurial inflammatory process.[13,5,4] A direct effect of *Mycobacterium leprae* on either Schwann cell or on the axons prior to the establishment of the inflammatory process can also be considered. Damage to the Schwann cells elicited by the presentation of *M. leprae* antigens to T cells *in vitro* has also been reported by Spierings *et al*.[21]

The aim of this study is to obtain an integrated understanding of the pathogenesis of neuropathy in patients with pure neural leprosy (PNL) by analysis of semithin sections, together with the clinical, electroneuromyographic and laboratory data. A morphological study of 19 nerve biopsies of leprosy patients with the pure neural form of the disease is presented. The three main changes that occur in nerve trunks affected by leprosy, nerve fibre loss, axonal degeneration and regeneration, as well as remyelination (an indirect sign of demyelination), were assessed.

Patients and methods

The case definition of pure neural leprosy used in this study was clinical evidence of nerve deficit, as sensory cutaneous or motor impairment, numbness, paraesthesia, nerve pain, possible nerve thickening with or without tenderness with no signs of skin inflammation or history of skin patch(es). However, PNL can only be diagnosed definitively by peripheral nerve biopsy. Excluded from the study were patients with evidence of any skin patches, infiltration or a history of skin lesion(s) as well as those with other potential cause for nerve damage such as diabetes mellitus, alcoholism, hepatitis B or C, HIV or HTLV-I infections, rheumatological/rheumatic diseases, in addition to toxic, drug-induced or hereditary neuropathies.

As related above, nerve enlargement was not critical for the diagnosis of PNL. We found nerve enlargement in 69% of our PNL patients and 36% it was associated with nerve pain.

Patients in whom PNL was suspected were evaluated at the Leprosy Outpatient Clinic, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, which is a National Leprosy Reference Centre, so a high number of PNL cases are seen here.

Follow-up of the PNL patients of this study spanned 3 years, from August 1999 to April 2002.

Based on these criteria, detailed clinical and electroneuromyographic examination, recording the number and distribution of affected nerves, were carried out in nineteen patients (five females, 14 males, average age: 35·9 years ± SD 12·9) in order to establish the neural leprosy diagnosis. We examined the patients for nerve thickening and for the presence of cyanosis, erythroderma on the palms and soles and for spontaneous or touch-induced nerve pain. In addition, sensory impairment, motor deficit, disability and deformity status were assessed using standard methods. In brief, tactile sensation was tested by way of an aesthesiometer[17] and thermal sensation by utilizing cold and warmed metal objects. A safety pin was used to assess pain perception. Individual muscle power was graded according to the method described by the Medical Research Council (MRC) of London. The median duration of the patients' symptoms was 18 months (SD ± 28·4, minimum 4 and maximum 120).

Electroneuromyographical testing by means of the Nihon-Koden Neuropack 2 EMG system followed standard procedures. The results of conduction studies were used to determine the electrodiagnostic status, and the patients were classified as follows: a) normal; b) axonal lesion defined when there is a reduction of compound muscle action potentials (CMAPs) and/or sensory nerve action potentials (SNAPs), the amplitude being more than 30% of reference values and the sensory and/or motor conduction velocity above 70% of reference value; c) demyelination lesions (defined when the CMAP and/or SNAP latency longer than reference value and reduction of sensory and/or motor conduction velocity below 85% of reference value); combined (when there are axonal and demyelinating lesions).[3,8,11]

NERVE BIOPSIES AND HISTOLOGICAL EVALUATION

Both sensory dorsal cutaneous ulnar branch (68·4%), and sural nerve at the ankle level (31·5%) were selected for diagnostic biopsies whenever they showed clinical or electrophysiological alteration. All patients selected were untreated at the time of diagnostic biopsy. No leprosy skin lesions were found on dermatological examination and the skin smears were *M. leprae* negative.

The peripheral nerve biopsies of the neural leprosy patients were processed for routine diagnostic procedures with haematoxylin–eosin, Wade and Gomorís trichrome staining. Small pieces of the specimens were separated for epon embedding followed by semithin sectioning and staining with toluidine blue. For this purpose, the small pieces of nerves were fixed overnight, at 4°C, in glutaraldehyde (EMS, USA), and post-fixed in 2% osmium tetroxide, washed in cacodylate-sucrose buffer, dehydrated in pure acetone, embedded in epon (EMS, USA). Sections (1 μm) of epon blocks were placed on glass slides and stained with toluidine blue–borax solution. The slides were observed in a Nikon Eclipse E400 microscope.

