Irreversible Disability and Tissue Loss in Multiple Sclerosis

A Conventional and Magnetization Transfer Magnetic Resonance Imaging Study of the Optic Nerves

Matilde Inglese, MD; Angelo Ghezzi, MD; Stefania Bianchi, MD; Simonetta Gerevini, MD; Maria Pia Sormani, PhD; Vittorio Martinelli, MD; Giancarlo Comi, MD; Massimo Filippi, MD

Objectives: To assess, by magnetic resonance imaging, the volumes and magnetization transfer ratio (MTR) values of optic nerves (ONs) from patients with multiple sclerosis (MS) who had incomplete or no visual recovery after optic neuritis; and to compare these quantities with those derived from ONs from patients with MS who showed a marked clinical recovery after optic neuritis, ONs from healthy volunteers, and ONs from patients with Leber hereditary optic neuropathy (LHON).

Methods: Conventional and magnetization transfer magnetic resonance images of the ONs were obtained from 30 patients with MS, 18 healthy volunteers, and 10 patients with LHON. The ON from patients with MS were classified as clinically unaffected (n=18); clinically affected with recovery (n=20; visual acuity ≥20/25 at least 6 months after optic neuritis); and clinically affected with incomplete or no recovery (n=22; visual acuity <20/25 at least 6 months after optic neuritis). The ON volumes and MTR values were measured.

Results: Volumes (P=.002) and MTR values (P<.001) of the ONs from patients with MS and incomplete or no recovery were both lower than those of the ONs from patients with MS and recovery, but not different from those of the ONs from patients with LHON. Volumes and MTR values of the affected ONs from patients with MS and recovery did not differ from those of clinically unaffected ONs, which were similar to those of healthy volunteers.

Conclusion: These findings suggest that, in patients with MS, neurodegeneration is associated with persistent functional deficits secondary to incomplete recovery from relapses.

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The factors leading to irreversible neurologic disability in multiple sclerosis (MS) are still poorly understood because of the heterogeneity of the pathologic substrates of the disease and the structural and functional complexity of the brain and the spinal cord. In this context, the optic nerve (ON) is of special interest, because it might provide a model for improving our understanding of how MS causes irreversible disability. There are several reasons for this. First, the ON is a typical site of MS lesions. Second, and different from what happens for other structures of the central nervous system, the ON subserves a specific function, which can be assessed reliably by clinical and neurophysiologic methods. Finally, several recent studies have shown that magnetic resonance (MR) imaging techniques enable us not only to obtain high-quality images of the ON, but also to quantify reliably the extent of ON tissue damage and loss.

Although conventional MR imaging is very sensitive for the detection of MS lesions, it lacks specificity to the most destructive aspects of the disease (ie, severe and irreversible demyelination and axonal loss). Also, conventional MR imaging is unable to detect the subtle changes known to occur in the normal-appearing tissue. These limitations have been at least partially overcome by the use of modern MR imaging technology, including magnetization transfer imaging, and by measuring the volumes of different portions of the central nervous system. Although neither of these 2 approaches provides specific information about demyelination and axonal loss, they allow estimates to be obtained of overall tissue damage in MS, which are thought to reflect more closely destructive pathologic changes of MS within and outside T2-visible lesions than conventional MR imaging does. Consistent with this, MS-related disability increases with decreasing brain and cord volumes and magnetization transfer ratio (MTR) values, and a postmortem study of patients with MS has found a strong correlation be-
PATIENTS AND METHODS

