J Neurol (2002) 249 : 902–910
DOI 10.1007/s00415-002-0758-5

ORIGINAL COMMUNICATION

P. Sarchielli
O. Presciutti
R. Tarducci
G. Gobbi
A. Alberti
G. P. Pelliccioli
P. Chiarini
V. Gallai

Localized ^{1}H magnetic resonance spectroscopy in mainly cortical gray matter of patients with multiple sclerosis

Received: 18 January 2001
Received in revised form: 16 January 2002
Accepted: 29 January 2002

Paola Sarchielli, MD (✉) · A. Alberti, MD · V. Gallai, MD
Neurologic Clinic
Neuroscience Department
Policlinico Monteluce
Via E. Dal Pozzo
06126 Perugia, Italy
Tel.: +39-075/5783568
Fax: +39-075/5783583
E-Mail: neuro@netemedia.net

O. Presciutti, PhD · R. Tarducci, PhD · G. Gobbi, PhD
Department of Medical Physics
Perugia General Hospital
Perugia, Italy

G. P. Pelliccioli, MD · P. Chiarini, MD
Department of Neuroradiology
Perugia General Hospital
Perugia, Italy

Abstract The brain water fraction (R), the brain water transverse relaxation time (T2), the atrophy index (α) and the absolute concentration of the principal brain metabolites (NAA, Cho and Cr) were measured by localized proton magnetic resonance spectroscopy in the occipito-parietal cortex (mainly gray matter) of 15 relapsing-remitting (R-R) multiple sclerosis (MS) patients, 15 secondary progressive (SP) MS patients and 8 healthy subjects.

Significantly lower values of N-acetylaspartate (NAA), creatine (Cr) and the NAA/Cr ratio in the occipito-parietal cortex were detected in SP MS patients than in R-R MS and control subjects ($p < 0.01$). Moreover, MS patients showed shorter T2 water relaxation times and reduced brain water fraction compared with controls. Higher atrophy indices were also detected in the mainly occipito-parietal gray matter of MS patients, particularly in those with the progressive form.

These findings suggest that the pathological process in MS is not limited to either white matter lesions or normal-appearing white matter but extends into the cortical gray matter (occipito-parietal), particularly in the progressive form of the disease. This can involve changes in neural metabolism or neural shrinkage and neuron loss. The significant increase in atrophy indices could be the expression of the relatively higher cerebrospinal fluid signal from the occipito-parietal cortex, even in the absence of obvious cortical atrophy.

Key words proton magnetic resonance spectroscopy · brain metabolites · absolute quantification · gray matter · multiple sclerosis

Introduction

Magnetic resonance spectroscopy (MRS) has proved to be a useful and appropriate technique for evaluating the biochemical changes occurring in the brains of patients affected by multiple sclerosis (MS). It provides direct information concerning the integrity of both myelin and axons in acute and chronic lesions and also in normal-appearing white matter (NAWM). The most relevant findings in MS concern the reduction in the N-acetylaspartate (NAA) peak which is more consistent, but partially reversible, in large acute lesions. This reduction is present and maintained in chronic lesions and has also been detected in NAWM [1, 2, 3, 13, 14, 15, 17, 20, 22, 24, 27, 30, 32, 33, 34, 35, 36, 40, 42, 43, 46, 47, 50, 53]. The decrease in the values of this neural marker has been interpreted either as an index of loss of neural viability and therefore of potential neural recovery, particularly in acute lesions, or as an index, when persisting, of neural loss and therefore accumulation of irreversible disease, especially in chronic demyelinating lesions and

JON 758

NAWM. Changes in mobile lipids, inositol (Ins) [27, 40] and an increased choline (Cho)/Creatine (Cr) ratio, correlated with a decrease in NAA, have also been demonstrated by MRS and suggested as markers of myelin breakdown or remodelling, even in the absence of clear demyelination [47].

Despite its relatively low resolution, MRS can play an important role in studies of the natural history of MS and in treatment trials, particularly when absolute quantification techniques are used in single voxel spectroscopy.

The reflection of the pathological process of MS on the gray matter of affected patients has been little investigated until now. It has been proposed that the lesional load, that is, both detectable and undetectable by conventional MRI, has an impact on gray matter, even in the absence of clear atrophy and when distant from demyelinating lesions.

A recent serial MRI study suggests that MS lesions can also involve the cerebral cortex arising mainly within the territory of the principal cortical veins, and a post-mortem study demonstrated that small cortical lesions are common in MS and are under-reported on MRI [26].

The aim of the present research was to investigate the biochemical characteristics of occipito-parietal mainly gray matter voxels in MS patients by estimating, with the aid of localized ^{1}H MRS, the absolute in vivo metabolite concentrations of NAA, Cho and Cr, thereby avoiding the use of metabolite ratios which, in some cases, can cause misinterpretation of the spectroscopic results.

Patients

MRS was performed on a single voxel in the parieto-occipital lobe of 30 patients diagnosed as having definite MS according to the Poser criteria [39]. The course of the disease was relapsing-remitting (R-R) in 15 of the patients and secondary progressive (SP) in the remaining 15. The expanded disability status scale (EDSS) of each patient was calculated according to Kurtzke [29].

None of the R-R patients was in clinical relapse nor was clinical evidence of acute optic neuritis detectable in any of the patients. They underwent pattern reversal, visual evoked potentials (VEPs). P100 latencies and amplitude were measured and compared with laboratory reference values to assess visual system abnormalities even in the absence of a clear, acute demyelinating process involving the optic nerve. A previous history of optic neuritis was reported by 7 out of 15 R-R MS patients and 9 out of 15 SP MS patients. None of the patients were treated with immunosuppressive therapy at the beginning of the study, and those who had undergone previous treatment had suspended immunosuppressive drugs at least 6 months prior to the study. None of the patients had received corticosteroid or adrenocorticoid treatment during the 2 months prior to the study. The characteristics of these patients are reported in Table 1.

