Neuroradiology (1999) 41: 738–743

A. Tourbah
J.-L. Stievenart
A. Abanou
M.-T. Iba-Zizen
H. Hamard
O. Lyon-Caen
E. A. Cabanis

Normal-appearing white matter in optic neuritis and multiple sclerosis: a comparative proton spectroscopy study

Received: 19 January 1999
Accepted: 23 March 1999

A. Tourbah (✉) · J.-L. Stievenart · A. Abanou · M.-T. Iba-Zizen · E. A. Cabanis
Service de Neuroradiologie,
Centre Hospitalier National d'Ophtalmologie des XV-XX,
28 rue de Charenton, F-75012 Paris, France
Tel.: + 33-1-40021655,
Fax: + 33-1-43408785

A. Tourbah · J.-L. Stievenart · A. Abanou · M.-T. Iba-Zizen · E. A. Cabanis · O. Lyon-Caen
Faculté de Médecine Pitié-Salpêtrière, Paris, France

A. Tourbah · O. Lyon-Caen
Fédération de Neurologie,
Hôpital de la Salpêtrière, Paris, France

A. Tourbah
CJF 97-11, Pathologie de la Myéline,
Hôpital de la Salpêtrière, Paris, France

L. Stievenart
Service de Biophysique et Médecine Nucléaire, Hôpital Beaujon, Clichy, France

H. Hamard
Sercie d'Ophtalmologie II,
Centre Hospitalier National d'Ophtalmologie des XV–XX, Paris, France

Abstract We investigated neurochemical abnormalities in the normal-appearing white matter (NAWM) on MRI of patients with optic neuritis (ON) and compared them to those of patients with multiple sclerosis (MS). Patients with ON (42) were classified into three groups according to abnormalities on brain MRI. Patients with MS (55) were devided in two groups: relapsing remitting MS (RRMS) and secondary progressive MS (SPMS). All patients underwent MRI of the brain and localised proton magnetic resonance spectroscopy (MRS) of NAWM. The results were compared to those of 15 controls. Patients with MS had significant abnormalities compared with controls and with patients with ON. Patients with RRMS and those with ON had comparable MRS parameters, while patients with SPMS had significant spectroscopic abnormalities in comparison with controls, but also with patients with RRMS. These changes consisted of a decrease in *N*-acetyl-aspartate, a neuronal marker, which may reflect axonal dysfunction and/or loss. MRS abnormalities were detected in 14 patients with ON (27%). The main abnormalities consisted of a decrease in *N*-acetyl-aspartate, an increase in choline-containing compounds at long echo times, and the presence of free lipid peaks at short echo times. MRS of the NAWM on MRI may prove useful for detecting neurochemical brain abnormalities in ON not visible on MRI.

Key words Magnetic resonance spectroscopy · Optic neuritis · Multiple sclerosis

Introduction

MRI has a profound impact on the diagnosis, follow-up and prognostic assessment of patients with multiple sclerosis (MS). It also appears to be the most powerful tool for predicting the occurrence of MS in isolated syndromes, especially optic neuritis (ON). Before MRI, this risk was reported to vary from 13% to 88% [1], with a median time of occurrence ranging from 2 to 5 years [2].

Patients with a single attack of ON and abnormal white matter on MRI have an increased risk of devel-