SUBJECTS

We studied 30 patients with relapsing-remitting and secondary progressive MS. All patients had to have a single previous episode of acute optic neuritis confirmed by a complete and standardized neuro-ophthalmologic assessment (including color vision and visual field testing) and to have been clinically stable for at least 6 months. In addition, they had had neither relapses nor corticosteroid treatment during the preceding 3 months. Optic nerves from patients with MS were classified as follows: (1) clinically affected with incomplete or no recovery when best-corrected visual acuity (VA), assessed with wall charts, was less than 20/25 at least 6 months after the attack; (2) clinically affected with recovery when best-corrected VA was equal to or greater than 20/25 at least 6 months after the attack; and (3) clinically unaffected. Two control groups were identified. The first consisted of 18 sex- and age-matched healthy volunteers, with no history of neurologic diseases and with normal results of neurologic and ophthalmologic examinations. The second control group consisted of 10 age-matched patients with LHON documented by one of the 3 primary mitochondrial DNA mutations (7 patients had the 11778, 2 the 3460, and 1 the 14484 mutation). A complete ophthalmologic evaluation was performed by one physician (S.B.), who was unaware of the MR imaging results, in all subjects. In patients with MS, disability was measured with the Expanded Disability Status Scale. Additional demographic and clinical characteristics of the subjects studied are reported in Table 1. Local ethical committee approval and written informed consent from all the subjects were obtained before study initiation.

MR IMAGE ACQUISITION AND PROCESSING

The ONs of all subjects were imaged in a single session, by means of a standard head coil and a scanner operating at 1.5 T. The following sequences were obtained: (1) T2-weighted turbo spin echo (repetition time [TR], 4230 milliseconds; echo time [TE], 119 milliseconds; echo train length, 15; 15 coronal slices with 3-mm thickness; interslice gap, 0.3 mm; matrix size, 180 × 512; and field of view [FOV], 156 × 250 mm); (2) T1-weighted spin echo (TR, 500 milliseconds; TE, 14 milliseconds; 15 coronal slices with 3-mm thickness; interslice gap, 0.3 mm; matrix size, 224 × 512; and FOV, 156 × 250 mm); and (3) 2-dimensional gradient echo (TR, 640 milliseconds; TE, 12 milliseconds; flip angle, 20°; 15 contiguous coronal slices with 5-mm thickness; matrix size, 256 × 256; and FOV, 250 × 250 mm), with and without an off-resonance radiofrequency saturation pulse (offset frequency, 1.5 kHz; gaussian envelope duration, 7.68 milliseconds; flip angle, 50°). During ON imaging, all subjects were asked to close their eyes and possibly avoid eye movements. We also obtained dual-echo turbo spin-echo (TR, 3300 milliseconds; TE, 16-98 milliseconds; echo train length, 5; 24 contiguous axial slices with 5-mm thickness; matrix size, 192 × 256; and FOV, 188 × 250 mm) images of the brain from all subjects.

The volumes of the ON were measured on T1-weighted images. A single observer (M.I.), unaware of subjects’ identity, calculated the areas of the ON sections from 11 consecutive 3-mm-thick slices, starting from the first slice showing the chiasm and moving backward (ie, of the P100 wave of the pattern-reversal VEP after an episode of optic neuritis is considered to be compatible with residual demyelination within the ON. Thus, we postulated that if irreversible demyelination is not a critical factor contributing to fixed deficits in MS, the P100 latency of ONs from patients with MS should be independent of the degree of clinical recovery.

Table 1. Demographic and Clinical Characteristics of Healthy Volunteers and Patients With MS and LHON*

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Healthy Volunteers</th>
<th>LHON</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>34.3</td>
<td>33.2</td>
<td>34.4</td>
</tr>
<tr>
<td>Range</td>
<td>23-51</td>
<td>17-50</td>
<td>23-52</td>
</tr>
<tr>
<td>Sex, No. M/F</td>
<td>6/12</td>
<td>10/0</td>
<td>10/20</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>2.5</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1-26</td>
<td>1-20</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2.0</td>
<td>0.0-6.5</td>
<td></td>
</tr>
</tbody>
</table>

*MS indicates multiple sclerosis; LHON, Leber hereditary optic neuropathy; EDSS, Expanded Disability Status Scale; and ellipses, not applicable. For further details, see “Subjects” subsection of the “Patients and Methods” section.

