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, PhD,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, MD1

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.

M

otor 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% re-diagnosed 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 hematocritical 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 1Department of Neurology, INSPE and Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy; 2Department of Clinical Neurosciences, San Raffaele Turro Hospital, Milan, Italy; 3Department of Clinical Neurosciences, San Raffaele Scientific Institute, Milan, Italy; 4Neurology, IRCCS Istituto Clinico Humanitas, Milan University, Milan, Italy; 5Hand Surgery and Microsurgery Unit, IRCCS Istituto Clinico Humanitas, Milan, Italy; 6Università 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 performed. Nerve morphometric analysis included fiber density and g-ratio (axonal/fiber diameter); the regeneration parameter was calculated as the number of regenerating clusters per mm² (cluster density [CD]) and as the ratio of clusters to fibers. 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).

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 MN). 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/neuropathological abnormalities of lower limbs at time of biopsy.

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

Follow-Up: Clinical Diagnosis
MND or peripheral neuropathy/MN diagnosis was performed according to standard criteria.

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.

(Morphometric studies showed increased CD in MN patients nerves (p < 0.001), a border-line reduction of g-
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-nerervation mechanisms in fact take place in MN and LMND.\(^{14}\) Collateral re-nerervation 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...
### Acknowledgments

This research was supported by Istituto Superiore di Sanità and 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.

---

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

<table>
<thead>
<tr>
<th></th>
<th>Present Study</th>
<th>Corbo et al.</th>
<th>Present Study + Corbo et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diagnosis</td>
<td>Diagnosis</td>
<td>Diagnosis</td>
</tr>
<tr>
<td></td>
<td>MN</td>
<td>MND</td>
<td>MN</td>
</tr>
<tr>
<td>Patients (n)</td>
<td>9</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Fiber density (mm²)</td>
<td>4819 (1443)</td>
<td>4350 (933)</td>
<td>4684 (1714)</td>
</tr>
<tr>
<td>G ratio</td>
<td>0.60 (0.02)</td>
<td>0.57 (0.03)</td>
<td>0.56 (0.03)</td>
</tr>
<tr>
<td>Cluster density</td>
<td>62.5 (33.7)</td>
<td>9.4 (6.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cluster/fiber ratio</td>
<td>145.3 (94.6)</td>
<td>23.4 (17.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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**
- Recent onset lower motor neuron syndrome
- Informative biopsy

**Pathologic Diagnostic Criteria**
- Signs of axonal pathology
- Regeneration parameter
  - Low regeneration: cluster density < 22.4 mm² (cluster/fiber ratio < 0.52%)
  - Intermediate regeneration: cluster density between 22.4 and 42.2 mm² (cluster/fiber ratio between 0.52% and 0.89%)
  - High regeneration: cluster density > 42.2 mm² (cluster/fiber ratio > 0.89%)
- Signs of demyelination/remyelination, pathologic deposits, or other potential causes of neuropathy

**Pathologic Diagnostic Categories**
1. Definite MN
2. Probable MN
3. Possible MN (LMND not excluded)

**Pathologic Diagnostic Criteria Required**
- a. Low regeneration: cluster density < 22.4 mm²
- b. Intermediate regeneration: cluster density between 22.4 and 42.2 mm²
- c. High regeneration: cluster density > 42.2 mm² (cluster/fiber ratio > 0.89%)

---

**ANNALS of Neurology**

4 Volume 000, No. 000

---

**Potential Conflict of Interest**

Nothing to report.
References