Motor Score of the Unified Parkinson Disease Rating Scale as a Good Predictor of Lewy Body–Associated Neuronal Loss in the Substantia Nigra

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Background: How well the motor symptoms assessed by the motor section of the Unified Parkinson Disease Rating Scale (UPDRS3) reflect the neuronal loss observed in the substantia nigra is not known.

Objective: To study the relationships among the motor symptoms assessed by the UPDRS3, Lewy body–associated neuronal loss in the substantia nigra, and duration of disease.

Design: Longitudinal, prospective, clinicopathological study.

Setting: Long-term care facility of a university hospital.

Patients: Eighteen elderly patients with a parkinsonian syndrome, studied prospectively but selected post mortem on the basis of the presence of Lewy bodies, and 5 age-matched control subjects.

Methods: One map of a section of the substantia nigra, indicating the location of all the nucleolated neuronal profiles, was drawn for each case. Neuronal density was estimated using a tessellation method. The relationship between time and neuronal loss and between neuronal loss and motor symptoms (assessed by the UPDRS3) was studied by means of regression analysis, using linear and exponential models.

Results: The neuronal density was linearly linked with the UPDRS3 score ($r = -0.83 \ [P < .001]$). Each point added to the UPDRS3 score corresponded to an estimated loss of 25 neurons/mm$^3$. The density of neuronal profiles in the substantia nigra decreased exponentially with time ($r = -0.73 \ [P < .001]$). Extrapolation of the curve suggested a presymptomatic phase of 5 years.

Conclusion: The UPDRS3 score is linearly linked to neuronal density, which, in Lewy body diseases, decreases exponentially with time at a similar pace in this series of elderly patients and in the younger patients described in the literature.

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**METHODS**

STUDY SUBJECTS

The patients, hospitalized in a long-term care facility, were studied prospectively from January 1, 1993, to December 31, 1999. The clinical inclusion criterion was a parkinsonian syndrome of progressive onset identified by trained neurologists (M.V. and A.-M.B.). Included patients were examined at least once a year. The UPDRS3 was used to assess the motor impairment. The Mini-Mental State Examination was repeated each year. Clinical diagnosis of probable IPD and probable DLB relied on the criteria of Hughes et al and McKeith et al. re-
The same way. To the density of neuronal profiles, from high to low. Shades of gray indicate disease and dementia with Lewy bodies. The maps were ranked according to the density of neuronal profiles, from high to low. Shades of gray indicate the neuronal density in neurons per cubic millimeter. Each map is oriented in the same way.

spectively. The year of the first motor symptom was determined by asking the patient or the patient’s family.

The patients underwent autopsy after consent from their authorized legal representatives. The diagnosis of Lewy body disease (LBD) was made regardless of the clinical information as soon as Lewy bodies were revealed by α-synuclein immunohistochemistry. Only patients with LBD underwent analysis in this study. Five age-matched control cases (mean age, 84.4 years) without signs or symptoms of IPD, with normal substantia nigra, and without Lewy bodies after α-synuclein immunohistochemistry were selected retrospectively in the Laboratory of Neuropathology Raymond Escourroule, Pitie-Salpetriere Hospital, Assistance Publique Hôpitaux de Paris, Paris, France.

LABORATORY METHODS

One hemisphere, randomly chosen as the right or the left, was fixed in formaldehyde for at least 2 months. After paraffin embedding, single sections were obtained from the upper surface of the mesencephalon at the level of the superior colliculus and stained with hematoxylin-eosin. Immunohistochemistry was performed with primary antibodies directed against α-synuclein (mouse monoclonal antibody; clone LB509; CliniSciences, Montreuil, France) and tau (rabbit polyclonal antibody; batch 122; Dako, Carpenteria, Calif.).

Sections from the hippocampus, entorhinal cortex, and isocortex were immunostained by Aβ (mouse monoclonal; clone 124; Dako) and tau (rabbit polyclonal; batch 122; Dako) antibodies to stage the cases according to the criteria of Braak and Braak.10

Maps of the pars compacta of the substantia nigra were manually drawn using Mercator software (provided by Explora Nova, La Rochelle, France).11 All of the profiles bearing a nucleus with a nucleolus were identified as neuronal (so that, actually, the procedure consisted of counting nucleoli).