Continued on next page
RESULTS

In the MS population, there were 18 clinically unaffected ONs, 20 affected ONs with recovery (VA was 20/25 in 5 patients and 20/20 in 15 patients), and 22 affected ONs with incomplete or no recovery (VA was light perception in 1 case, counting fingers in 2, 20/100 in 5, 20/80 in 2, 20/70 in 1, 20/40 in 2, and 20/30 in 9). In patients with LHON, VA was 20/400 bilaterally in 3 patients, counting fingers OD and 20/100 OS in 2 patients, light perception OD and hand movements OS in 1 patient, counting fingers bilaterally in 2 patients, and hand motions bilaterally in 1 patient.

No brain or ON lesions were detected on any of the MR images obtained from healthy volunteers and patients with LHON. All patients with MS had abnormal brain MR images and met the criteria of Fazekas et al.31 The MR images of 2 ONs from patients with MS (1 with no or incomplete recovery and 1 with recovery) were of suboptimal quality, and, as a consequence, they were not considered in the following analyses. Five MS ONs with incomplete or no recovery (24%) and 3 of those with recovery (16%) had a hyperintense lesion on the dual-echo images. The lengths of these lesions ranged from 3 to 9 mm. Seven of them were located in the intracanalicular portion, while 1 was located in the intraorbital portion of the ON.

**Table 2** gives the mean volumes and MTR values from the ONs of all the subjects. Volumes and MTR values of the affected MS ONs with incomplete or no recovery were both significantly lower ($P=0.002$ and $P<0.001$, respectively) than the corresponding quantities of the MS ONs with recovery, whereas both of these quantities did not differ from those of the ONs from patients with LHON. Also, volumes and MTR values of the affected MS ONs with recovery did not differ from the corresponding quantities of clinically unaffected ONs, which in turn had volumes and MTR values similar to those of the ONs from healthy volunteers. The same results were obtained when a VA cutoff of 20/50 was used for incomplete recovery (data not shown).

Visual evoked potentials were obtained from 25 patients with MS (14 clinically unaffected ONs, 18 affected ONs with recovery, and 18 affected ONs with incomplete or no recovery). The VEPs could not be obtained from 9 ONs with incomplete or no recovery. The mean P100 latencies were 99.6 milliseconds (SD, 4.5 milliseconds) for healthy control subjects, 114.1 milliseconds (SD, 19.6 milliseconds) for clinically unaffected ONs ($P=0.008$ vs control subjects), 126.7 milliseconds (SD, 23.2 milliseconds) for affected ONs with recovery ($P<0.001$ vs control subjects), and 154.5 milliseconds (SD, 20.3 milliseconds) for affected ONs with incomplete or no recovery.

VISUAL EVOKED POTENTIALS

A black-and-white checkerboard pattern on a square television screen, reversing every 0.7 milliseconds, was used to obtain pattern-reversal VEPs. The entire stimulating field was 11°; 30° check size was used. The contrast was kept constant at 70%. The cortical responses were recorded from Oz referred to Cz. The bandwidth was 1 to 250 Hz. An average of 100 responses were used to obtain the VEP. The P100 latency was considered abnormal if it was more than 2.5 SDs above the mean of the healthy volunteers.