Table 1 Clinical details of multiple sclerosis patients

	Relapsing-remitting patients	Secondary progressive patients
Number	15	15
Males	6	5
Females	9	10
Age (years) (mean ± SD)	33.48 ± 5.4	40.52 ± 7.61
Duration of the disease (years) (mean ± SD)	5.9 ± 3.11	8.64 ± 6.50
Expanded disability status scale (mean ± SD)	2.25 ± 0.71	4.87 ± 0.94
Range	1.5–3.5	3–5.5
Lesional load score (mean ± SD)	30.91 ± 6.43	44.51 ± 5.94
Patients with Gd-enhancing lesions	8	0
Gd-enhancing lesions (mean ± SD)	3 ± 1	0

MRS was also performed on 8 age-matched control subjects (mean age = 32.84 ± 5.15 years, 5 females and 3 males) with no systemic or neurological diseases. The study was approved by the Scientific Ethics Committee of the Perugia Municipality (Region of Umbria). Informed written consent was obtained from all the patients and control subjects.

Magnetic resonance imaging (MRI)

To quantify the lesional load and number of active lesions, an MRI examination was performed in a separate session preceding MRS using a 1.5 Tesla whole body MRI system (Signa Advantage, GE Medical System). A standard head coil was used for imaging, which was performed by a fast spin-echo sequence with an echo train of 8, repetition time (TR) = 4000 ms, echo time (TE) = 18 ms and 100 ms, a 5 mm slice thickness and a 1 mm gap between slices for complete coverage of the brain, field of view (FOV) = 24 × 24 cm and acquisition matrix = 256 × 256. Moreover, T1-weighted (TR/TE 650/15 ms), proton density (TR/TE 2000/15 ms) and T2-weighted (TR/TE 2000/70 ms) images were obtained on the axial plane. Gd-DTPA (gadolinium-diethylenetriaminpentaacetic acid) was administered intravenously at 0.2 ml/kg body weight (0.1 mmol/kg) followed by a post-injection flush of 10 ml saline. T1-weighted sequences were performed starting 5 to 10 minutes after Gd-DTPA administration.

The quantification of MRI abnormalities was obtained according to the method proposed by Ormerod et al. [37]. Assessment of lesions was performed on 15

anatomically defined brain sites (eight at a distance from the ventricles and seven periventricular). An arbitrary scoring system weighted for lesion size was used to estimate the regional T2-weighted lesional load: 1 point was given for each lesion with a diameter of ≤ 5 mm; 2 points for lesions with a 6–10 mm diameter and 3 points for those larger than 10 mm. Confluent lesions were given 1 extra point. The scores of the fifteen sites were added up to determine a cumulative lesional load score. Areas of markedly increased signal intensity, unrelated to a physiologically enhancing structure and consisting of at least three pixels, were considered as Gd-enhancing lesions.

MRS Data acquisition and Processing

Using the same magnetic resonance system, spectroscopy assessments were carried out after a short imaging session which was used to define the voxel of interest (VOI) for spectra recording. Sagittal T1-weighted sequence was performed as a reference for an axial, fast spin-echo sequence with the echo train = 8, TR = 4000 ms, TE = 18 ms and 100 ms, a 5 mm slice thickness, a 1 mm gap between slices and the FOV = 24 × 24 cm.

For each patient and control individual, the spectra acquisition was carried out in a selected area in the occipito-parietal cortex (mainly gray matter). Fig. 1 shows the typical VOI locations for spectra acquisition. Sagittal views were used for an accurate and reliable VOI positioning.

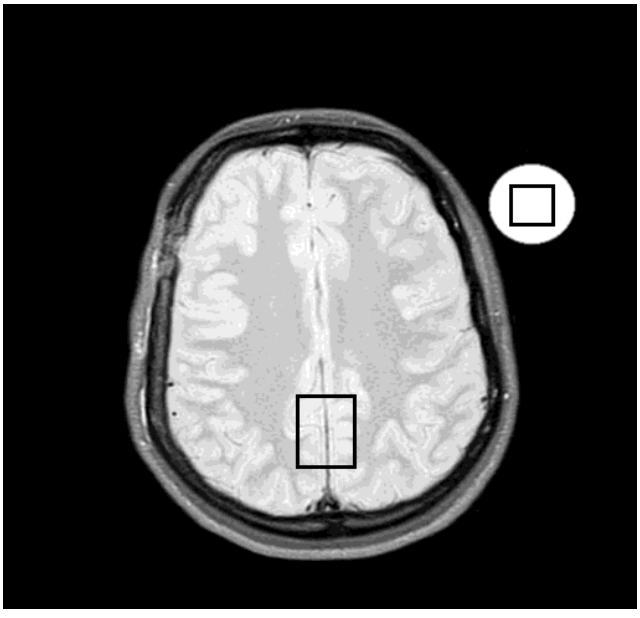

Fig. 1 Mainly gray matter VOI chosen in the occipo-parietal cortex of an MS patient and VOI in the external standard of pure water.

The typical VOI size ranged from 6 to 7 cc. The STEAM (stimulated echo acquisition mode) sequence was used for spectra acquisition, with the CHESS (chemical shift selective) pre-sequence for water peak suppression (if required) and after local shimming for magnetic field homogeneity optimization. We used the STEAM sequence instead of PRESS (point resolved spectroscopy), although STEAM has an inherently lower signal-to-noise ratio than a PRESS sequence. This choice was because the STEAM sequence, implemented in our tomograph, is very accurate in localizing the voxel of acquisition with an extremely low contamination of the signal from the external tissue, as we verified with studies carried out in the voxel image both of the phantom and in vivo from human brain.

Common parameters for all acquisitions were: bandwidth = 2500 Hz, number of points acquired = 4096, middle time (TM) = 13.7 ms, and number of averages for the unsuppressed water peak = 1.

Absolute quantification of the signal intensities of NAA, Cho and Cr was obtained using a method based on water compartmentation [18, 28] where a vial of pure water, placed at the side of the patient's head, was the external standard [46]. This method was set up thanks also to the experience acquired during the participation in the European multicenter trial of protocols for absolute metabolite quantification by *in vivo* NMR spectroscopy [16, 25].

