Motor Nerve Biopsy: Clinical Usefulness and Histopathological Criteria

Nilo Riva, MD,[1] Sandro Iannaccone, MD,[2] Massimo Corbo, MD,[3] Chiara Casellato, MD,[4] Barbara Sferrazza, MD,[2] Alberto Lazzerini, MD,[5] Marina Scarlato, MD, PhD,[1] Federica Cerri, MD,[1] Stefano C. Previtali, MD, PhD,[1] Eduardo Nobile-Orazio, MD, PhD,[4] Giancarlo Comi, MD,[1,6] and Angelo Quattrini, MD[1]

Early differential diagnosis of motor neuropathies (MN) and lower motor neuron diseases (LMND) is important, as prognosis and therapeutic approaches are different. We evaluated the diagnostic contribution of the biopsy of the motor branch of the obturator nerve and gracilis muscle in 21 consecutive patients in which, after proper clinical and neurophysiological studies, the differential diagnosis was still open. At baseline, motor biopsy was performed; diagnostic confirmation was obtained by 2-year clinical follow-up. Our results support the usefulness of this diagnostic procedure for selected cases of MN and LMND.

ANN NEUROL 2010;00:000–000

Motor neuron disease (MND) indicates a group of neurological disorders characterized by degeneration of motor neurons. Amyotrophic lateral sclerosis (ALS) is the most common form, involving both lower motor neurons (LMN) and upper motor neurons (UMN), whose biological, psychological, and social impacts are devastating.[1] ALS diagnosis is generally fairly simple,[2] but may be less certain in patients presenting with sporadic progressive disease of LMN. These patients were diagnosed as "suspected ALS" according to the 1994 El-Escorial criteria but this category no longer exists in the 2000 revised criteria.[3] The term lower MND (LMND) is more appropriately used to indicate this heterogeneous group of diseases, which includes progressive muscular atrophy (PMA). A substantial proportion of PMA patients develop ALS or have an "ALS-like" disease course.[4] Notably, the reported percentage of misdiagnosis is 19% for PMA,[5] and up to 10% for ALS (1% rediagnosed as neuropathy).[6–8] Therefore, in some cases, only follow-up can lead to a certain diagnosis.

Motor neuropathies (MN) are an heterogeneous group of diseases primarily affecting the motor nerves. In most MN cases the absence of UMN signs and demyelinating features at nerve conduction studies lead to a straightforward diagnosis. However, demyelinating features may not always be demonstrated and purely axonal electrophysiologic findings are found in selected cases, some responding to intravenous immunoglobulin therapy.[9–11]

Early differential diagnosis between LMND and MN is important, as prognosis and therapeutic approach are different; moreover, current and future therapies might be more effective in the first stages of disease.[12]

The morphological aspects of the motor branch of the obturator nerve have been shown to differ in patients with a definite diagnosis of MN or MND; however, the clinical value and potential diagnostic contribution of this investigation in the early stages of disease are still unknown and are therefore the focus of our study.[13]

Patients and Methods

Patients

We studied 21 consecutive patients over about 900 screened (neuropathies ≈ 630; MND ≈ 270). Patients provided informed consent to the study, approved by the local ethic committee. We included patients presenting with sporadic, recent-onset LMN syndrome in which, after extended clinical, neurophysiological and hematochemical examination, a conclusive diagnosis could not be reached. Neurophysiological findings were consistent with pure motor axonal neuropathy/neuronopathy at limbs. Follow-up neurological examination was performed at 6, 12, 18, and 24 months (see Supplementary Material and Supplementary Table S1 and S3).

From the [1]Department of Neurology, INSPE and Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy; [2]Department of Clinical Neurosciences, San Raffaele Turro Hospital, Milan, Italy; [3]NEuroMuscular Omnicentre (NEMO), Niguarda Ca Granda Hospital, Milan, Italy; [4]2° Neurology, IRCCS Istituto Clinico Humanitas, Milano University, Milan, Italy; [5]Hand Surgery and Microsurgery Unit, IRCCS Istituto Clinico Humanitas, Milan, Italy; [6]Università Vita e Salute San Raffaele Milan, Milan, Italy.