The intensity of the morphological alterations varied from 'absent' to 'intense', but for the purposes of evaluation in this study, only the presence or absence of the alteration were considered.

LABORATORY TESTS

Polymerase chain reaction (PCR) for specific *M. leprae* DNA was also carried out.[19] In brief, nerve samples were processed according to the recommendations of Chemoulli *et al*. with few modifications. Briefly, a small fragment (1 mm) of the biopsy sample was incubated with 50 μl NaOH at room temperature for 10 min, neutralized with 1 M NaH_2PO_4, and centrifuged. The supernatant was removed and the pellet suspended in 100 μl of 60 mM Tris buffer, pH 8·8. The samples were treated with 60 μg of proteinase K at 50°C for 30 min, followed by enzyme inactivation at 95°C for 5 min. Samples were then submitted to a thermal shock procedure consisting of three consecutive cycles of 10 min boiling and snap-freezing. Amplification of *M. leprae* specific DNA was achieved using the primers ML S 5′-GCACGTAAGCCTGTCGGTGG-3′ and ML AS 5′-CGGCCGATCCTCGATGCAC-3′ hybridization conditions were performed as described elsewhere.

The diagnosis of PNL was confirmed in patients who presented at least some of the histological and/or molecular biology criteria adopted in the study of Jardim *et al*.[12] These are as follows: definite group = clinical presentation consistent with leprosy/inflammatory

infiltrate with AFB on histopathological examination/*M. leprae* specific DNA detected with PCR; probable group = clinical presentation consistent with leprosy/epithelioid granulomatous neuritis/mononuclear cell endoneuritis/fibrosis.

As Brazil is a leprosy endemic country with the second highest prevalence in the world, the finding of either mononeuropathy (multiple or simplex) together with a granulomatous or mononuclear inflammatory endoneuritis is accepted as highly suggestive of leprosy, justifying a therapeutic trial of anti-leprosy drugs with post-treatment follow-up. Other inflammatory neuropathies such as vasculitic neuropathy, inflammatory demyelinating neuropathy and sarcoidosis were ruled out by clinical and laboratory procedures. These, together with the satisfactory response to specific therapy, made the diagnosis of leprosy highly reliable.

Statistical analysis for correlation of clinical data of the patients with the presence of neuritis on the analysis of the semithin sections was carried out using the χ^2 test. Significant results were ascribed to *P*-values lower than 0·05.

Results

Clinical data for the patients are shown in Table 1. We found a high incidence of sensory impairment (78·9%) and paraesthesia (73·6%). Erythrocyanosis was present in 63·1%, nerve enlargement (68·4%), nerve pain in 42·1% and paresis in 78·9%. The average duration of these symptoms from their appearance to the time of the first visit to the medical outpatient service was 18 months (28·4, minimum = 4, maximum = 120). The electroneuromyographic study revealed a predominance of axonal pattern of nerve lesion (94·7%) and 52·6% of the patients were classified as multiplex mononeuropathy, 10·5% as mononeuropathy and 10·5% as sensory–motor polyneuropathy (see Table 2). The patient with a demyelinating pattern in the electroneuromyographic study (0·5%) did not demonstrate significant clinical neurological alteration; only nerve enlargement was detected (Table 3).

ANALYSIS OF THE H–E, WADE AND GOMORÍS TRICHROME STAINED SECTIONS

In this analysis, 13 (68·4%) nerve biopsies showed a variable intensity of inflammatory infiltrate, which ranged from focal perivascular cuffs to massive occupation of the three nerve compartments (epineurium, perineurium and endoneurium). Subperineurial infiltration surrounding the microvessels that cross the perineurium into the endoneurium and forming a subperineurial collar was also observed. The inflammatory infiltrate was composed of either epithelioid granulomas in seven biopsies (42·1%), occupying the whole endoneurial area of the section and sparing no myelinated fibres, or bacteria-loaded macrophages with foamy change, and interspersed with lymphocytes in four biopsies. Thus, only one biopsy exhibited a lepromatous inflammatory infiltrate, three had AFB-positive mononuclear infiltrate without foamy change and seven displayed tuberculoid appearance of the infiltrate. One biopsy had a normal histological appearance and four biopsies were devoid of any active inflammatory process, showing only fibrosis in the nerve compartments (the diagnosis of this case was confirmed with the PCR). In two nerve biopsies we detected the concomitant presence of epithelioid granulomas and bacteria-loaded macrophages.