Water content (S) from the selected VOI in the occipito-parietal mainly gray matter was obtained with 10 acquisitions with TR = 10 s and TE = 10, 20, 68, 92, 136, 272, 500, 1000 and 2000 ms. The water peak area was calculated for each free induction decay (FID) with fast Fourier transform (FFT) and Lorentzian fit (Levenberg-Marquardt method) [21]. The data were elaborated with a bi-exponential fit:

$$S(TE) = S_{bw}(0)\ \exp(-TE/T2_{bw}) + S_{csf}(0)\ \exp(-TE/T2_{csf})$$

in order to separate the contribution of CSF (S_{csf}, $T2_{csf} \cong 1$s) from that of cerebral water (S_{bw}, $T2_{bw} \cong 80$ ms) and to obtain the value of the cerebral water signal intensity S_{bw} for TE = 0.

For the external standard water, 5 acquisitions were carried out with TR = 10 s and TE = 10, 20, 40, 68 and 136 ms. The water peak area was calculated for each FID with FFT and Lorentzian fit. The data were processed with a mono-exponential fit to obtain the value of signal intensity of pure water (S_h) for TE = 0:

$$S_h(TE) = S_h(0)\ \exp(-TE/T2_h).$$

For cerebral metabolites, four acquisitions (number of averages = 128; phase cycle = 8) were carried out with TR = 4 s and TE = 68, 136, 204 and 272 ms. The acquisition of the unsuppressed water peak (number of averages = 8, phase cycle = 8) was also performed for eddy current corrections. The metabolite peak areas were cal-

culated for each FID using Lorentzian-Gaussian apodization, FFT and Gaussian fit. No correction was made for the baseline. The data for each peak were processed with a mono-exponential fit to obtain the metabolite signal intensity (S_{met}) for TE = 0:

$$S_{met}(TE) = S_{met}(0)\ \exp(-TE/T2_{met}).$$

The resonance effect of macromolecules with a short T2 was negligible due to the long echo times used.

For clarity and completeness, details of the procedure used to quantify brain metabolites are reported. The VOI chosen from volume V is subdivided into two compartments, *a)* the properly named cerebral tissue with which the signal S_b is associated (directly measurable) and *b)* the cerebrospinal fluid with which the signal S_{csf} (measurable) is associated in cerebral tissue. Two subcompartments were then separated: brain water with signal S_{bw}, which is measurable, and structural material, which is invisible on MR, whose signal S_{dry} is calculated by introducing the signal S_h. This is the signal, derived by using an external reference, which would be obtained by substituting the brain VOI with a VOI of pure water. The following formula is derived:

$$S_{dry} = S_h - S_{bw} - S_{csf} \qquad S_b = S_{bw} + S_{dry} = S_h - S_{csf}$$

The sizes of each compartment can therefore be summarized as follows:

Compartment signal	Relative signal	Volume	Mass
Cerebral tissue S_b	$s_b = S_b/S_h$	$V_b = s_b{*}V$	$m_b = V_b{*}\rho_b$
Cerebral water S_{bw}	$s_{bw} = S_{bw}/S_h$	$V_{bw} = s_{bw}{*}V$	$m_{bw} = V_{bw}{*}\rho_w$
Structural material S_{dry}	$s_{dry} = S_{dry}/S_h$	$V_{dry} = s_{dry}{*}V$	$m_{dry} = m_b - m_{bw}$
Cerebrospinal fluid S_{csf}	$s_{csf} = S_{csf}/S_h$	$V_{csf} = s_{csf}{*}V$	$m_{csf} = V_{csf}{*}\rho_w$

where ρ_I represents the density of tissue *i*.

The main hypothesis on which the quantification is based is that for each metabolite *met*, the signal associated with S_{met} is proportional, through the constant K which accounts for the experimental conditions, to the number of moles of protons n_p responsible for resonance $S_{met} = K{*}n_p$. This can also be written as: $S_{met} = K{*}n_{met}/\nu_{met}$, where n_{met} is the number of metabolite moles and ν_{met} the number of protons per molecule contributing to the resonance.

From the previous hypothesis, the following formula can be derived:

1. $n_{met}/n_{bw} = S_{met}/S_{bw} * \nu_{bw}/\nu_{met} = S_{met}/S_{bw} * 2/\nu_{met}$

The mass of cerebral tissue m_b ($m_b = m_{bw} + m_{dry}$) can be written as:

$$m_b = \rho_b{*}s_b{*}V = (r_b{*}s_b){*}(m_{bw}/(\rho_{bw}{*}s_{bw})) = (\rho_b/\rho_{bw}){*}(S_b/S_{bw}){*}(n_{bw}{*}M/10^3)\ \ (Kg)$$

where M is the molecular weight of water.

The moles of metabolite per mass unit of brain tissue, with the exclusion of cerebrospinal fluid, in the volume of acquisition can be obtained with the previous formula:

$$n_{met}/m_b = n_{met}/n_{bw} * \rho_{bw}/\rho_b * S_{bw}/S_b * 10^3/M$$

Using (1) we found:

2. $n_{met}/m_b = S_{met}/S_{bw} * \rho_w/\rho_b * R * 10^3/M * 2/\nu_{met}$ (mmoles/kg wet weight),

where:

n_{met} = number of metabolite moles, m_b = brain mass, $R = S_{bw}/S_b = S_{bw}/(S_h - S_{csf})$, ρ_w and ρ_b = water and brain density ($\rho_b = 1.047$ g/cm^3), M = molecular weight of water and ν_{met} = number of protons per molecule which contribute to the resonance signal ($\nu_{met} = 3$ for NAA and Cr, $\nu_{met} = 9$ for Cho).

When calculating the brain water fraction R, the different localization of the VOIs in the acquisition of cerebral water and the external pure water standard was taken into account, using previous measurements on phantoms.

We also calculated the atrophy index α defined as: $\alpha = [1 - S_{bw}/(S_{bw}+S_{csf})]{*}100$.

We decided not to quantify the Ins resonance, because Ins features a strongly coupled, complex spectrum which could lead to questionable results. Neither lactate nor lipids were detectable in the spectra of either MS patients or control individuals.

Further considerations should be given concerning the method of quantification used.

It automatically accounts for the presence of CSF and therefore enables the metabolite concentration to be measured independently of the quantity of CSF present in the VOI.

The gray matter VOIs examined in our study were 'contaminated' by the presence of a small quantity of white matter. Careful examination of these VOIs showed a white matter percentage never exceeding 18% of the total brain tissue in both patients and control subjects. We did not make any correction for this contamination and therefore it is clear that the metabolite concentrations in our study do not strictly refer to "pure" gray matter.

