IS NERVE BIOPSY USEFUL IN DIAGNOSIS OF CIDP?

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ABSTRACT

Chronic inflammatory demyelinating polyradiculopathy is mainly based on clinical and electrophysiological criteria. If electrophysiological findings remain questionable for the diagnosis of CIDP and/or clinical presentation is atypical, nerve biopsy may assist the diagnosis. We present a case in which the nerve biopsy was helpful in diagnosis of CIDP and revealed the peculiar presence of tomacula.

Key words: CIDP, demyelination, tomacula, nerve biopsy

INTRODUCTION

When the practitioner has to deal with of a clinical picture suggestive of polyneuropathy is important to obtain rigorous data about onset and clinical evolution without ignoring family history.

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an acquired neuropathy of presumed autoimmune etiology and it should be taken into account in any patient who develops insidiously over weeks (at least 8 weeks) to months, sometimes years, motor and sensory deficits in all 4 limbs. Often motor signs predominate and are symmetrical with a variable degree of weakness in muscles of the shoulder and hip girdle which are frequently accompanied by weakness in the forearms and hands and distal legs and feet. Deep tendon reflexes are commonly absent or markedly reduced. Of all sensory modalities, those mediated by large myelinated fibers are most severely affected.

CIDP diagnosis is mainly based on clinical and electrophysiological criteria. If electrophysiological findings remain questionable for the diagnosis of CIDP and/or clinical presentation is atypical, cerebrospinal fluid (CSF) examination, gadolinium-enhanced MRI of spinal roots, brachial or lumbar plexus, nerve biopsy and a trial of immunotherapy with objective assessment of endpoints may assist the diagnosis.

Nerve biopsy can provide supportive evidence for the diagnosis of CIDP. Supportive features for the diagnosis of CIDP are macrophage-associated demyelination, onion bulb formation, demyelinated and to a lesser extent remyelinated nerve fibres, endoneurial oedema, endoneurial mononuclear cell infiltration, and variation between fascicles.

We present a case in which the nerve biopsy was helpful in diagnosis of CIDP.

MATERIAL AND METHODS

Sural nerve biopsy. A sural nerve biopsy was performed. A nerve segment at least 4 cm in length was obtained. It was cut into 3 sections, to be

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processed for frozen and semithin sections, and for fiber teasing. The first nerve specimen was frozen in isopentane cooled in liquid nitrogen, then cut in 8 μm thickness cryosections, stained with H&E and modified trichrome Gomori. The immunohistochemistry for myelinic proteins PMP22 (Peripheral myelin protein 22) and MPZ (Myelin protein zero) was also performed on cryosections, after blocking with incubation in BSA in PBS 3%. The primary antibodies were provided by SIGMA-ALDRICH: monoclonal anti-PMP22, Clone CF1, produced in mouse for human and monkey PMP22; polyclonal anti-MPZ (211-225), produced in rabbit for human MPZ. The dillutions utilised were: 2,5:1000 for anti-PMP22 and 1:250 for anti-MPZ. For the secondary antibody we utilised a Dako LSAB2 System-HRP Kit, provided by DAKO. The second nerve specimen was prepared for Epon embedding. We obtained transversal semithin sections, stained with toluidine blue 1%. The morphometry analysis was made on light microscope (Nikon Eclipse TE300) digital images. The 3rd nerve specimen was prepared for teasing. Approximately 100 nerve fibers were teased and analysed on light microscopy.

Gastrocnemius muscle biopsy. It was performed also a muscle biopsy at the site of nerve biopsy, for additional data concerning the disease process. The muscle fragment was cooled in isopentan in liquid nitrogen and cut in 8 μm thickness cryosections, stained with H&E, modified trichrome Gomori, SDH, NADH.

CASE PRESENTATION

The patient is a 64-year-old right handed man whose disease started 9 months before admission in our department. Onset was insidious by paresthesia of both distal lower limbs and after that weakness was added, in a multifocal manner, in all limbs (first in left leg than, after a month, in right leg than left arm and finally in right arm) in a period of 3-4 months. From the personal history we retain: irradiated pituitary adenoma, high blood pressure, osteopenia, knee joint pain.

There was no family history for neuromuscular disorder.

Cervical and lumbar spine MRI examination was performed before admission in our department and showed minimal median disc herniation L4-L5.