The polygon that was automatically drawn around each neuronal profile delineated the space that was free of any neighbor neuron (the computer program is available on the Internet at http://www.u106.eu.org/~charles/tel.htm). These polygons, each containing only 1 neuronal profile, tessellated the substantia nigra. In the density maps of Figure 1, the small polygons were painted in black to highlight areas of high density where contiguous neurons are close to each other. Large polygons, painted in clear shades, meant low density.12 An estimate of the neuronal density per unit area was determined by dividing 1 by the mean size of the polygons.11,12 To reduce the importance of the border effects on the size of the polygons, an “erosion,”13 defined as taking out all of the profiles touching the peripheral border of the substantia nigra, was performed before measuring the neuronal density. The density per unit volume was estimated by the following formula by Abercrombie14:

Density per Volume = Density per Area/ (Section Thickness + Diameter of Nucleolus).

The section thickness was measured by a transducer (Heidenhain Corp., Schaumburg, Ill) fixed on the moving stage of the microscope along the z-axis with a step of 0.5 µm. The mean value (6.23 µm; coefficient of error [CE], 3%), in a sample of 6 cases, was used as an estimate of the section thickness for the whole population. There was no “case” factor explaining variability in the measurement (analysis of variance, F = 0.7 [P = .60]). We measured 102 nucleoli in 5 IPD cases and 45 in 3 controls, using a micrometric scale (objective, × 100; optic aperture, 1.25). The mean diameter was 3.23 µm (CE, 2%) in the patients and 3.84 µm in the controls (CE, 2%). These values were statistically different (unpaired, 2-tailed, t test, −4.6 [P < .001]). The neuronal density per cubic millimeter was therefore estimated using different values for the patients (3.23 µm) and controls (3.84 µm).

STATISTICAL ANALYSIS

We used the Pearson correlation coefficient r to compare 2 quantitative values. Differences between patient groups were analyzed using analysis of variance and Fisher protected least significant difference. To illustrate the shape of the relationship between the duration of disease on one hand and UPDRS3 score or neuronal loss in the substantia nigra on the other, a moving average was taken between 5 successive values, and a curve was drawn between these values.

RESULTS

CLINICAL DATA

We examined 103 cases for study inclusion. Of these, 76 had died at the end of the study. The family had allowed the autopsy in 36 cases. Eighteen cases had Lewy bodies at the postmortem examination and were included in the study (Table). They had been followed up for a mean ± SEM of 24.7 ± 0.3 months. In all the cases, even in those identified as DBL, motor symptoms were given by the patient or the patient’s family as the first symptom, sometimes contemporarily with the cognitive deficit (3 cases). The UPDRS3 scores on the left and right sides of the body were not significantly different. The prevalence of tremor was low (5 cases of IPD). Rigidity and akinesia were the main motor symptoms in all cases. All of the patients, except 1, were cognitively impaired.

NEUROPATHOLOGICAL DATA

Lewy bodies and neurites, stained by α-synuclein, were, by definition, present in all cases. One case was at Braak neurofibrillary stage I, 1 at stage II, 4 at stage III, 5 at stage IV, 4 at stage V, and 3 at stage VI.
Maps of the substantia nigra in the 18 cases and 5 controls are shown in Figure 1. The mean±SEM density of neurons was 788±67, 1124±176, and 1974±249 neurons/mm³ in the 14 IPD cases, 4 DLB cases, and 5 controls, respectively. Analysis of variance indicated that the density was dependent on the diagnosis (F=21.4 [P<.001]). Fisher protected least significant difference showed that the controls were different from the DLB (P=.004) and IPD (P<.001) cases but did not indicate a significant difference between the IPD and DLB cases.

Neuronal density in the substantia nigra was inversely correlated with UPDRS3 score (r=−0.83 [P<.001]) (Figure 2) when using the following equation:

\[ \text{UPDRS3} = 77.9 - 0.04 \times \text{Density of Neurons (No. of Neurons/mm}^3) \]

where each point added to the UPDRS3 score was related to the loss of 25 neurons/mm³. The correlation remained significant when the controls were taken out of the computation (r=−0.65 [P=.004]).

The density of the neuronal profiles was correlated with the rigidity subscore of UPDRS item 22 (r=−0.73 [P<.001]) and the bradykinesia subscore of UPDRS item 31 (r=−0.76 [P<.001]). It was not correlated with the tremor subscore of UPDRS item 20 (r=−0.15; P=.49).

Neuronal loss (r=−0.61 [P<.003]) and UPDRS3 (r=0.67 [P<.001]) were correlated with the duration of disease.