In any case, we tried to establish how much variation in the metabolite concentrations of MS patients compared with control subjects could be attributed to an abnormality of NAWM in MS patients. In this regard we referred to the results of one of our previous studies [46] demonstrating an NAA content in NAWM that was about 10% and 20% lower in R-R patients and SP patients respectively, than in control subjects, without significant differences in the other brain metabolites. NAWM was studied in that research in the parietal area and therefore not strictly in the same location of the present study, but we assumed that these data were accept-

able to obtain a maximum error estimate. Translating the findings concerning NAWM to the volume fraction of NAWM present in the VOI examined in the current study (18%), we estimated, using a simple proportion, that the NAA concentration in the mainly gray matter VOI from the occipito-parietal cortex can be at most underestimated in 2% of R-R MS patients and in 4% of SP MS patients compared with control subjects, and therefore any eventual residual variation could only be due to an abnormality of the gray matter.

Statistics

The data were expressed as a mean ± standard deviation (SD). ANOVA was used to compare the mean values of the cerebral metabolites of the control group with those of both R-R and SP patient groups. Fisher's least significant difference (LSD) was also calculated to compare the main effect mean in the ANOVA.

Five percent was chosen as the level of statistical significance.

The Spearman rank correlation coefficient was also calculated between the cortical gray matter metabolite concentrations and the duration of the disease and the lesional load in both patient groups.

Results

The mean R value (corrected for the ratio between the density of pure water and brain tissue) for all control subjects was $R = 0.73 \pm 0.02$ (mean ± SD) which is similar to that calculated by Ernst et al. [18] (0.745 ± 0.015). In contrast, biochemical methods have revealed greater values of R (0.82) in normal gray matter [18, 31] compared with Ernst et al. and our findings. This discrepancy could be attributed to a possible uncorrected measurement of the blood water content in gray matter and eddy current effects in the signal acquisition [18].

We found a significant difference between the cortical gray matter water fraction R of controls ($R = 0.75 \pm 0.02$, mean ± SD) and that of MS patients with the R-R form ($R = 0.70 \pm 0.019$, $p < 0.01$) and SP form ($R = 0.67 \pm 0.027$, $p < 0.01$).

Metabolite concentrations and brain water T2 values obtained in the occipito-parietal gray matter of control subjects were comparable with values from the literature [11, 28, 41, 52], with some slight differences which may depend on the differing methodological approaches.

In Table 2 the mean values of NAA, Cr and Cho measured with absolute quantification by ^{1}H-MRS in the occipito-parietal gray matter of control subjects and of both R-R and SP patient groups are shown. Significantly lower values of NAA were detected in SP MS patients with respect to R-R MS and also control subjects, whereas a slight, but not statistically significant reduction in NAA peak values was observed in the occipito-parietal gray matter of R-R MS patients compared to control subjects. The data were not corrected for the effects of NAWM in the patient group; effects that gave, in any case, an underestimation of the NAA concentration of 2% (R-R) and 4% (SP) compared with control individuals, and did not modify the statistical significance of our findings.

Table 2 Metabolite concentrations (mM/kg wet weight) in mainly gray matter in the occipito-parietal cortex of MS patients and controls

	NAA (Mean ± SD)	Cho (Mean ± SD)	Cr (Mean ± SD)
R-R MS Patients	10.84 ± 0.68	1.57 ± 0.24	8.77 ± 0.81
SP MS Patients	8.66 ± 0.89*	1.41 ± 0.18	7.64 ± 0.61*
Control Subjects	11.36 ± 0.70	1.51 ± 0.15	8.98 ± 0.75

* $p < 0.01$ versus control subjects and versus R-R MS patients

No significant difference was observed between the NAA levels of patients with P100 increased latencies ($n = 18$) and those without ($n = 12$), nor in patients with a previous history of optic neuritis ($n = 16$) and those without ($n = 14$).

Significantly lower values of Cr were also detected in SP MS patients compared with control subjects and R-R MS patients.

No significant differences were found in the mainly gray matter Cho peak values between MS patients and control subjects, nor between R-R MS patients and SP MS patients.

As further proof of the MRS findings, we analysed the statistical difference between the NAA/Cr and NAA/Cho and Cr/Cho ratios. The NAA/Cho and Cr/Choratios in SP MS patients were significantly lower than in control subjects ($p < 0.01$ and $p < 0.03$, respectively). The NAA/Cratio also appeared to be lower in the group of SP MS patients, even if at a less statistically significant level ($p = 0.049$). This is due to the low concentration of both NAA and Cr in the SP MS patients.

Figures 2A, 2B and 2C show the MR spectra obtained from an occipito-parietal gray matter VOI of a control subject, an R-R MS patient and an MS patient with the SP form.

No significant correlation was found between the duration of the disease, the lesional load score and metabolite peak values in either patient group.

The brain water T2 values appeared to be significantly shorter in MS patients both with the R-R form ($T2 = 81.1 \pm 12$ ms, mean ± SD) and SP form ($T2 = 79.0 \pm 11$ ms) than in control individuals (86.0 ± 5 ms) ($p < 0.01$ and $p < 0.02$ respectively). In contrast no significant difference was found for the metabolite T2 estimation between controls ($T2_{NAA} = 385 \pm 30$ ms,