Neurological exam found asymmetrical weakness in all four limbs, predominating in the lower limbs (left lower limb being more severely affected than right lower limb) and hip girdle muscles, paresthesia in fourth and fifth hands digits and in a "socks" manner in legs; no deep tendon reflexes was obtained.

Blood tests revealed: slight bicytopenia (anemia and thrombocytopenia), hepatitis with virus C without cryoglobulinemia.

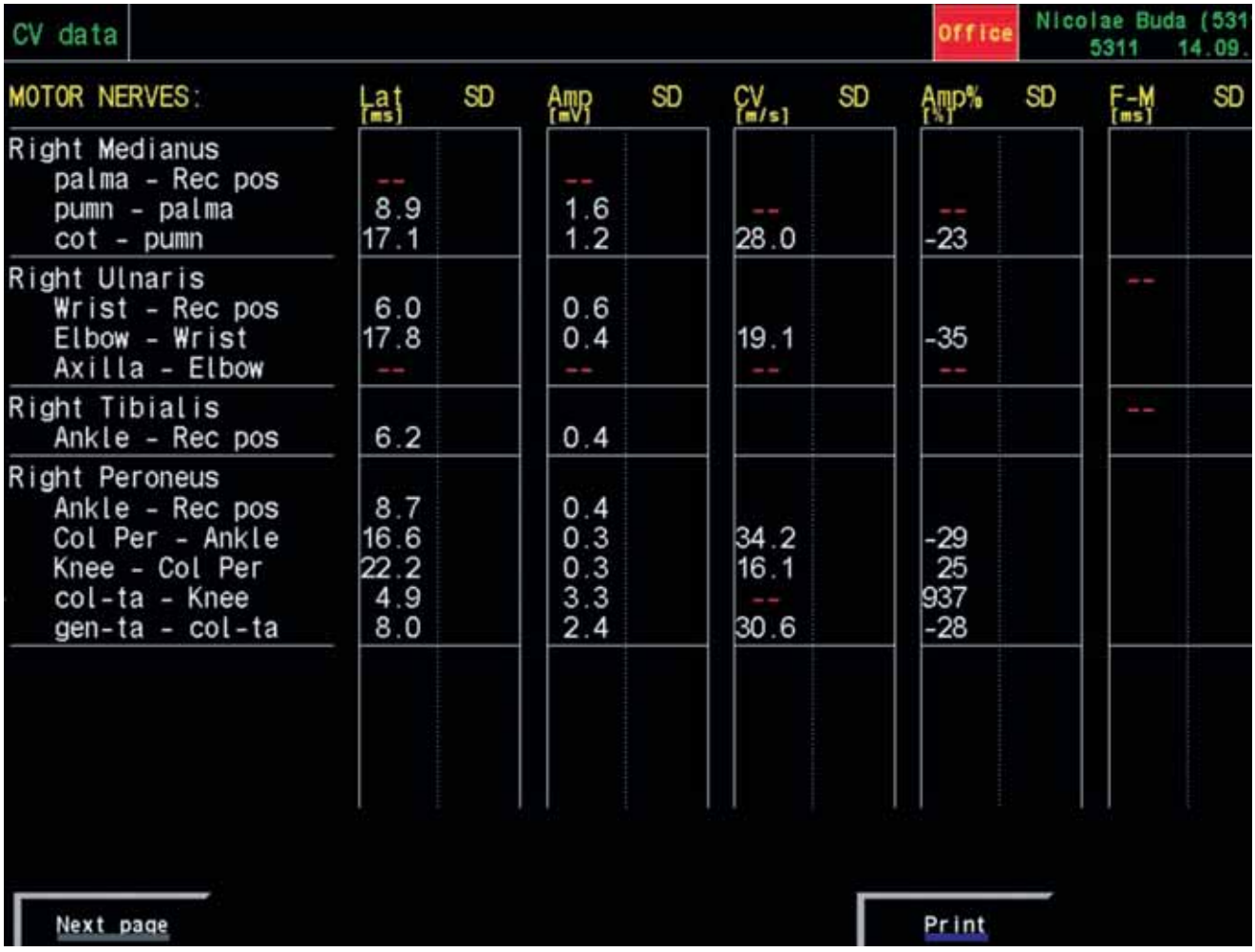
Electrophysiological studies were performed and the nerve conduction study on the motor nerves showed changes suggestive of extensive demyelinating process, but uneven, along nerves (see Table 1 and Figures 1 and 2):