The epineurial matrix showed fibrosis in 10 biopsies (57·8%) and the perineurial and the endoneurial fibrosis were present in 78·9% and 73·6%, respectively. Fibrosis appeared as an increase of green-stained extracellular matrix with the Gomorís trichrome in the nerve

Table 1. Clinical data for the patients

Patients	Paresthesia	Erythrocyanosis	Nerve enlargement	Nerve pain	Paresia	Sensorial impairment	ENMG pattern	Type of neuropathy
1	+	+	−	−	+	+	Axonal	MM
2	−	−	+	−	−	−	Demyelin	SM PNP
3	+	−	−	−	−	+	Axonal	MM
4	+	+	+	−	+	+	Axonal	MM
5	+	+	+	+	+	+	Axonal	MONO
6	−	+	+	−	+	+	Axonal	MM
7	+	+	+	−	+	+	Axonal	MM
8	+	+	−	−	−	−	Axonal	MM
9	−	−	−	−	+	+	Axonal	MM
10	+	−	+	−	+	+	Axonal	MM
11	+	+	+	+	+	+	Axonal	MM
12	+	−	+	+	−	−	Axonal	MM
13	+	+	−	+	+	+	Axonal	SM PNP
14	+	+	+	+	+	−	Axonal	MONO
15	−	−	+	−	+	+	Axonal	MM
16	+	−	+	+	+	+	Axonal	MM
17	−	+	+	−	+	+	Axonal	MM
18	+	+	+	+	+	+	Axonal	MM
19	+	+	−	+	+	+	Axonal	MM
Total	14 (73·6%)	12 (63·1%)	13 (68·4%)	8 (42·1%)	15 (78·9%)	15 (78·9%)		

ENMG: electroneuromyographic pattern, Demyelin: demyelinating pattern, MM: multiple mononeuropathy, MONO: mononeuropathy SM PNP: sensory-motor polyneuropathy; +: present; −: absent.

compartments. Its intensity tended to increase showing a hyaline aspect in three nerves (15·7%) leaving almost no intact fibres.

One nerve biopsy displayed a normal histological appearance on light microscopy.

ANALYSIS OF THE SEMITHIN SECTIONS

This examination showed changes corresponding to those found in the haematoxylin–eosin-stained sections, except in four biopsies, which were discrepant regarding the inflammation, showing only alterations of the nerve fibres. The presence of epithelioid granuloma correlated with the heaviest loss of myelinated fibres, while in two biopsies few myelinated fibres could be observed among the AFB-positive lepromatous infiltrates. No intimate association of the inflammatory cells with the nerve fibres could be seen in the endoneurial space. In the biopsies with AFB-negative inflammatory infiltrate, the predominant cell was the lymphocyte, accompanied by epithelioid granulomas, whereas the AFB-positive lepromatous infiltrates exhibited easily identifiable macrophages.

Semithin sections revealed a complete or partial loss of the large and small myelinated nerve fibres (94·7%) (Figures 1, 2, 4 and 5) and the space among the remaining fibres was occupied by extracellular matrix, which in some cases was heterogeneously hyalinized. Remyelination was detected in 42% of the biopsies in low or moderate intensity (Figure 3). Active axonal degeneration was found in only two patients (10·5%). Axonal regeneration instead was noticed in 31·5% of the patients (Figure 5), two of which showed concomitant endoneurial fibrosis.