Address correspondence to Dr. Riva, Department of Neurology, INSPE and Division of Neuroscience, San Raffaele Scientific Institute, via Olgettina 48, 20132 Milan, Italy. E-mail: riva.nilo@hsr.it

Received Feb 3, 2010, and in revised form May 27, 2010. Accepted for publication May 28, 2010.

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/ana.22110

Additional Supporting Information can be found in the online version of this article.

Neuropathology

All patients underwent biopsy of the motor branch of the obturator nerve and gracilis muscle.

Light and electron microscope examinations were perfomed.[14,15] Nerve morphometric analysis included fiber density and g-ratio (axonal/fiber diameter); the regeneration parameter was calculated as the number of regenerating clusters per mm^2 (cluster density [CD]) and as the ratio of clusters to fibers.[16]

Nerve and muscle morphological examination were performed by 3 blinded independent examiners. Inter-reader agreement was evaluated with Cohen's kappa index.

Criteria for nerve biopsy analysis:

1. Signs of myelin pathology: nerve fiber demyelination/remyelination, onion bulbs.
2. Signs of axonal pathology: reduction of myelinated fibers, signs of active axonal degeneration.
3. Pathological signs suggesting a known cause of neuropathy.[15]
4. Nerve regeneration parameter. The only previous study on the obturator nerve found a mean CD of $19.2/mm^2$ in patients with a definite clinical diagnosis of MND (standard deviation (SD)= 8.4).[13] CD $<27.6/mm^2$(mean + SD) was chosen as a supportive criterion for LMND (CD $>$ $27.6/mm^2$ for MN).

Based on these findings, patients were divided into 2 groups: 1) Group I, suspected LMND; and 2) Group II, suspected MN.

Follow-Up: Clinical Diagnosis

MND or peripheral neuropathy/MN diagnosis was performed according to standard criteria.[3,5,17]

Statistical Analysis and Development of Neuropathological Diagnostic Criteria

Statistical analysis of morphometric data was performed after clinical diagnostic confirmation at 2 years of follow-up using SPSS software (Chicago, IL); for group comparisons, the Mann-Whitney U-test was applied (statistical significance threshold: $p < 0.05\%$).

We propose neuropathological criteria for motor nerve biopsy interpretation. CD (and cluster/fiber ratio) reference intervals have been defined as follows:

- Upper CD limit for LMND: mean LMND-CD + SD;
- Lower CD limit for MN: mean MN-CD − 1SD.

(CD obtained from compound descriptive statistic analysis including morphometric data from our previous study performed in patients with a definite clinical diagnosis at biopsy).[13]

Results

Baseline: Histopathological Diagnosis

Inter-reader agreement was 100%(Cohen's kappa).

Twelve patients (1–12; mean age: 51.8 years, range: 43–62 years) were classified in Group I (suspected LMND). Morphological examination showed reduction of myelinated fibers, sometimes associated with signs of active axonal degeneration, poor/no signs of nerve regeneration, and no signs of demyelination/remyelination and/or inflammatory cell infiltration. Notably, the reduction in fiber density tended to be a focal/multifocal distribution among and within fascicles (Fig 1).

Eight patients (14–21; mean age: 55.6 years; range: 45–65 years) were classified in Group II (suspected MN). Morphological examination showed a reduction of myelinated fibers uniformly distributed within and between the fascicles; in 6 patients (16–21) the diagnosis was supported by high CD, in 3 patients (19, 20, and 21) it was associated with signs of demyelination/remyelination. In 2 patients (14 and 15) nerve biopsy showed low-moderate signs of nerve regeneration, associated in one (14) with amyloid deposits on Congo-red staining, and in the other (15) with sign of demyelination/remyelination.

Muscle biopsy did not help in differentiating MN from LMND, in all patients showing small angulated denervated fibers, variable degree of type grouping, type II fiber hypertrophy in 2 patients (10 and 12) and fiber I hypertrophy in 3 (14, 17, and 18).

In 1 patient (13) nerve and muscle biopsy showed normal pathological findings. This patient did not have clinical/neurophysiological abnormalities of lower limbs at time of biopsy.

Differences in clinical characteristics between group I and II were unremarkable.

Follow-Up: Clinical Diagnosis

Two years of clinical follow-up confirmed the baseline histopathological diagnosis in all patients (see Supplementary Table S1 and S2).