- prolongation by more than 50% of distal motor latency for median nerve, ulnar and peroneal;
- absence of F waves for ulnar and tibial nerves;
- motor nerve conduction velocities severely increased, with more than 30%, for the median nerve, ulnar and peroneal;
- increased duration of distal compound motor action potential for median (9.0 ms) and ulnar (8.0 ms);
- motor conduction bloc at elbow for ulnar nerve.

Other findings on motor nerve conduction study were small amplitudes and some irregular motor action potentials. Sensory nerves were severely affected with no sensory potential.

RESULTS OF BIOPSIES

Sural nerve. The histological data showed a moderate loss of large diameter axons; some nerve fibers with thick myelin sheath are present. No vasculitis picture was seen (Figure 3). Immunohistochemistry for the myelinic proteins PMP22 and MPZ demonstrated the presence of the proteins in the myelinic sheath (Figure 4). Semithin sections showed some denuded axons (demyelination), some axons with tomacula and thickening of myelin, some axons with uncompacted myelin sheath. Some small thinly myelinated axons and clusters of small axons (regenerating) are seen. Few nerve fibers with axonal degeneration (myelinic bulae or a degenerative process) were present. There are some areas without nerve fibers or only with Schwann cells nuclei (Figure 5). Myelinic fibers density was



MOTOR NERVES:	Lat [ms]	SD	Amp [mV]	SD	CV [m/s]	SD	Amp% [%]	SD	F-M [ms]	SD
Right Medianus										
palma - Rec pos	--		--							
pumn - palma	8.9		1.6		--		--			
cot - pumn	17.1		1.2		28.0		-23			
Right Ulnaris									--	
Wrist - Rec pos	6.0		0.6							
Elbow - Wrist	17.8		0.4		19.1		-35			
Axilla - Elbow	--		--		--		--			
Right Tibialis									--	
Ankle - Rec pos	6.2		0.4							
Right Peroneus										
Ankle - Rec pos	8.7		0.4							
Col Per - Ankle	16.6		0.3		34.2		-29			
Knee - Col Per	22.2		0.3		16.1		25			
col-ta - Knee	4.9		3.3		--		937			
gen-ta - col-ta	8.0		2.4		30.6		-28			

Table 1. *Electrophysiological parameters*

5.443 nerve fibers/mm2, showing a moderate axonal degeneration (the normal fibers density is 7.000-11.000 nerve fibers/mm2). No inflammatory cells were seen. The myelinic fibers histogram is unimodal, with the pick at 3 microns diameter fibers; this means a loss of large axons. The axons of 9-16 microns are, in fact, the hypermyelinated axons (Figure 6). Teased fibers were: 65% normal fibers; 22% demyelinating fibers (8% segmental demyelination and 14% remyelinating fibers), 13% fibers with axonal degeneration. Tomacula were present in 21% teased fibers, and 41% teased fibers presented hypermyelinisation (myelin thickenings) (Figure 7). Tomacula refer to the focal sausage-shaped swellings of myelin sheaths.

Skeletal muscle. The muscle biopsy presented neurogenic modifications: important variability of muscle fibers diameter especially by the presence of atrophic fibers, singles or in small clusters, a few angulated fibers, and some internalised nuclei. A small fascicle with very atrophic muscle fibers in a connective tissue mass and inflamatory infiltrate is present in the biopsy. Muscle fiber type grouping with the predominance of tpye II, moth eaten fibers, and targetoid fibers with oxidative enzymes stainings (NADH, SDH) are typical neurogenic aspects (Figure 8).