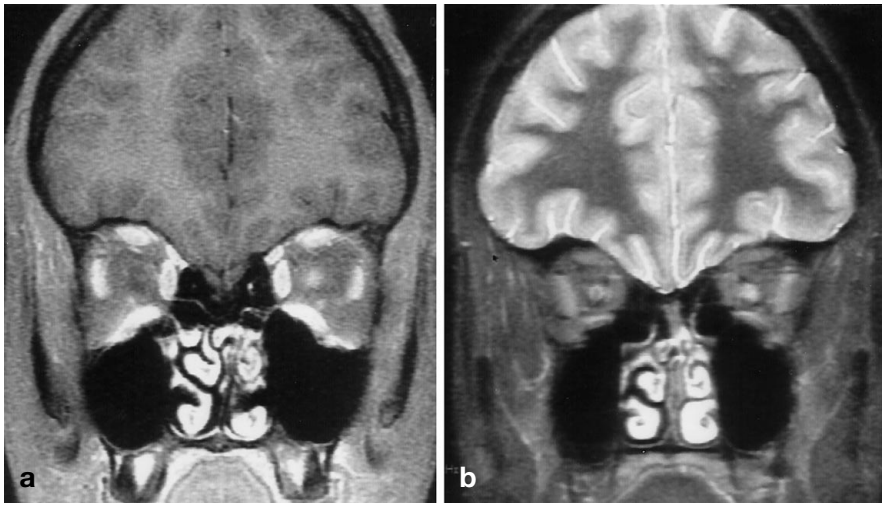

Fig. 1 Abnormalities of signal located in the intraorbital portion of the left optic nerve in a patient with optic neuritis, in a coronal plane. Hyperintensity on T2 weighted inversion recovery sequence (**a**); contrast enhancement on T1 weighted sequence with fat suppression (**b**)

oping MS. In the Optic Neuritis Treatment Trial, MRI was the single most important predictor of the development of clinically definite MS (CDMS). The risk of developing CDMS was estimated at 27 % after 5 years, ranging from 16 % in patients with normal brain MRI to 51 % in patients with at least three periventricular white matter lesions measuring 3 mm or more [3]. Magnetic resonance spectroscopy (MRS) has emerged as a new method for investigating brain disease, giving insights into the neurochemistry of the lesions [4–8]. In white matter diseases, it permits assessment of the biochemical characteristics of a localised volume of the brain, showing abnormalities in the normal-appearing white matter (NAWM) on MRI [9–11]. In MS, it gives information on the biochemistry of the lesions and their temporal evolution [12, 13]. Localised MRS has proven routine use and is a reproducible method.

We used localised MRS to study NAWM in patients with ON and compared the data with those found in a group of patients with CDMS and in a control group. Our aim was to investigate whether localised MRS is useful for detecting damage not visible on conventional MRI, by sampling the centrum semiovale, and to underline the possible role of MRS in the assessment and monitoring of parameters of MS.

Methods

The 15 controls were normal healthy volunteers or patients undergoing MRI for headaches who were otherwise neurologically intact and had normal conventional MRI. Patients with ON (42) had a single isolated recent (2 days to 2 weeks) acute episode of ON with no other past neurological history. Clinical features and visual evoked responses were consistent with the diagnosis, and no other metabolic, toxic or vascular disorder could be found. They were divided into three groups according to the presence or absence of lesions on T2-weighted images using the criteria of Lee et al [14]: group 1: no lesions on MRI (14 patients), group 2: nonspecific lesions (11 patients); group 3: lesions suggestive of MS (17 patients). The 55 patients with CDMS [15] were divided into two groups according to the course of the disease: relapsing-remitting (RRMS) (40 patients) or secondary progressive (SPMS) (15 patients). All patients with CDMS had areas of high signal suggestive of the disease on T2-weighted images.

MRS was carried out immediately a conventional MRI study performed as part of diagnosis and/or for assessment of the activity of the disease. We used a 1.5 T system with a standard quadrature head coil. For all patients, at least the following sequences covering the whole brain and optic nerves were undertaken: T1 weighted spin-echo (TR 440 TE 11 ms, matrix 512 × 256; thickness 5 mm; gap 1.5 mm; 2 excitations) in the sagittal plane; a fast spin-echo sequence (4500 17 ms; 512 × 256; 4 mm; 0.4 mm; 2) in the neuro-optical plane; a fast spin-echo sequence in the coronal plane (5000 102 ms; 512 × 256; 5 mm; 0.5 mm; 2); a fast FLAIR (fluid attenuated inversion recovery) sequence in an axial plane parallel to the neuro-optical plane (11 000 144 ms, inversion time 2600 ms; 256 × 256; 4 mm; 0.4 mm; 1); and a T2-weighted inversion-recovery sequence in the coronal plane for the optic nerves (4000-17-140; 512 × 256; 5 mm interleaved; 4).