Group I: in 8 patients (1, 3, 4, 5, 8, 10, 11, and 12) the final diagnosis was ALS. Four patients (2, 6, 7, and 9) developed diffuse LMN signs and a rapidly progressive disease leading to respiratory failure: LMND was diagnosed.

Group II: in 5 patients (14, 15, 18, 19, and 21) a final diagnosis of motor-sensory axonal neuropathy was made, while in 3 patients (16, 17, and 20) the final diagnosis was axonal MN. The patient with amyloid neuropathy died within 1 year.

Patient 13 developed ALS.

We obtained an overall sensibility for disease detection of 0.95 (95% confidence interval: 0.74–0.99). No patient complained of adverse symptoms related to biopsy.

Morphometric Analysis and Neuropathological Diagnostic Criteria

Morphometric studies showed increased CD in MN patients nerves ($p < 0.001$), a border-line reduction of g-

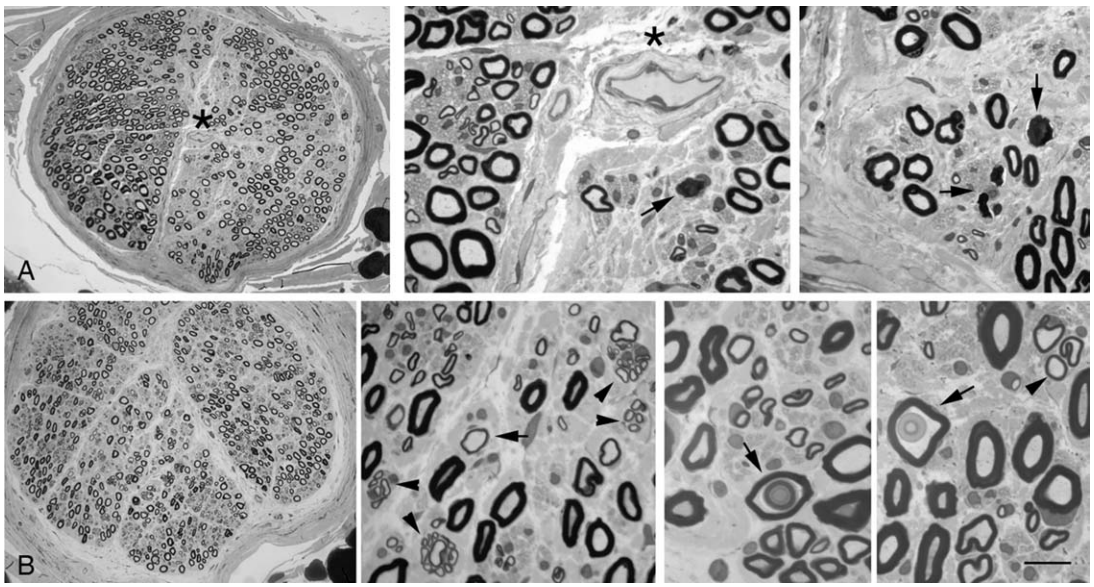

FIGURE: Representative neuropathological cases. Transverse semi-thin sections of biopsy of motor nerve from case 1 (A: MND) and 19 (B: MN). Focal decreased density of myelinated nerve fibers (A*) is evident. In A, axonal degeneration is present at higher magnification *(arrows)*. (B) Mild reduction of large myelin nerve fibers is present in representative sections from patients with definite diagnosis of MN. There are many clusters of small myelinated fibers *(arrowheads)* indicating axonal regeneration. In addition a thinly myelinated nerve fiber *(arrow)*, indicating remyelination, and poliglucosan bodies inclusions *(white arrows)* are present. Bar: 50 μm; high magnification: 15 μm. MN = motor neuropathies; MND = motor neuron diseases.

ratio in LMND ($p < 0.05$) but no group differences in myelinated fiber density (Table 1).

The proposed pathological diagnostic criteria should be applied in the appropriate clinical context (namely, recent-onset LMN syndromes)(Table 2; Supplementary Table S4 and S5).

Signs of axonal pathology and low CD should lead to suspicion of LMND, which is also suggested, in our experience, by focal fiber loss. Signs of demyelination/remyelination and/or high CD support a pathological diagnosis of MN; rarely, specific findings can be demonstrated, such as pathologic deposits or axonal inclusions.