DISCUSSION

We present here a case with electrophysiological findings that respect the EFNS electrodiagnostic criteria for CIDP (2) for a patient with a polyneuropathy with progressive multifocal evolution with asymmetric weakness and hepatitis C virus infection. Atypical presentations of CIDP have been recognized where the weakness is focal or multifocal and is often asymmetrical (1). In these cases an acquired multifocal motor and sensory demyelinating neuropathy is supported by the electrophysiological findings of slow motor and sensory conduction velocities and of persistent partial conduction block in single nerves indicating focally accentuated demyelination (1-3). However, it is important

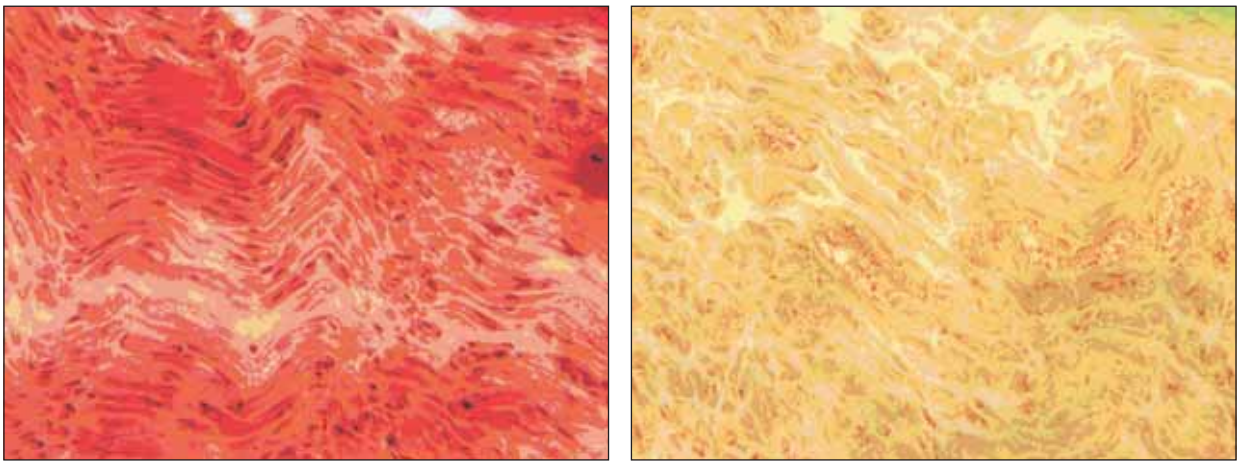
Figure 1. *Motor nerve conduction study for right median shows prolonged distal latency, increased duration of compound muscular action potential (CMAP), decreased motor conduction velocities and small CMAP*

to keep in mind that similar clinical presentation may be seen with ischemic mononeuropathies caused by a leukoclastic vasculitis associated with chronic hepatitis C and cryoglobulinemia, or by a systemic or nonsystemic necrotizing vasculitis. The differential diagnosis should also include the granulomatous or infiltrative pathologies (1). This was the reason to perform pathology study of a nerve biopsy apart from electrophysiological examination.

In CIDP the characteristic lesions are a combination of fiber and interstitial alterations. They are multifocal affecting proximal and distal levels of nerves to a variable degree. The typical fiber changes are segmental demyelination, which is the most characteristic lesion, and remyelination, axonal degeneration and fiber loss, and reactions to these events (onion-bulb formation, fiber loss, axonal sprouting). The interstitial alterations include edema, inflammatory cell infiltrates and scarring (1, 2, 3). Because CIDP is due to a multifocal interstitial process that usually affects roots and proximal to distal levels of peripheral nerve, a sural nerve biopsy may, or may not, show the worst or typical lesions of CIDP, but it is important to perform it rather to rule out other disease such as amyloidosis, necrotizing vasculitis and sarcoidosis(1).