Spectra were acquired with the PROBE-SV module. A stimulated-echo acquisition mode (STEAM) sequence was used (TE 136 and 18, TR 1500 ms, mixing time = 13.7 min.) [16]. The total time for MRS acquisition was 15 min. For the NAWM investigation, the parallelopiped volume of interest was graphically prescribed on a T2-weighted axial image, in such a way that neither abnormal appearing white matter nor grey matter was included. The volume of interest varied from 5 to 8 ml and was placed in the centrum semiovale. The raw data were transferred to a workstation with modified SAGE data analysis software. Four main peaks appeared as singlets: myoinositol (Myo) 3.56 ppm, choline (Cho) 3.22 ppm, creatine (Cr) 3.03 ppm, *N*-acetylaspartate (NAA) 2.02 ppm. At short echo times, we looked for broad peaks centred at 0.89 and 1.3 ppm containing mobile moieties of cytosolic proteins and short-chain fatty acids (CH_3 or CH_2 compounds) and a lactate doublet at 1.3 ppm with two peaks 7 Hz apart (J-coupling).

Comparisons of peak area ratios of Myo/Cr, Cho/Cr, NAA/Cr and NAA/Cho were performed with a variance analysis using Scheffe's method. A p level of 0.05 was considered as significant.

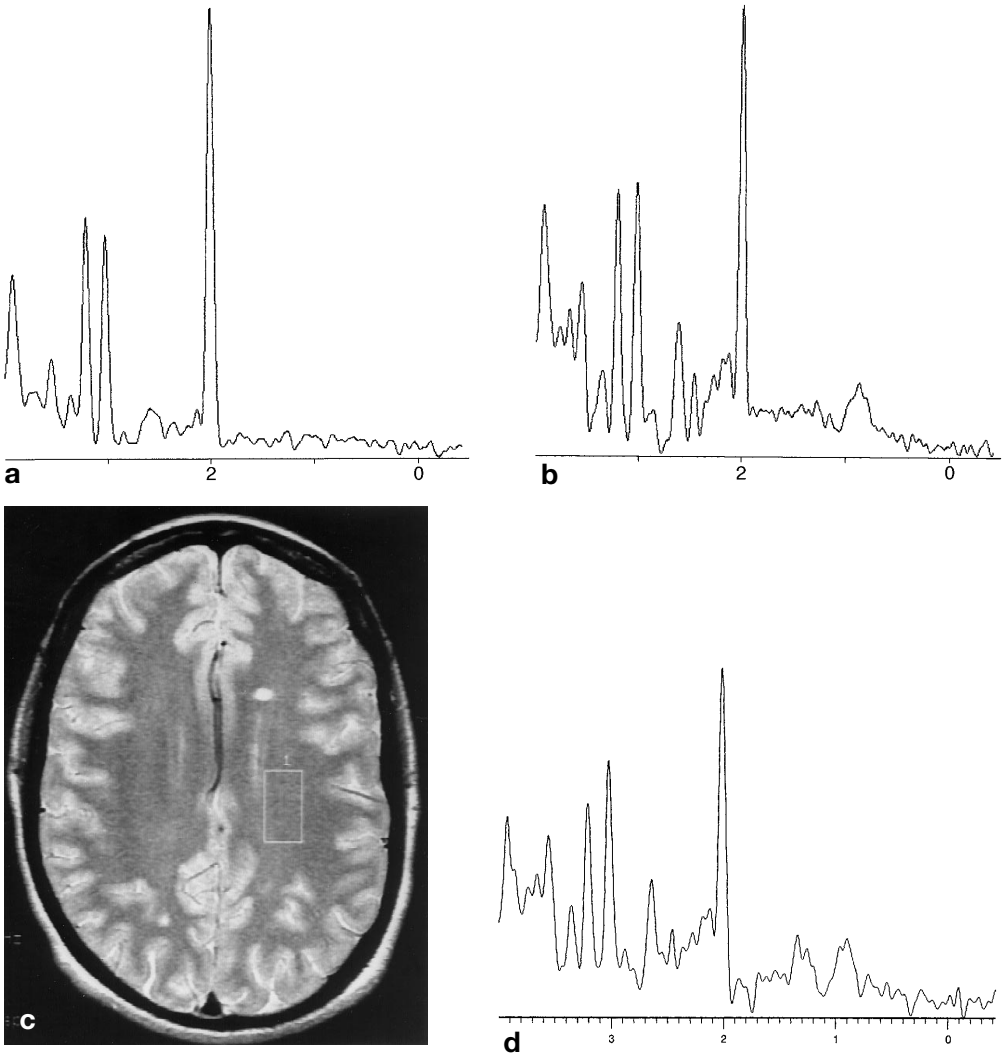

Fig. 2 a–d Average control spectra at **a** TE 136 **b** TE 18 ms. Selection of the volume of interest in the normal-appearing centrum semiovale on MRI in a patient with optic neuritis and high-signal foci on T2-weighted images suggestive of multiple sclerosis, **c** clinically definite multiple sclerosis and **d** its corresponding spectrum at TE 18 ms, showing increase in resonances at 0.9 and 1.3 ppm

Results