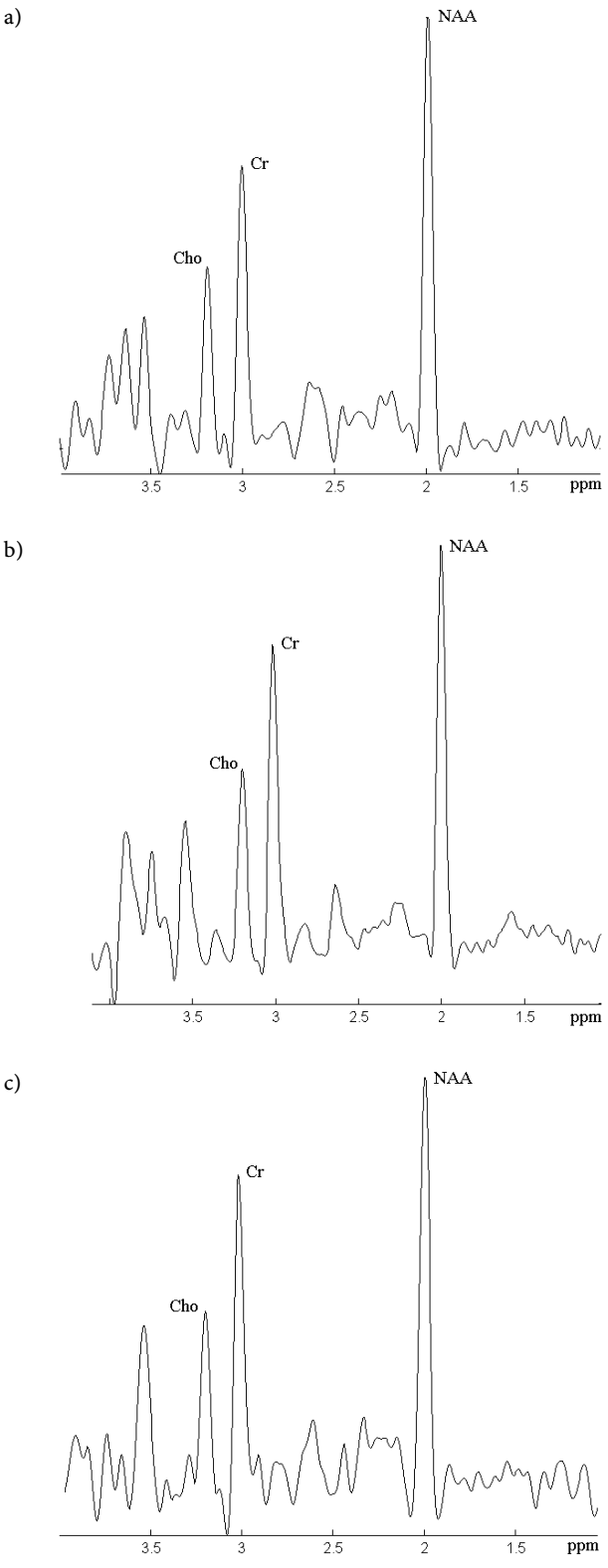

Fig. 2 MR spectra obtained from a mainly gray matter VOI in the occipito-parietal cortex of a control subject (**a**), an R-R MS patient (**b**) and an MS patient with the SP form (**c**).

$T2_{Cr} = 202 \pm 20$ ms, $T2_{Cho} = 356 \pm 45$ ms), SP MS patients ($T2_{NAA} = 361 \pm 40$ ms, $T2_{Cr} = 197 \pm 21$ ms, $T2_{Cho} = 327 \pm 54$ ms), and R-R MS patients ($T2_{NAA} = 368 \pm 34$ ms, $T2_{Cr} = 211 \pm 23$ ms, $T2_{Cho} = 332 \pm 64$ ms).

The atrophy index α appeared to be significantly higher in the occipito-parietal cortical mainly gray matter of patients with the SP form (22.2 ± 8.5) compared with control individuals (13.4 ± 5.1) ($p < 0.02$). Greater values of this index were also observed in MS patients with the R-R form (17.5 ± 7.4) but in this case the difference was not statistically significant.

Discussion

Although gray matter is not primarily affected by MS, some evidence suggests that it can be influenced and be part of its pathological process.

The extent of this involvement has not been clearly defined. Using the conventional, T2-weighted, spin-echo sequence, the presence of demyelinating plaques was demonstrated in the gray matter of some MS patients [48]. The localization of the demyelinating process of MS in the deep gray matter, in particular the thalamus, also emerged in an early-onset case of multiple sclerosis [4]. Furthermore, there is a case report of an R-R MS patient with an initial presentation of myelopathy who had demyelinating lesions located in spinal cord gray matter [54].

A recent MR imaging quantification study showed that lesions in the gray matter of MS patients represent less than 6 % of the total lesional load in patients with the R-R form without a significant correlation with the neurocognitive function and disability status [8, 9, 12]. The total cortical and subcortical disease burden was, on the contrary, associated with cognitive impairment in a research of Rovaris et al. [44]. The authors also demonstrated that subtle changes, undetectable by conventional MRI but revealed by lower magnetization transfer ratio (MTR) histographic values, are relevant in determining MS cognitive impairment in MS. The distribution of gray matter lesions in MS was furthermore investigated by Kidd et al. [26] who also demonstrated in a post-mortem study small cortical lesions, often under-reported on MRI, the majority of which arise in the territory of the principal cortical veins and the remaining quarter in the territory of the small branch and superficial veins.

Low signal intensity on long-repetition-time MR sequences has been observed in deep gray matter structures in some patients affected by multiple sclerosis. According to Russo et al. [45], this T2 shortening most likely represents a non specific degenerative process. The same authors found T2 shortening in the pericentral cortical gray matter as well as in the subcortical white matter in a patient with severe MS and postulated that this finding is a further manifestation of neural de-

generation. MRI T2 shortening was also detected by Bakshi et al. [6] in the thalamus, putamen, caudate and to a lesser extent in the Rolandic cortex in MS patients, particularly those with a secondary progressive course. This MRI shortening was related to an abnormal brain deposition of iron.

All the above observations prompted us to investigate the mainly cortical gray matter in MS using MRS, with the aim of testing for evidence of neural injury or loss in the cortex in this predominantly subcortical, white matter disease.

We chose the occipito-parietal cortical region because this region shows a variety of tissue types without consistent overlapping areas between gray and white matter, with a greater prevalence of the former.

Although the MRS voxel used is relatively large, the contribution of NAWM to changes in the gray matter metabolite values of the patient group with respect to the control group is small and can be estimated to range from 2 to 4%, and therefore did not modify the statistical significance of our findings.

The MRS method used allows us to exclude the contribution of CSF signals, particularly in SP MS patients in which it may be more relevant due to the greater reduction in brain size rather than in R-R MS patients.