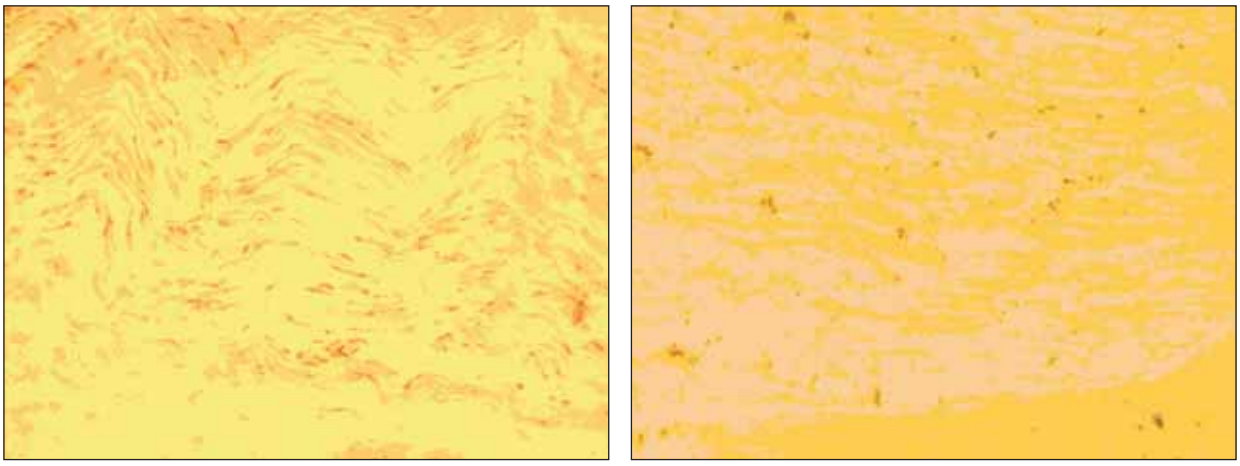
Peripheral neuropathy associated with hepatitis C virus is mainly characterized by axonal damage and it is often associated with cryoglobulinemia. The peripheral neuropathy is a common complication of cryoglobulinemia associated with hepatitis