Neither Schwann cells of non-myelinated fibres nor denervated Schwann cells were

Table 2. Clinical, histopathological and semithin section analysis

Clinical data	Histopathology	Semithin sections
Paresthesia: 14 (73·6%)	Epi inf: 10 (52·6%)	LSMF: 18 (94·7%)
Erythrocyanosis: 12 (63·1%)	Per inf : 11 (57·8%)	LLMF: 18 (94·7%)
Nerve enlargement: 13 (68·4%)	End inf: 10 (52·6%)	Ax reg: 6 (31·5%)
Nerve pain: 8 (42·1%)	Mn inf: 13 (68·4%)	Ax deg: 2 (10·5%)
Paresis: 15 (78·9%)	End eg: 8 (42·1%)	Remyel: 8 (42%)
Sensory impairment: 15 (78·9%)	Vac macr: 4 (21%)	
MM: 10 (52·6%)	Epi fib: 10 (57·8%)	
MONO: 2 (10·5%)	Per fib: 15 (78·9%)	
SM-PNP: 2 (10·5%)	End fib: 14 (73·6%)	
Axonal ENMG: 18 (94·7%)	AFB: 4 (21%)	
Demyel ENMG: 1 (0·5%)		
Serum anti-PGL1 abs: 5 (26·5%)		
PCR on the nerve: 7 (36·7%)		

AFB = acid fast bacilli positivity in the nerve biopsy; ax deg = axonal degeneration; ax reg = axonal regeneration; ax = axonal (electroneuromyographic pattern); Ax ENMG pattern = axonal electroneuromyographic pattern; Demyelin ENMG pattern = demyelinating (electroneuromyographic pattern); End eg = endoneurial epithelioid granuloma; Epi fib = epineurial fibrosis; Per fib = perineurial fibrosis; End fib = endoneurial fibrosis; Epi inf = epineurial infiltrate; Per inf = perineurial infiltrate; End inf = endoneurial infiltrate; LLMF = loss of large myelinated fibers; LSMF = loss of small myelinated fibers; MM = multiple mononeuropathy; MONO - = mononeuropathy simplex; Mn inf = mononuclear infiltrate; PCR = polymerase chain reaction on the nerve; PGL-1 = phenolic glycolipid-1; Remyel = morphological signs of remyelination; SM PNP = sensory-motor polyneuopathy; Vac macr = vacuolated macrophages.

reliably identified among the inflammatory infiltrate. In one biopsy, Schwann cells were surrounding the thinly myelinated axons, showing the onion bulb appearance, which corresponds to repetitive attempts at remyelination.

Gomorís trichrome stained slides proved better to detect fibrosis than toluidine blue stained semithin sections. It seemed as if a replacement of the lost myelinated fibres for the extracellular matrix occurred in the endoneurium.

No significant correlation ($P > 0{\cdot}05$) was found between clinical parameters such as nerve enlargement, nerve pain, sensory alteration, motor impairment, and the presence of specific inflammatory infiltration in the nerves.

The electroneuromyography partially matched with the morphological findings. Most of them (94·7%) showed an axonal electroneuromyographic pattern associated with axonal loss in the analysis of semithin sections. In addition, the only patient with a demyelinating electroneuromyographic pattern also exhibited a matched demyelination in the histopathological study. Despite remyelination being present in 8 (42%) of the patients at the histopathological level, only one showed a corresponding demyelinating pattern on electroneuromyography. (For anatomo-clinical correlation, see Tables 2 and Table 3).

Discussion

According to Sridharan,[22] the histological findings of nerve biopsies vary according to the leprosy type; however, this particular group of neural leprosy patients can exhibit either