The MRS method used enables an absolute quantification of brain metabolites of interest to be obtained, thus avoiding all the problems inherent in the relative quantification of the ratios between metabolite peak areas. However, this is characterized by a greater complexity of protocols for spectra acquisition and elaboration, as well as by a longer duration of the MRS study compared with that needed for relative quantification. In the method section we described in detail the theoretical background of our method and calibration, as well as the checking procedures which we followed, with the aim to eliminate, or in any case to minimize, the potential errors expected in the final quantification. They include the errors due to the stability of the tomograph in the course of the examination, the accuracy of the theoretical and actual size of the VOI, the accuracy in the calibration with an external reference etc.

Moreover, there are other simplifying assumptions required in our absolute quantification of cerebral metabolites, such as the two components attributed to T2 decay for the brain water signal and the limited number of measurements used to obtain the exponential fit. The latter are essentially due to the need to perform the examination in a period of time tolerated by the patients.

We believe, however, that the method of absolute quantification used in the present study is sufficiently accurate, as shown from the comparison between values obtained in the control group and those reported in the literature. Finally, and above all, this method can be easily applied in clinical practice as a "routine" examination to monitor and follow-up neurological diseases.

In the present study a significant decrease in the concentration of N-acetyl moieties emerged in occipito-parietal mainly gray matter areas of MS patients with the progressive form. A slight, but not significant, decrease in the peak of this metabolite was also evident in the gray matter of R-R MS patients compared with control subjects.

The decrease in the NAA content is a consistent finding of both acute and chronic demyelinating lesions in MS patients and also of NAWM. This reduction has been interpreted as a consequence of axonal damage which involves not only lesions but also white matter areas which do not appear to be affected by the demyelinating process observed on conventional MRI [20, 24, 50, 46].

We demonstrated that the decrease in this brain metabolite also extends into occipito-parietal mainly gray matter areas which seem to be physically unrelated to the MS lesional process on conventional MRI, particularly in patients with an SP form. This involvement does not seem to be associated with previous or actual occurrence of a demyelinating process directed against the optic nerves, as revealed by the absence of a significant difference between the NAA values detected in the occipito-parietal gray matter of patients with or without previous or present optic neuritis, abnormal P100 latencies demonstrated with VEP, or involvement of an MS process in other structures of the visual pathways.

The finding of a decrease in NAA concurs with the results of a previous study by Bruhn et al. [10], in which a reduction in NAA was shown in cortical gray matter, particularly in relation to neighboring multiple sclerosis lesions. Further evidence that gray matter is affected by axonal dysfunction or loss emerges in a study of ^{1}H spectroscopic MR imaging, carried out by Pan et al. [38], who demonstrated an average increase in the Cr/NAA ratio Cho/NAA ($p < 0.01$) and a slightly elevated Cho/NAA ratio, which was, however, statistically insignificant.

It cannot be argued from our study whether the NAA decrease in MS cortical mainly gray matter is only an expression of changes in metabolism or whether it expresses a secondary neural shrinkage and loss as a consequence of Wallerian degeneration which is not confined to projection pathways. These are disconnected from their origin as a result of axonal transection [19, 51], and may also strike nerve cell bodies located in gray matter.

The NAA decrease could also be an indirect expression of brain volume loss in MS. Unfortunately, in our study no direct measures of brain atrophy, especially concerning the brain parenchyma fraction, are available, which can furnish a further marker of MS disease progression, probably reflecting a destructive and irreversible pathological process in MS, even in the early stages of the disease.

Moreover, we cannot exclude a little contribution to

the reduced NAA values in the cortical mainly gray matter due to small cortical lesions that are not detectable in any of our patients on conventional MRI.

The reduction in Cr could be a further expression of brain volume loss in MS but this cannot be argued by our study, owing to the lack of a direct measure of atrophy.

On the other hand, brain Cr levels have been associated with the amount of cytosolic and mitochondrial creatine kinase, and therefore ATP metabolism [23]. Its reduction has been proposed as an index of metabolic dysfunctioning in MS.

The reduction in absolute Cr values found by our group in the occipito-parietal mainly gray matter of SP MS patients, could therefore be interpreted as an indirect expression of reduced brain metabolism, as shown in previous studies [5, 49, 7] using different methods, even though this hypothesis is only speculative and needs to be clearly documented with MRS.

Further proof of subtle changes in the occipito-parietal mainly gray matter of MS patients derives from our finding of shorter T2 water relaxation times compared with controls, which could be considered an additional index of degeneration or persistent axonal dysfunctioning. This T2 shortening is particularly evident in MS patients with an SP form. This can be interpreted as an index of more tightly bound water and may also explain the reduced R values revealed by our study in both MS forms. On the other hand, the reduction in the latter values does not exclude a real decrease in water content in apparently unaffected gray matter of MS patients.

The T2 shortening revealed in the cortical mainly gray matter of MS patients, especially in those with the SP form, is coupled with the detection of high atrophy indices, measured according to Kreis et al. [28]. This significant increase could reflect the relatively high CSF signal from the occipito-parietal cortex which significantly exceeded the values of age-matched control subjects, even though clear evidence of cortical atrophy is not available and this index is not potentially interpretable in terms of tissue volume change.