Figure 2. *Motor conduction block at elbow on right ulnar nerve*



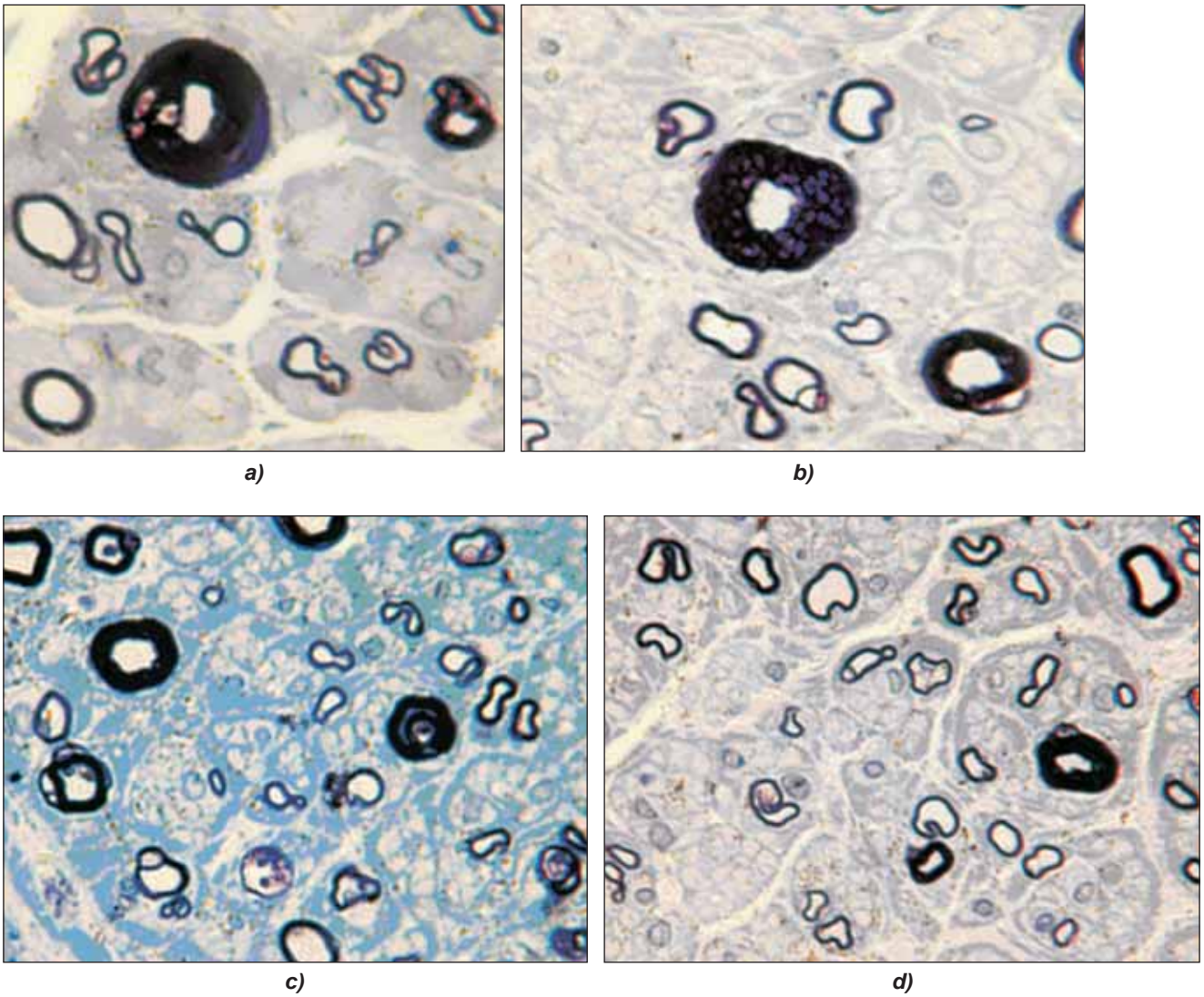
a) ***b)***

Figure 3. *Histological aspects on cryosections: moderate loss of large axons, the presence of nerve fibers with tomacula and myelin thickenings, small area depleted of axons (a. H&E, Ob. x10; b. trichrome Gomori modified, Ob. x10)*



a) b)

Figure 4. *Immunohistochemistry: a) the presence of PMP22 in the compacted myelin (anti-PMP22 antibody; HRP labelling, Ob. x10); b) the presence of MPZ in the myelin sheath (anti-MPZ antibody; HRP labelling, Ob. x10)*



a) b)

c) d)

Figure 5. *Semithin sections: tomacula presence (a, b, d), thinly myelin sheath, loss of large axons, uncompacted myelin sheath (b), clusters of small axons (regenerating) are seen (b), nerve fiber with axonal degeneration (myelinic bulae and a degenerative process) (c), some areas with a deplesion of nerve fibers (b, c, d) (toluidine blue, Ob. x60 immersion)*

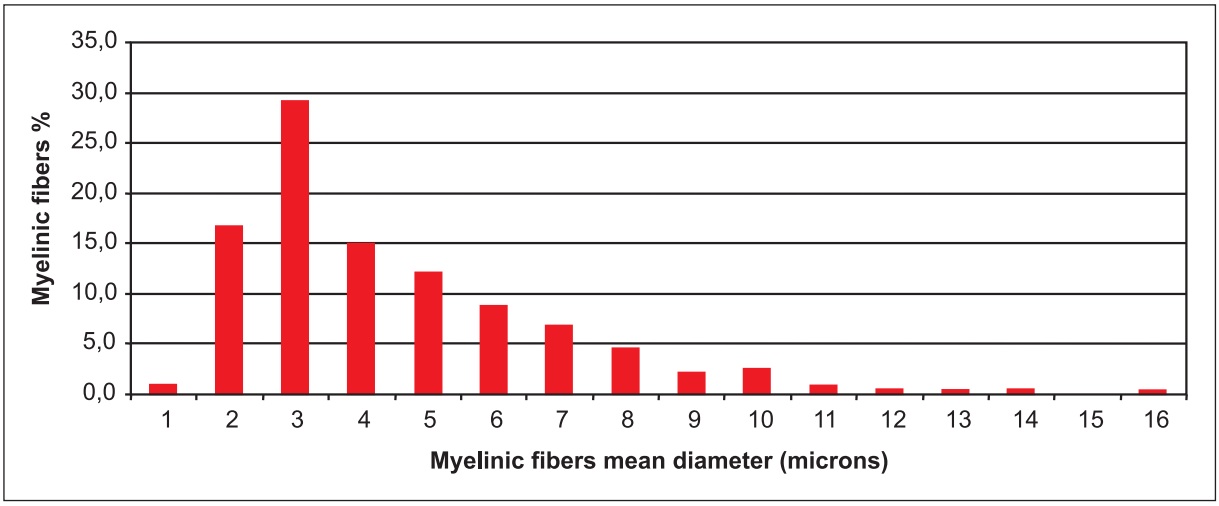
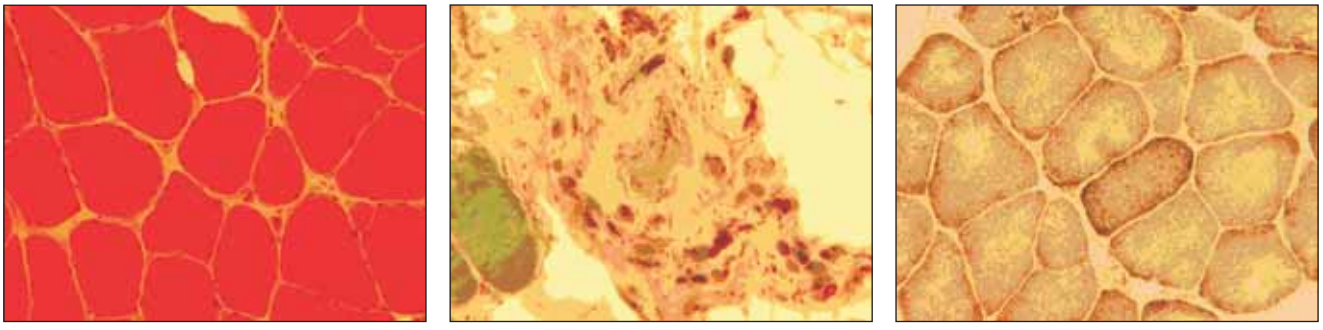