Table 3. Clinicopathological correlation

Patient no.	Nerve selected; symptom and its duration, ENMG pattern; type of neuropathy	Histopathological alterations	Alterations found on semithin sections	Defining diagnostic criteria
1	Ulnar, paresthesia; 6 m; ax; MM	End mn inf; end fib	LLMF; LSMF; remyel	Fib; NII
2	Sural; paresthesia, 60 m; Demyelin; SM PNP	Per mn inf; epi/per fib	LLMF; LSMF; ax reg; remyel	PCR
3	Ulnar; paresthesia ax; 12 m; MM	–	Remyel	PCR
4	Sural; parethesia; 48 m; ax; MM	AFB+????	LLMF; LSMF	NII; PCR; PGL1 ab
5	Ulnar; paresthesia; 60 m; ax;	End eg; per/end fib; AFB+	LLMF; LSMF	BAAR; NII; PCR; PGL1 ab
6	Ulnar; amyotrophy; 24 m; ax; MM	End EG	LLMF; LSMF	BAAR; eg;
7	Ulnar; paresis; 120 m; ax; MM	Per fib	LLMF; LSMF; ax deg; ax reg; remyel	PCR
8	Ulnar; paresthesia; 24 m; ax; MM	End eg; epi/per/end fib	LLMF; LSMF; ax reg	eg; fib
9	Ulnar; amyotrophy; 48 m; ax MM	Epi fib	LLMF/LSMF	PCR
10	Ulnar; paresthesia; 14 m; ax; MM	End eg; end fib	LLMF; LSMF	Eg
11	Sural; paresthesia; 12 m; ax; MM	Foamy cells ; AFB+	LLMF; LSMF remyel	PCR
12	Ulnar; nerve pain; 24 m; ax; MM	Epi mn inf; per/end fib	LLMF; LSMF	Fib; PGL1 ab
13	Sural; paresthesia; 6 m; ax; SM PNP	End eg; epi/per/end fib	LLMF; LSMF	BAAR; eg; fib
14	Sural; paresis; 12 m; ax; MONO	MM	LLMF; LSMF ax reg; remyel	Eg
15	Ulnar; paresthesia; 12 m; ax; MM	MM	LLMF; LSMF ax reg; remyel	NII; fib
16	Ulnar; paresthesia; 4 m; ax; MM	MM	LLMF; LSMF ax reg	EG; Fib; PGL1 ab
17	Ulnar; amyotrophy; 10 m; ax; MM	MM	LLMF; LSMF; ax deg; ax reg; remyel	Fib; PGL1 ab
18	Sural; paresthesia; 18 m; ax; MM	MM	LLMF; LSMF; ax deg; ax reg	Eg; fib
19	Ulnar; paresthesia; 20 m; ax; MM	SM PNP	LLMF; LSMF; ax deg; ax reg	EG; fib

Patients who had their diagnosis confirmed with the clinicoepidemiological data and therapeutic proof; −: absent; AFB+ = acid fast bacilli positivity in the nerve biopsy; ax deg = axonal degeneration; ax reg = axonal regeneration; ax = axonal electroneuromyographic pattern; ax ENMG pattern = axonal electroneuromyographic pattern; demyelin ENMG pattern = demyelinating electroneuromyographic pattern; eg = endoneurial granuloma; End eg = endoneurial epithelioid granuloma; epi fib = epineurial fibrosis; epi mn inf = epineurial mononuclear infiltrate; end mn inf = endoneurial mononuclear infiltrate; end fib = endoneurial fibrosis; per fib = perineurial fibrosis; per mn inf = perineurial mononuclear infiltrate; fib = fibrosis; LLMF = loss of large myelinated fibers; LSMF = loss of small myelinated fibers; m = month; MM = multiple mononeuropathy; MONO = mononeuropathy simplex; Mn inf = mononuclear infiltrate; PCR = polymerase chain reactioin; per fib = perineurial fibrosis; per inf = perineurial mononuclear infiltrate; PGL-1 ab = serum anti-phenolic glycolipid-1 antibodies; remyel = morphological signs of remyelination; SM PNP = sensory-motor polyneuopathy; Vac macroph = vacuolated macrophage.

lepromatous or tuberculoid infiltrates in nerve histology. Uplekar and Antia[24] found a narrow range (TT to BB) for the classification of the leprosy infiltrate in pure neural leprosy.

Leprosy patients may also exhibit discrepant histological appearances in the skin and in the nerve trunks, suggesting that the immunoinflammatory response in the nerve does not depend on that occurring in the skin. Despite the difficulties to employing the Ridley–Jopley

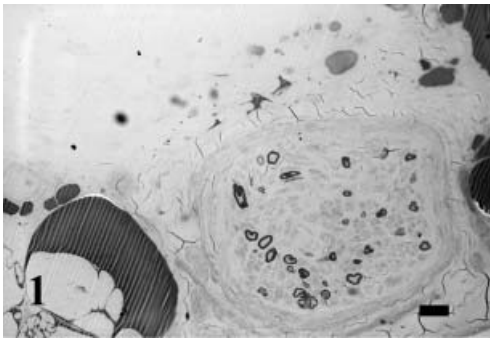

Figure 1. A small nerve fascicle of a neuritic leprosy patient with large and small myelinated fibre loss. Toluidine blue-stained semithin section. Sensory dorsal cutaneous ulnar nerve. Patient 7 of Table 2. Scale bar: 12 μm.