**STATISTICAL ANALYSIS**

Volumes and MTR values among the 5 groups of ONs (MS affected, with no or incomplete recovery; MS affected, with recovery; MS unaffected; normal; and LHON) were compared by means of a random effect model (random intercept), which allows correlations existing for any of the variables tested between the 2 ONs from the same individual to be accounted for. Four contrasts were decided a priori on the basis of the available degrees of freedom and biological considerations. The following were the a priori contrasts we tested: (1) normal ON vs MS clinically unaffected ON; (2) MS clinically unaffected ON vs MS affected ON with recovery; (3) MS affected ON with recovery vs MS affected ON with incomplete or no recovery; and (4) MS affected ON with incomplete or no recovery vs LHON ON. Univariate correlations were assessed with the Spearman rank correlation coefficient, with $P$ values corrected to account for the correlations existing between the 2 ONs from the same individual. A multivariable linear regression model was used to generate a composite MR score to be correlated with the VA in patients with MS. This was computed by means of a linear combination of ON volumes (reflecting the extent of tissue loss) and MTR values (reflecting the intrinsic abnormality of the residual tissue). The weight of each MR parameter resulted from the coefficients estimated by the regression model. The magnitude and the significance of the correlation was evaluated by a nonparametic Spearman correlation analysis. Pairwise comparisons were performed with a Student $t$ test for unpaired data, again correcting for the correlation existing between the 2 ONs of the same individual.
For further details and statistical analysis, see "Results" section.

MS.34-36 Accumulation of disability in MS can be second-
correlated with the degree of disability in patients with
moderately correlated (covery and ONs from patients with LHON.
values were similar in MS ONs with incomplete or no re-
percentage of ONs with atrophy and abnormal MTR val-
ties are reported for each of the categories studied. The
atrophy, abnormal MTR values, and abnormal P100 la-
covery after acute relapses.27 Previous studies have been
mainly focused on understanding the progressive accumu-
lation of disability,32 whereas the factors related to in-
complete recovery after an acute injury are still to be elu-
cidated. This lack of knowledge is secondary to the fact
that, because of the anatomic and functional complexity
of the brain and the spinal cord and the multifocality of
the disease, it is virtually impossible to define the exact
role of regional tissue damage in determining fixed neu-
rologic deficits in MS. In this respect, the assessment of
MS-related tissue damage in the ON can be viewed as an
excellent model to investigate the effect of the various com-
ponents of MS abnormality on the clinical manifestations
of the disease. Until recently, however, MR imaging of the
ON was limited by its small size, motion artifacts, and the
effect of surrounding cerebrospinal fluid, lipid, and bony
structures.37 Although MR imaging of the ON remains tech-
cically challenging, several studies have shown that with
careful optimization, it is possible to obtain high-quality
T1- and T2-weighted images, as well as reliable quantita-
tive measurements of MR quantities, including the MTR.3-10
Against this background, the present study was per-
formed to increase our understanding of the factors asso-
ciated with irreversible disability after MS relapses. This
study has demonstrated a reduction in the volume and
MTR values of the ON from patients with MS, which, af-
after an episode of optic neuritis, showed no or only an
incomplete functional recovery. The volume and MTR
reductions found in these ONs were not different from
those found in patients with LHON, a condition in which
there is loss of neurons and axons.20 In contrast, af-

whose P100 could be elicited (P<.001 vs control subjects).
In Table 3, the numbers and percentages of ON with
atrophy, abnormal MTR values, and abnormal P100 la-
tency are reported for each of the categories studied. The
percentage of ONs with atrophy and abnormal MTR val-
ues were similar in MS ONs with incomplete or no re-
covery and ONs from patients with LHON.

In patients with MS, ON volumes and MTR were
moderately correlated (r=0.46, P=.001). The VA was
correlated with ON volume (r=0.39, P=.01), MTR values
(r=0.49, P=.001), and P100 latency (r=−0.57, P<.001).
No statistically significant correlation was found be-
tween P100 latency and MTR values (r=−0.10), whereas
P100 latency and ON volumes were moderately corre-
lated (r=−0.31, P=.05). A moderate correlation was also
found between the VA and the MR composite score
(r=0.53, P<.001).