Figure 6. *Unimodal myelinated fibers histogram*

Figure 7. *Teasing images: segmental demyelination (a), tomaculum (b), tomaculum and myelin thickenings (c) (Ob. x10)*



a) ***b)*** ***c)***

Figure 8. *Muscle biopsy: variability of fibers diameter, atrophic fibers, singles or in small clusters (a), or a whole fascicle (b), angular fibers (a), some internalised nuclei (a); one muscular fascicle with few fiber very atrophied in a collagen mass (b); moth eaten fibers and type II muscle fiber predominance (c). (a. H&E, Ob. x10; b. trichrome Gomori modified, Ob. x10; c. Succinic dehydrogenase, Ob. x10).*

C virus infection and it is thought to be attributable to nerve ischemia secondary to epineurial vessels changes caused by occlusion or vasculitis induced by longstanding cryoglobulin precipitation with complement fixation and rheumatoid factor deposition (6,7).

So, in our case the lack of inflammation of the walls of microvessels, as well as the lack of ischemic changes, hemosiderin and neovascularization rules out other diseases such are necrotizing vasculitis.

Some mentions must be made about the presence of tomacula in our findings in nerve biopsy. Although tomacula is the hallmark of hereditary neuropathy with liability to pressure palsies (HNPP), it is not found exclusively in this kind of polyneuropathy. Tomacula defined as thickening of myelin sheath has as possible causes hypermyelination, redundant myelin loop formation, second mesaxon, transnodal myelination, myelin sheath formed by two Schwann cells. Anti-MAG neuropathy, CIDP, CMT (Charcot-Marie-Tooth polyneuropaty) 1A, CMT 4B, HMSN (hereditary motor sensory neuropathy) are other kind of polyneuropathy associated with tomacula (10). The general electrophysiologic correlations with presence of tomacula are: multiple mononeuropathy, sensory and motor involvement and demyelination. In HNPP there is a background of demyelinating neuropathy independent of entrapment neuropathy proven by diffuse distribution of sensory nerve conduction velocities slowing (11). Also, in contrast to widespread sensory nerve conduction velocities slowing, slowing of motor nerve conduction velocities in segments of nervs outside the usual entrapment sites are less common and tend to be minor (11). In our case apart of severe sensory nerve involvement and prolongation of distal motor latencies we found significant slowing of motor nerve conduction velocities, which is not seen in HNPP.

CONCLUSIONS

The clinical presentation of our patient with a polyneuropathy with progressive multifocal evolution with asymmetric weakness associated to hepatitic virus C infection usually is associated with ischemic mononeuropathies caused by a leukoclastic vasculitis that is seen with chronic hepatitis C and cryoglobulinemia, or by a systemic or nonsystemic necrotizing vasculitis. This clinical picture was surprisingly associated with an asymmetric pattern of demyelination after nerve conduction studies. So we decided to perform a nerve biopsy. The benefit of biopsy was, beside of gaining supportive arguments in favor of the CIDP diagnosis, to eliminate other diseases.

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