References

1. Arnold DL, Matthews PM, Francis G, Antel J (1990) Proton magnetic resonance spectroscopy of human brain in vivo in the evaluation of multiple sclerosis: assessment of the load of disease. Magn Reson Med 14 (1): 154–159
2. Arnold DL, Matthews PM, Francis GS, O' Connor J, Antel JP (1992) Proton magnetic resonance spectroscopic imaging for metabolic characterization of demyelinating plaques. Ann Neurol 31 (3): 235–241
3. Arnold DL, Riess GT, Matthews PM, Francis GS, Collins DL, Wolfson C, Antel JP (1994) Use of proton magnetic resonance spectroscopy for monitoring disease progression in multiple sclerosis. Ann Neurol 36 (1): 76–82
4. Asai K, Inagaki M, Maegaki Y, Yamamoto T, Suzaki I, Ohta S (1994) An early-onset case of multiple sclerosis with thalamic lesions on MRI. Acta Paediatr Jpn 36 (4): 431–434
5. Bakshi R, Miletich RS, Kinkel PR, Emmet ML, Kinkel WR (1998) High-resolution fluorodeoxyglucose positron emission tomography shows both global and regional cerebral hypometabolism in multiple sclerosis. J Neuroimaging 8 (4): 228–234
6. Bakshi R, Shaikh ZA, Janardhan V (2000) MRI T2 shortening ('black T2') in multiple sclerosis: frequency, location, and clinical correlation. Neuroreport 11 (1): 15–21
7. Blinkenberg M, Jensen CV, Holm S, Paulson OB, Sorensen PS (1999) A longitudinal study of cerebral glucose metabolism, MRI, and disability in patients with MS. Neurology 53 (1): 149–153
8. Blinkenberg M, Rune K, Jensen CV, Ravnborg M, Kyllingsbaek S, Holm S, Paulson OB, Sorensen PS (2000) Cortical cerebral metabolism correlates with MRI lesion load and cognitive dysfunction in MS. Neurology 54 (3): 558–564
9. Blinkenberg M, Rune K, Jønsson A, Holm S, Jensen CV, Paulson OB, Sørensen PS (1996) Cerebral metabolism in a case of multiple sclerosis with acute mental disorder. Acta Neurol Scand 94 (5): 310–313
10. Bruhn H, Frahm J, Merboldt KD, Hanicke W, Hanefeld F, Christen HJ, Kruse B, Bauer HJ (1992) Multiple sclerosis in children: cerebral metabolic alterations monitored by localized proton magnetic resonance spectroscopy in vivo. Ann Neurol 32 (2): 140–150
11. Cady EB (1996) Quantification of metabolites in human brain by proton magnetic resonance spectroscopy: methodologies, limitations, and achievements. In: Podo F, Bovée WMMJ, de Certaines JD, Henriksen O, Leach MO, Leibfritz D (eds) Eurospin Annual 1995–1996. Rome: Istituto Superiore di Sanità pp. 17–40
12. Catalaa I, Fulton JC, Zhang X, Udupa JK, Kolson D, Grossman M, Wei L, McGowan JC, Polansky M, Grossman RI (1999) MR imaging quantitation of gray matter involvement in multiple sclerosis and its correlation with disability measures and neurocognitive testing. Am J Neuroradiol 20 (9): 1613–1618
13. Davie CA, Barker GJ, Webb S, Tofts PS, Thompson AJ, Harding AE, McDonald WI, Miller DH (1995) Persistent functional deficit in multiple sclerosis and autosomal dominant cerebellar ataxia is associated with axon loss. [Published erratum appears in Brain 1996, 119 (P + 4): 1415]. Brain 118 (P + 6): 1583–1592
14. Davie CA, Hawkins CP, Barker GJ, Brennan A, Tofts PS, Miller DH, McDonald WI (1994) Serial proton magnetic resonance spectroscopy in acute multiple sclerosis lesions. Brain 117 (P + 1): 49–58
15. Davies SE, Newcombe J, Williams SR, McDonald WI, Clark JB (1995) High resolution proton NMR spectroscopy of multiple sclerosis lesions. J Neurochem 64 (2): 742–748
16. De Beer R, Barbiroli B, Gobbi G, Knijn A, Kugel H, Langenberger KW, Tkac I, Topp S (1998) Absolute metabolite quantification by in vivo NMR spectroscopy: III. Multicentre ^{1}H MRS of the human brain addressed by one and the same data-analysis protocol. Magn Reson Imaging 16(9): 1107–1111
17. De Stefano N, Francis G, Antel JP, Arnold DL (1993) Reversible decreases of N-acetylaspartate in the brain of patients with relapsing-remitting multiple sclerosis. Proc Soc Magn Reson Med 12: 280
18. Ernst T, Kreis R, Ross BD (1993) Absolute quantitation of water and metabolites in the human brain. I. Compartments and water. J Magn Reson B 102: 1–8