There is increasing appreciation that neurodegeneration
is the major factor underlying the accumulation of dis-
ability in MS and that inflammatory demyelination alone
is not sufficient to explain the neurologic dysfunction in
this disease.32 Although neurodegeneration has been known
to occur in MS from the early descriptions of Charcot, the
major pieces of evidence suggesting the importance of this
aspect of the disease for disability in MS come from MR
spectroscopy studies,32 showing that the level of brain N-
acetyl-aspartate, a marker of axonal integrity,33 is strictly
correlated with the degree of disability in patients with
MS.34-36 Accumulation of disability in MS can be second-
ary to a progressive disease course or to incomplete re-
covery after acute relapses.27

### Table 3. Optic Nerves With Atrophy, Abnormal MTR Values, and Abnormal P100 Latency*

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>LHON</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Clinically</td>
<td>ON With</td>
</tr>
<tr>
<td>No. (%) of ONs</td>
<td>Unaffected ON</td>
<td>Recovery</td>
</tr>
<tr>
<td>ON with atrophy</td>
<td>6/18 (33)</td>
<td>7/19 (37)</td>
</tr>
<tr>
<td>ON with abnormal MTR values</td>
<td>3/18 (17)</td>
<td>4/19 (21)</td>
</tr>
<tr>
<td>ON with abnormal P100 latency</td>
<td>8/14 (64)</td>
<td>18/18 (100)</td>
</tr>
</tbody>
</table>

*ON indicates optic nerve; MTR, magnetization transfer ratio; MS, multiple sclerosis; and LHON, Leber hereditary optic neuropathy. For further details and statistical analysis, see “Results” section.

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fect ONs that recovered after the disease injury had volumes and MTR values higher than those of ONs with incomplete or no recovery and similar to those of clinically unaffected ONs and of ONs from healthy volunteers. We also showed a graded relationship between these 2 MR quantities and VA in patients with MS. Although neither ON volume reductions nor MTR decreases are specific markers of axonal loss, we interpret these findings as an indication that loss of axonal integrity is associated with persistent functional deficit in MS due to incomplete recovery from relapses.

Several previous studies have shown that brain and spinal cord atrophy occurs in MS and correlates with the degree of disability.12-18 Although the pathologic basis of atrophy in MS is not fully elucidated, axonal loss is likely to be an important contributing factor, since axons represent the largest proportion of the white matter volume,19 and there is evidence of considerable axonal damage in MS lesions and normal-appearing tissue.20-22 All of this is in keeping with the findings of this study showing that a similar average degree of ON volume and a similar frequency of ON atrophy are detectable in the ON with incomplete or no recovery from patients with MS and in those from patients with LHON.

The same considerations apply to the interpretation of the MTR changes seen in this study. Low MTR values correspond to areas where the relative proportion of water bound to macromolecules is reduced, indicating a loss of microstructural tissue integrity. Consistent with this, several studies have shown that brain and spinal cord MTR changes are correlated with disability in patients with MS.18-24 Axonal loss is likely to be an important contributor to MTR decreases in MS for several reasons. First, a postmortem study of patients with MS has shown that a strict correlation exists between MTR values and percentage amounts of residual axons.25 Second, MTR reduction has been found to correlate well with N-acetyl-aspartate– creatine ratio measured in MS lesions.42 Third, low MTR values have been found in animal models of wallerian degeneration43 and diffuse axonal injury.44

Given that ON volume and MTR reductions can also be related to persistent demyelination, we obtained VEPs from patients with MS. Because an increased P100 latency weeks or months after an acute episode of optic neuritis is considered to be a marker of residual demyelination,2,3 we focused our analysis on this component of the VEP. Although the ONs with incomplete or no recovery were those in which the P100 was more frequently abnormal, we also found that a large proportion of clinically unaffected ONs (69%) and affected ONs with subsequent recovery (89%) had increased P100 latencies. The mean P100 latencies of clinically unaffected ONs and affected ONs with subsequent recovery were also significantly increased compared with those from healthy subjects. These findings, which agree with those of previous studies,2,3 support the concept that demyelination per se is not sufficient to explain fixed neurologic symptoms after acute damage.

In this study, we also showed significant correlations between VA and ON volumes and MTR values. The composite MR score, based on a measure reflecting the amount of tissue loss (ON volume) and on a measure reflecting the integrity of the residual tissue (ON MTR), was also corre-