Discussion

As main result of this study, motor nerve pathologic examination was helpful for early differential diagnosis of LMN syndromes. At morphometric examination, CD was the best parameter for differentiating MN from MND patients and was also a prognostic factor, independently from the diagnosis. As expected from neurophysiological inclusion criteria, neuropathological examination showed scant signs of demyelination/remyelination in MN. These results are consistent with a previous study, which was performed, unlike the present study, on patients with a definite diagnosis of MND or MN.[13] We observed a tendency toward a relatively focal/patchy fiber loss in MND motor nerves. However, this observation was not included as a diagnostic criterion for 2 reasons. First, this observation has never been reported before. This might be explained by the different timing of motor nerve biopsy, performed in this study at an early stage, while in previous reports in patients at an advanced stage of disease or postmortem.[13,18,19] Second, a varying degree of focal/multifocal fiber loss is seen in a variety of neuropathies, including vasculitis or demyelinating neuropathies and has recently been described in biopsies of upper limb nerves obtained at sites of conduction block from long-lasting cases of MMN.[20]

In this study, muscle biopsy, considered by the El-Escorial criteria as a possible diagnostic investigation for ALS, did not help in differentiating MN from MND.[3] Type grouping percentage was similar, in spite of the marked increase in CD observed in MN patients (see Table 2). Different re-enervation mechanisms in fact take place in MN and LMND.[14] Collateral re-enervation through sprouting of surviving distal motor fibers can be observed both in MN and MND, underlying type grouping formation on muscle pathological inspection and increased motor unit potential amplitude on needle examination, while cluster formation underlies nerve regeneration, requires a vital LMN, and is more prominent in MN.

We avoided the introduction of the pathologic diagnostic category "definite LMND" because for a pathologic confirmative diagnosis, an extensive central nervous system examination, including motor neuron cell

TABLE 1: Results of Morphometric Studies and Comparison with Previous Literature Results

	Present Study			Corbo et al.[13]			Present Study + Corbo et al.[15]		
	Diagnosis			Diagnosis			Diagnosis		
	MN	MND	*p*	MN	MND	*p*	MN	MND	*p*
Patients (n)	9	12		6	9		25	21	
Fiber density (mm^2)[a]	4819 (1443)	4350 (933)	NS	4684 (1714)	4788 (1291)	NS	4761 (1502)	4358 (1093)	NS
G ratio[a]	0.60 (0.02)	0.57 (0.03)	0.02	0.56 (0.03)	0.51 (0.04)	0.03	0.58 (0.03)	0.54 (0.04)	0.02
Cluster density (mm^2)[a]	62.5 (33.7)	9.4 (6.7)	<0.001	92.7 (25.8)	19.2 (8.4)	<0.001	75.4 (33.2)	13.6 (8.8)	<0.001
Cluster/fiber ratio (%)[a]	145.3 (94.6)	23.4 (17.4)	<0.001	208.4 (53.4)	43.0 (20.4)	<0.001	172.4 (83.4)	31.8 (20.8)	<0.001

[a]Values are mean (SD).
MN = motor neuropathy; MND = motor neuron disease; NS = not significant; SD = standard deviation.

TABLE 2: Proposed Neuropathological Diagnostic Criteria for Motor Nerve Biopsy

Inclusion Criteria	
1. Recent onset lower motor neuron syndrome	
2. Informative biopsy	
Pathologic Diagnostic Criteria	
1. Signs of axonal pathology	
2. Regeneration parameter	
a. Low regeneration: cluster density < $22.4mm^2$ (or cluster/fiber ratio < 0.52%)	
b. Intermediate regeneration: cluster density between 22.4 and $42.2mm^2$(or cluster/fiber ratio between 0.52% and 0.89%)	
c. High regeneration cluster density > $42.2\ mm^2$ (or cluster/fiber ratio > 0.89%)	
3. Signs of demyelination/remyelination, pathologic deposits, or other potential causes of neuropathy	
Pathologic Diagnostic Categories	**Pathologic Diagnostic Criteria Required**
Definite MN	1 + 2b + 3
	1 + 2c + 3
	2b + 3
	2c + 3
	3
Probable MN	1 + 2 c
Possible MN (LMND not excluded)	1 + 2b
Probable LMND	1 + 2a

bodies, should be performed.[3] LMND remains, therefore, a diagnosis of exclusion because a disease morphological marker in the peripheral nerve is still lacking. However, a definite diagnosis of MN is possible.