classification to the infiltrates of leprosy neuritis, we can state that regarding the cellular composition of the leprosy infiltrates and the presence of AFB found in the nerves of this study, the range was from BT to BL. Two patients presented both epithelioid granulomas and AFB, suggesting a neural reaction with pain in the nerves. This morphological analysis seems to confirm that a narrower range of immunoinflammatory response can be seen in the nerves affected by pure neural leprosy. The characterization of polar forms in the nerve is made somewhat more difficult because of the obvious impossibility of considering visualization of inflammatory invasion of epidermis (TT form) and of the clear zone (LL form), as seen in the skin.

Leprosy patients may also exhibit discrepant histological appearances in the skin and in the nerve trunks,[18] suggesting that the immunoinflammatory response in the nerve does not depend on that occurring in the skin.

The axonal electroneuromyographic pattern matched with the loss of myelinated fibres found in 94·7% of the biopsies. However, remyelination detected in eight biopsies was not accompanied by a corresponding electroneuromyographic alteration, perhaps because the remyelination found in these patients could be responsible for a possible recovery of their velocity of conduction.

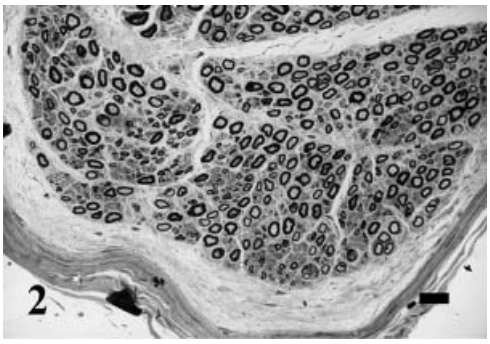

Figure 2. A nerve fascicle from a neuritic leprosy patient showing enlarged subperineurial space and mild loss of myelinated fibres. Sensory dorsal cutaneous ulnar nerve. Patient 14 of Table 2. Toluidin blue-stained semithin section. Scale bar: 12 μm.

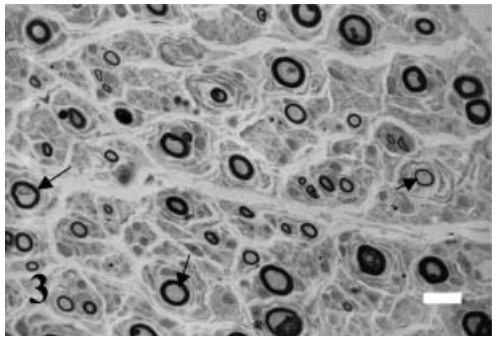

Figure 3. Endoneurial compartment of a neuritic leprosy patient showing some remyelinating fibres accompanied by concentric Schwann cell proliferation surrounding the fibres, forming onion bulbs (arrows). Sural nerve. Patient 2 of Table 2. Toluidin blue-stained semithin section. Scale bar: 5 μm.

Tzourio *et al.*[23] have reported a closer association of disturbances of velocity conduction with axonal loss rather than with demyelination in patients with sensory loss, and thus concluded that the slowing of nerve conduction was more related to axonal degeneration than to demyelination. This interpretation could also be applied to our results, which showed disturbances of velocity conduction on some nerves but which did not allow the definition of a demyelinating neuropathy, because there were important alterations in the amplitude of the axonal potential.

The discrepancy between a normal histological appearance of the nerve biopsy and the electroneuromyographic alterations found in the same nerve exhibited by one patient of this study could be explained by a failure to include the affected segment of the fascicle in the collection of the sampled nerve specimen,[8] or alternatively to exclusively functional disturbances of the nerve rather than damage to its structure.