The mean ages at which MRS was performed were: controls, 33 years (25–50 years); group 1, 30.8 years (20–38 years); group 2, 41.7 years (21–59 years); group 3, 33.52 years (22–47 years); RRMS, 34.9 years (19–46 years); SPMS, 38.4 years (26–49 years). The mean duration of the disease was 3.8 years in RRMS (0.4–26 years) and 10.5 years in SPMS (2–24 years).

On MRI, all patients with acute ON showed abnormalities of the intraorbital, intracanalicolar, and/or cisternal segment of the optic nerve (Fig. 1 a). Contrast-enhanced T1-weighted images in 12 patients showed enhancement of the optic nerve in 8 (Fig. 1 b).

On MRS, both echo times resulted in well-localised, water-suppressed spectra exhibiting several clearly defined peaks. Averaged control spectra at both echo times are shown in Figs. 2 a and 2 b. Signal-to-noise ratios were comparable for all groups at both echo times.

We first compared MRS in all patients with ON, all patients with MS and controls. At TE 136 ms, there was a significant decrease in NAA/Cr (P =0.007) in patients with MS compared with controls and patients with ON (Fig. 3 a). The ratio NAA/Cho was also lower in patients with MS than in controls (P = 0.0039), but not than in patients with ON (P = 0.08) (Fig. 3 b). In patients with MS the mean NAA/Cr ratio was significantly lower in patients with SPMS than in controls (P = 0.007), in the group of ON with a normal brain on MRI (P = 0.03) and in the RRMS group (P = 0.04) (Fig. 3 c). NAA/Cho ratio was significantly lower in SPMS than in controls, any group of patients with ON, or RRMS (P < 0.0001) (Fig. 3 d). The Cho/Cr ratio was higher in SPMS than in controls or RRMS, but the difference was not significant. No significant difference in any metabolite was found between the three groups of patients with ON, whatever the appearance of their white matter on MRI.