The biopsy of the motor branch of the obturator nerve should be considered as a potential diagnostic tool for early differential diagnosis of selected cases of LMND and MN.

Acknowledgments

This research was supported by Istituto Superiore di Sanità e Fondo per gli investimenti della Ricerca di Base TissueNet (to A.Q.) and by MoH RF-FSR-2007-637144 to S.I.

Potential Conflict of Interest

Nothing to report.

References

1. Carus R. Motor neurone disease: a demeaning illness. Br Med J 1980;280:455–456.
2. de Carvalho M, Dengler R, Eisen A, et al. Electrodiagnostic criteria for diagnosis of ALS. Clin Neurophysiol 2008,119:497–503.
3. Brooks BR, Miller RG, Swash M, Munsat TL. World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000;1:293–300.
4. Van den Berg-Vos RM, Visser J, Kalmijn S, et al. A long-term prospective study of the natural course of sporadic adult-onset lower motor neuron syndromes. Arch Neurol 2009;66:751–757.
5. Visser J, van den Berg-Vos RM, Franssen H, et al. Mimic syndromes in sporadic cases of progressive spinal muscular atrophy. Neurology 2002;58:1593–1596.
6. Scottish Motor Neuron Disease Research Group. The Scottish Motor Neuron Disease Register: a prospective study of adult onset motor neuron disease in Scotland. Methodology, demography and clinical features of incident cases in 1989. J Neurol Neurosurg Psychiatry 1992;55:536–541.
7. Davenport RJ, Swingler RJ, Chancellor AM, Warlow CP. Avoiding false positive diagnoses in motor neuron disease: lessons from the Scottish Motor Neuron Disease Register. J Neurol Neurosurg Psychiatry 1996;60:147–151.
8. Traynor BJ, Codd MB, Corr B, et al. Clinical features of amyotrophic lateral sclerosis according to the El Escorial and Airlie House diagnostic criteria. Arch Neurol 2000;57: 1171–1176.
9. Van Asseldonk JT, Franssen H, Van den Berg-Vos RM, et al. Multifocal motor neuropathy. Lancet Neurol 2005;4:309–319.
10. Katz JS, Barohn RJ, Kojan S, et al. Axonal multifocal motor neuropathy without conduction block or other features of demyelination. Neurology 2002;58:615–620.
11. Delmont E, Azulay JP, Giorgi R, et al. Multifocal motor neuropathy with and without conduction block: a single entity? Neurology 2006;67:592–596.
12. Shook SJ, Pioro EP. Racing against the clock: recognizing, differentiating, diagnosing, and referring the amyotrophic lateral sclerosis patient. Ann Neurol 2009;65:S10–S16.
13. Corbo M, Abouzahr MK, Latov N, et al. Motor nerve biopsy studies in motor neuropathy and motor neuron disease. Muscle Nerve 1997;20:15–21.
14. Dubowitz V, Sewry CA. Neurogenic disorders. In: Dubowitz V, Sewry CA, eds. Muscle biopsy. A practical approach. 3th ed. Philadelphia: Elsevier Saunders, 2007:275–292.
15. Dick PJ, Dyck PJB, Engelstad J. Pathologic alterations of nerves. In: Dyck PJ, Thomas PK, eds. Peripheral neuropathy. 4th ed. Philadelphia: Elsevier Saunders, 2005:733–830.
16. Previtali SC, Malaguti MC, Riva N, et al. The extracellular matrix affects axonal regeneration in peripheral neuropathies. Neurology 2008;71:322–331.
17. Grant IA, Benstead TJ. Differential diagnosis of polineuropathy. In: Dyck PJ, Thomas PK, eds. Peripheral neuropathy. 4th ed. Philadelphia: Elsevier Saunders. 2005:1163–1180.
18. Atsumi T. The ultrastructure of intramuscular nerves in amyotrophic lateral sclerosis. Acta Neuropathol 1981;55:193–198.
19. Mitsumoto H, Chad DA, Pioro EP. Neuropathology. In: Amyotrophic lateral sclerosis. Philadelphia: FA Davis Company, 1998:179–196.
20. Taylor BV, Dyck PJ, Engelstad J, et al. Multifocal motor neuropathy: pathologic alterations at the site of conduction block. J Neuropathol Exp Neurol 2004;63:129–137.