The best fit for the neuronal loss was obtained with a negative exponential curve (r=−0.61 [P<.003])

\[ \text{Density of Neurons (in Percentage of Controls)} = 10^{(1.85 - 0.03 \times \text{Duration of the Disease in Years})} \]

This equation shows that the neuronal density, in a given case and a given year, is the neuronal density of the previous year times 0.93 (i.e., 10⁻₀.₃). The percentage of neuronal loss occurring each year is 7% of the neuronal reserve at that time. The presymptomatic phase was calculated by extrapolating the curve to the point where neuronal loss was 0. It was found to last 5 years; 29% of

### Table. Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n = 5)</th>
<th>IPD Cases (n = 14)</th>
<th>DLB Cases (n = 4)</th>
<th>Statistical Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M:F</td>
<td>3:2</td>
<td>6:8</td>
<td>0:4</td>
<td>Fisher exact test, NS</td>
</tr>
<tr>
<td>Age at death, y</td>
<td>84.4 ± 3.1</td>
<td>77.9 ± 3.4</td>
<td>94.3 ± 1.7</td>
<td>F = 3.29 (P = .06); DLB vs IPD: P = .02</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>NA</td>
<td>8.5 ± 1.6</td>
<td>3.5 ± 0.3</td>
<td>t = 1.51 (P = .15)</td>
</tr>
<tr>
<td>MMSE score†</td>
<td>NA</td>
<td>10 ± 2.5</td>
<td>16 ± 2.5</td>
<td>t = 1.23 (P = .23)</td>
</tr>
<tr>
<td>UPDRS3 score‡</td>
<td>NA</td>
<td>53 ± 3.8</td>
<td>37 ± 7.4</td>
<td>t = 1.9 (P = .06)</td>
</tr>
</tbody>
</table>

*Abbreviations: DLB, dementia with Lewy bodies; IPD, idiopathic Parkinson disease; MMSE, Mini-Mental State Examination; NA, not applicable; NS, not significant; UPDRS3, motor section of the Unified Parkinson Disease Rating Scale.  
†Maximum value (best score) of 30.  
‡Maximum value (worst score) of 108.
the neurons were lost at the first symptom and 50% were lost after 5 years of symptomatic disease (Figure 3).

Comparison of the progression of the neuronal loss and the value of the UPDRS3 (Figure 4), using moving averages, showed that both increased dramatically within the first 5 years of symptomatic disease and then tailed off. It was quite remarkable and unexpected that the motor score was close to the decrease in neuronal density (expressed as a percentage of controls); eg, a UPDRS3 score of 57 corresponded to a decrease of 59% in neuronal density after a clinical course of 10 years.

These data suggest that the motor score of the UPDRS3, now in the process of being revised, is a good predictor of the neuronal loss. It also shows that the numerical value of the score is remarkably and unexpectedly parallel to the neuronal loss.

The evaluation of neuronal density was performed on a single section with an original mapping method. The neuronal density in a section is the result of 2 counter-acting effects: cell loss and atrophy of the anatomical structure. This atrophy, compensating for cell loss, buffers the decrease in neuronal density and tends to mask the loss. The general agreement between the single section count and the disector method, mentioned by Ma et al,15 indicates that the neuronal density truthfully reflects the total number of neurons because the atrophy of the substantia nigra remains modest.10

Rinne et al17 mentioned a negative correlation between bradykinesia, or rigidity, assessed by the Columbia University Rating Scale and neuronal loss in the lateral part of the pars compacta of the substantia nigra. Tremor was positively correlated with neuronal density. The absence of any correlation with tremor in our study may be due to the large number of DLB and IPD cases with dementia in the cohort, with both clinical forms characterized by a low prevalence of tremor.18

Our figures for the duration of the presymptomatic phase are in good agreement with data published by Fearnley and Lees.2 The extrapolation of the negative exponential relationship in that report suggested that the presymptomatic phase reached 4.7 years (our value was 5 years). These findings concurred in showing that the onset of Parkinson disease antedates the onset of symptoms by years.

Our results are also remarkably consistent with findings obtained with positron emission tomography. The symptoms became apparent when the neuronal loss reached 29% in this study and when the loss of dopaminergic fibers was 31% in the study by Hilker et al.3 These data are discrepant with the traditional view that 60% to 80% of the neurons have to be lost in the substantia nigra before the first symptoms are noticed.19

Hilker et al3 found a preclinical disease period of 5.6 years and an exponential progression of the disease. The slope of the curve in semi-log coordinates is 0.028, implying that the density of dopaminergic endings in the putamen is $10^{-0.028}$ times the density found in the preceding year, $10^{-0.028} = 0.93$, a value identical to the one we found with our neuronal counts. These convergent results, obtained by neuronal count (this study) and by an assessment of receptor density at the synaptic terminal,7 suggest that the proportion of lost nerve endings equals the proportion of lost neurons, the arborization of each neuron being probably and on average of similar range in the putamen. It also suggests that progression of the disease is similar in the relatively young patients studied by Hilker et al3 and in our cohort of elderly individuals.

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