The present study is consistent with Girdhar *et al.*'s reports,[8] in which the correlation between clinical and pathological data was not considered an aid to grading the severity of the leprosy neuropathy. Jacob and Mattai[9] also studied 77 neural leprosy patients and showed that 50% of them had histological evidence of leprosy, whereas 30% of the remainder showed demyelination with axonal loss, 60% no histological abnormality, 5% vasculitis and 5%

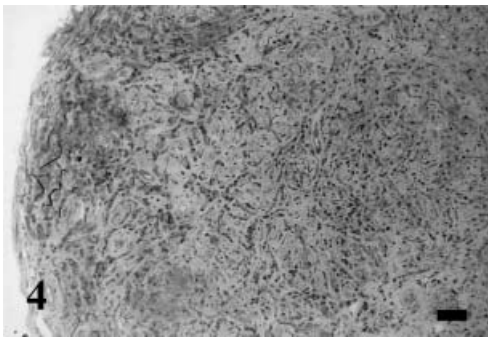

Figure 4. A nerve fascicle from a neuritic leprosy patient exhibiting complete occupation of the endoneurial space by epithelioid granuloma and scattered lymphocytes. The perineurial layers surrounding the fascicle are blurred by the inflammatory process. No nerve fibres can be observed in the endoneurium. Sural nerve. Patient 13 of Table 2. Toluidin blue-stained semithin section. Scale bar: 12 μm.

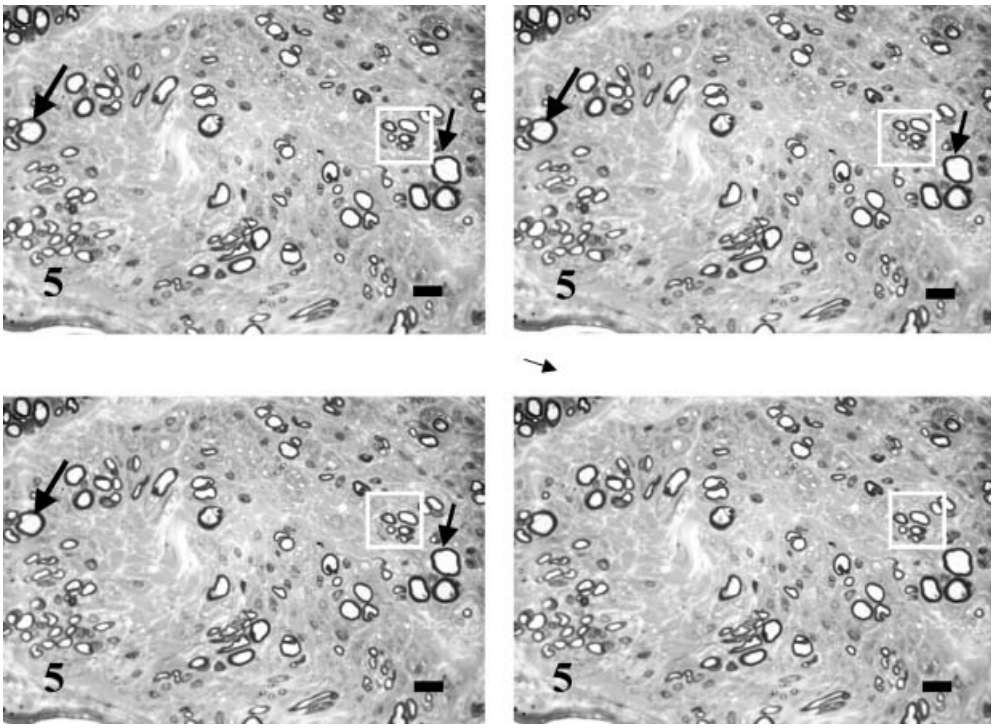

Figure 5. Detail of a fascicle of a neuritic leprosy patient with decreased number of fibres, showing morphological evidence of remyelination (large axon with thin myelin layer, arrows) and axonal sprouting, indicating regenerating axons (square area). Sensory dorsal cutaneous ulnar nerve. Patient 15 of Table 2. Toluidin blue-blue-stained semithin section. Scale bar: 5 μm.

neural hyalinization. Therefore, the correlation of the clinical data with histological analysis of the conventional and semithin sections will not reliably reflect the reality of the involvement of the peripheral nervous system in leprosy.