19. Ferguson B, Matyszak MK, Esiri MM, Perry VH (1997) Axonal damage in acute multiple sclerosis lesions. Brain 120 (P + 3): 393–399
20. Fu L, Matthews PM, De Stefano N, Worsley KJ, Narayanan S, Francis GS, Antel JP, Wolfson C, Arnold DL (1998) Imaging axonal damage of normal-appearing white matter in multiple sclerosis. Brain 121 (P + 1): 103–113
21. Gans P (1992) Data fitting in the chemical sciences: By the Methods of Least Squares. England: John Wiley & Sons Ltd pp. 59–87
22. Grossman RI, Lenkinski RE, Ramer KN, Gonzalez-Scarano F, Cohen JA (1992) MR proton spectroscopy in multiple sclerosis. Am J Neuroradiol 13 (6): 1535–1543
23. Holtzman D (1996) Brain creatine kinases and phosphocreatine: an update. Dev Neurosci 18 (5–6): 522–523
24. Husted CA, Goodin DS, Hugg JW, Maudsley AA, Tsuruda JS, de Bie SH, Fein G, Matson GB, Weiner MW (1994) Biochemical alterations in multiple sclerosis lesions and normal-appearing white matter detected by in vivo ^{31}P and ^{1}H spectroscopic imaging. Ann Neurol 36 (2): 157–165
25. Keevil SF, Barbiroli B, Brooks JC, Cady EB, Canese R, Carlier P, Collins DJ, Gilligan P, Gobbi G, Hennig J, Kugel H, Leach MO, Metzler D, Mlynarik V, Moser E, Newbold MC, Payne GS, Ring P, Roberts JN, Rowland IJ, Thiel T, Tkac I, Topp S, Wittsack HJ, Podo F, et al. (1998) Absolute metabolite quantification by in vivo NMR spectroscopy: II. A multicentre trial of protocols for in vivo localised proton studies of human brain. Magn Reson Imaging 16(9): 1093–1106
26. Kidd D, Barkhof F, McConnell R, Algra PR, Allen IV, Revesz T (1999) Cortical lesions in multiple sclerosis. Brain 122 (Pt 1): 17–26
27. Koopmans RA, Li DK, Zhu G, Allen PS, Penn A, Paty DW (1993) Magnetic resonance spectroscopy of multiple sclerosis: in-vivo detection of myelin breakdown products [letter]. Lancet 341 (8845): 631–632
28. Kreis R, Ernst T, Ross BD (1993) Absolute quantitation of water and metabolites in the human brain. II. Metabolite concentrations. J Magn Reson B 102: 9–19
29. Kurtzke JF (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33 (II): 1444–1452
30. Larsson HB, Christiansen P, Jensen M, Frederiksen J, Heltberg A, Olesen J, Henriksen O (1991) Localized in vivo proton spectroscopy in the brain of patients with multiple sclerosis. Magn Reson Med 22 (I): 23–31
31. Lentner C (1981) (ed) Geigy scientific tables, 8th ed. Ciba-Geigy, Basel pp. 222
32. Matthews PM, Francis G, Antel J, Arnold DL (1991) Proton magnetic resonance spectroscopy for metabolic characterization of plaques in multiple sclerosis. [Published erratum appears in Neurology 1991, 41 (II): 1828] Neurology 41 (8): 1251–1256
33. Matthews PM, Pioro E, Narayanan S, De Stefano N, Fu L, Francis G, Antel J, Wolfson C, Arnold DL (1996) Assessment of lesion pathology in multiple sclerosis using quantitative MRI morphometry and magnetic resonance spectroscopy. Brain 119 (P + 3): 715–722
34. Miller DH (1995) Magnetic resonance imaging and spectroscopy in multiple sclerosis. [Review] Curr Opin Neurol 8 (3): 210–215
35. Narayana PA, Doyle TJ, Lai D, Wolinsky JS (1998) Serial proton magnetic resonance spectroscopic imaging, contrast-enhanced magnetic resonance imaging, and quantitative lesion volumetry in multiple sclerosis. Ann Neurol 43 (I): 56–71
36. Narayanan S, Fu L, Pioro E, De Stefano N, Collins DL, Francis GS, Antel JP, Matthews PM, Arnold DL (1997) Imaging of axonal damage in multiple sclerosis: spatial distribution of magnetic resonance imaging lesions. Ann Neurol 41 (3): 385–391
37. Ormerod IE, Miller DH, Mc Donald WI, du Boulay EP, Rudge P, Kendall BE, Moseley IF, Johnson G, Tofts PS, Halliday AM, et al. (1987) The role of NMR imaging in the assessment of multiple sclerosis and isolated neurological lesions. A quantitative study. Brain 110 (P + 6): 1579–1616
38. Pan JW, Hetherington HP, Vaughan JT, Mitchell G, Pohost GM, Whitaker JN (1996) Evaluation of multiple sclerosis by ^{1}H spectroscopic imaging at 4.1 T. Magn Reson Med 36 (I): 72–77
39. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, Johnson KP, Sibley WA, Silberberg DH, Toortellotte WW (1983) New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 13 (3): 227–231
40. Posse S, Schuknecht B, Smith ME, van Zijl PC, Herschkowitz N, Moonen CT (1993) Short echo time proton MR spectroscopic imaging. J Comput Assist Tomogr 17 (I): 1–14
41. Pouwels PJ, Frahm J (1998) Regional metabolite concentrations in human brain as determined by quantitative localized proton MRS. Magn Reson Med 39 (I): 53–60
42. Roser W, Hagberg G, Mader I, Brunnschweiler H, Radue EW, Seelig J, Kappos L (1995) Proton MRS of gadolinium-enhancing MS plaques and metabolic changes in normal-appearing white matter. Magn Reson Med 33 (6): 811–817
43. Roser W, Mader I, Hagberg G, Kappos L, Radue EW, Seelig J (1993) Proton MRS of gadolinium-enhancing MS plaques. (abstract). Proc Soc Magn Reson Med 1: 278
44. Rovaris M, Filippi M, Minicucci L, Iannucci G, Santuccio G, Possa F, Comi G (2000) Cortical/subcortical disease burden and cognitive impairment in patients with multiple sclerosis. [see comments] Am J Neuroradiol 21 (2): 402–408
45. Russo C, Smoker WR, Kubal W (1997) Cortical and subcortical T2 shortening in multiple sclerosis. Am J Neuroradiol 18 (1): 124–126
46. Sarchielli P, Presciutti O, Pelliccioli GP, Tarducci R, Gobbi G, Chiarini P, Alberti A, Vicinanza F, Gallai V (1999) Absolute quantification of brain metabolites by proton magnetic resonance spectroscopy in normal-appearing white matter of multiple sclerosis patients. Brain 122 (P + 3): 513–521
47. Schiepers C, Van Hecke P, Vandenberghe R, Van Oostende S, Dupont P, Demaerel P, Bormans G, Carton H (1997) Positron emission tomography, magnetic resonance imaging and proton NMR spectroscopy of white matter in multiple sclerosis. Mult Scler 3 (I): 8–17
48. Shah M, Ross JS, VanDyke C, Rudick RA, Goodkin DE, Obuchowski N, Modic MT (1992) Volume T1-weighted gradient echo MRI in multiple sclerosis patients. J Comput Assist Tomogr 16 (5): 731–736
49. Sun X, Tanaka M, Kondo S, Okamoto K, Hirai S (1998) Clinical significance of reduced cerebral metabolism in multiple sclerosis: a combined PET and MRI study. Ann Nucl Med 12 (2): 89–94
50. Tourbah A, Stievenart JL, Iba-Zizen MT, Zannoli G, Lyon-Caen O, Cabanis EA (1996) In vivo localized NMR proton spectroscopy of normal appearing white matter in patients with multiple sclerosis. J Neuroradiol 23 (2): 49–55
51. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L (1998) Axonal transection in the lesions of multiple sclerosis [see comments]. N Engl J Med 338 (5): 278–285. Comment in: N Engl J Med 1998, 338 (5): 323–325
52. Wang Y, Li SJ (1998) Differentiation of metabolic concentrations between gray matter and white matter of human brain by in vivo ^{1}H magnetic resonance spectroscopy. Magn Reson Med 39 (1): 28–33
53. Wolinsky JS, Narayana PA, Fenstermacher MJ (1990) Proton magnetic resonance spectroscopy in multiple sclerosis. Neurology 40 (II): 1764–1769
54. Yamamoto T, Kawamura J, Hashimoto S, Nakamura M (1991) Extensive proliferation of peripheral type myelin in necrotic spinal cord lesions of multiple sclerosis. J Neurol Sci 102 (2): 163–169