The morphological findings of this study convey the idea that leprosy causes loss of myelinated and unmyelinated fibres without a conspicuously active axonal degeneration. Fibre loss may therefore be either a consequence of the inflammatory process elicited by *M. leprae* or by an *M. leprae*-induced degenerative process. In other words, we can ask whether axonal loss is an event that happens as a consequence of inflammation or there is another specific axonal degenerative process occurring in leprosy nerves. According to our results, axonal degeneration in leprosy seems to be a consequence of an endoneurial neuritic process, at least in the established inflammatory stage of this neuropathy, although no morphological evidence of a lymphocytic cytotoxic effect could be seen. Alterations posed by the inflammatory process such as modification of the endoneurial environment could likely contribute to axonal loss.

The attraction of the inflammatory cells to the endoneurial environment could be elicited by the presence of *M. leprae* or indirectly by an axonal degenerative process.[26] In our view, the former hypothesis is more plausible for explaining the axonal loss in the leprosy neuropathy, since intrinsic axonal defects directly induced by the presence of *M. leprae* are not likely and the inflammatory process induced by axonal degeneration is usually mild.[26]

Demyelination in leprosy is usually not conspicuous, being better detected by the examination teased fibre.[10] In a speculative way, the demyelinating process in leprosy could be masked by the axonal loss which follows the inflammatory cell invasion of the nerves. It is worth remarking that the only case that showed a demyelinating pattern in the electroneuromyographic assessment, exhibited on the semithin analysis noticeable signs of remyelination with the presence of onion bulb patterns. This case was confirmed to be leprosy with the detection of *M. leprae* DNA, using the polymerase chain reaction carried out in the nerve material.

Another morphological alteration strongly revealed in this study was fibrosis, which was present in up to 73·6% of the nerve biopsies, in the three compartments of the nerve [epineurium (57·8%), perineurium (78·8%) and endoneurium (73·6%)], varying in degree, however, from slight increase of extracellular matrix to more significant accumulation of hyaline matrix (present in only three patients). This high index of fibrosis is because we noticed that the greater the fibre loss, the more intense was the fibrosis. This alteration was better assessed with Gomorís trichrome staining than with semithin sections. Fibrosis in leprosy nerves was reported by Dastur *et al*.,[5] by Junqueira *et al*.,[14] Singh *et al*.[20] and Kajihara *et al*.[15] Junqueira also detected increased type I collagen replacing the normal endoneurial type III collagen in nerve biopsies of leprosy patients. Singh associated fibrosis in the leprosy nerves with an *M. leprae*-induced alteration of fibroblasts. Kajihara found lamellated periaxonal fibrosis surrounding myelinated and also unmyelinated fibres. Antia and Shetty[1] interpret fibrosis as a sequel of the neural inflammatory process and it seems that Schwann cells, besides local fibroblasts, play a role in this endoneurial matrix accumulation. Van Brakel[25] reports the involvement of fibrosis in silent neuritis. We consider two points regarding fibrosis in leprosy: first, it may be due to an inflammation-induced imbalance of cytokines,[2] regulating the assembly of the nerve connective tissue in the post-inflammatory repair and second, the impediment of the regenerating axon growth observed in leprosy[16] may be a consequence of endoneurial fibrosis.

Remyelination and fibrosis can be ascribed to the repairing capability of nerves and are, in the case of this study, concomitants to the damaging leprosy neuritis. We did not consider that both parameters can be interpreted as signals of self-cure as the average duration time of the symptoms before the first examination had been 29·3 months.

In conclusion, no significant correlation of the severity of clinical signs and symptoms with the histopathological findings was detected. This, however, does not mean that the impairment of nerve function has nothing to do with the inflammatory destruction of the nerves, as the biopsy of a segment of the nerve trunk obviously does not represent the whole status of the peripheral nervous system. By contrast, almost all the electroneuromyographic data widely matched with histology. The nerve fibre loss was the most striking finding on the nerve biopsies, but very low active axonal degeneration was observed in the histological sections, making an active axonal neuropathy unlikely. Therefore, a more logical hypothesis would be that fibre loss is a consequence of either the inflammatory process or of demyelination due to functionally impaired, M leprae-infected Schwann cell.

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