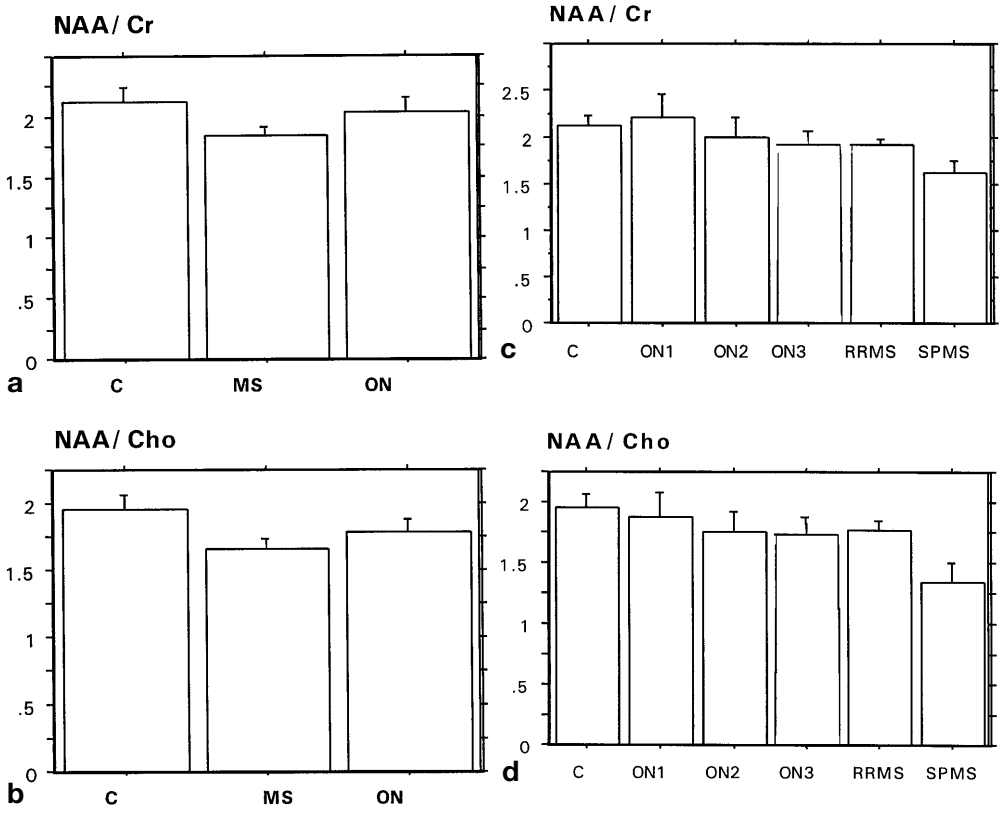

Fig. 3a–d Graphs showing the mean **a, c** NAA/Cr **b, d** NAA/Cho ratios in the different groups with their 95% confidence level at TE 136 ms. *C* controls; *MS* multiple sclerosis; *ON* optic neuritis; *ON1* optic neuritis and normal brain on MRI, *ON2* optic neuritis and lesions not suggestive of MS on MRI; *ON3* optic neuritis and lesions suggestive of MS on MRI; *RRMS* relapsing remitting multiple sclerosis; *SPMS* secondary progressive multiple sclerosis. **a** NAA/Cr is decreased in MS in comparison to controls and ON. **b** NAA/Cho is decresed in MS in comparison to controls. **c** NAA/Cr is decreased is SPMS in comparison to controls, ON1, and RRMS. **d** NAA/Cho is decreased in SPMS in comparison to controls, all ON groups and RRMS

At TE 18 ms, no significant difference could be detected between the groups. Myo/Cr was increased in SPMS, but this was not significant. Abnormal peaks were observed in the free-lipid resonances (0.89 and/or 1.3 ppm) in three patients with ON (Fig. 2 d), two with RRMS and two with SPMS. These peaks were not detected in control patients. No doublet suggesting lactates could be detected.

Even if no significant difference was found between patients with ON and controls, spectroscopic changes were observed in 14 patients with ON (27%). Thus six patients (43%) of group 1, three (27%) of group 2 and five (29%) of group 3 had an abnormal value of one or other of the three principal ratios compared to the values found in controls with 2 standard deviations (NAA/Cr <1.7; Cho/Cr > 1.3; NAA/Cho < 1.5). Of these 14 patients, 7 (50%) developed definite MS 2 years after their first visual attack (three belonging to group 2 and four belonging to group 3); 7 (25%) patients with no spectroscopic abnormalities, belonging to group 3, developed MS within the same period.

Discussion

We used localized proton MRS because of its routine feasibility in the context of a conventional MRI examination. Spectroscopic features were comparable in the three groups of patients with ON, and no major significant difference was found between them and controls. Patients with RRMS had a profile intermediate between those of controls and patients with SPMS. Significant changes were observed in patients with SPMS compared not only with controls but also with RRMS. Patients with ON and particularly those with MS-like lesions on MRI (group 3) had spectroscopic parameters comparable to those with RRMS.

NAA reflects neuronal function and/or density [17–22]. The significant decrease in NAA/Cr in the NAWM of patients with SPMS is consistent with axonal loss secondary to demyelination and/or axonal dysfunction. A significant difference in NAA/Cr was found between RRMS and SPMS, which may reflect the relative preservation of axons in the former, and damage to them in the secondary progressive form.

Increase in choline compounds has been described in inflammatory and/or demyelinating processes [3] and has been interpreted as an increase in cell membrane turnover. The Cho/Cr ratio was increased in patients with SPMS, but this was not significant. The significant

decrease in NAA/Cho in patients with SPMS compared with all other groups may indicate the presence of demyelination and/or inflammation associated with axonal loss and/or dysfunction in the NAWM of these patients. Spectroscopic abnormalities were found in individual patients with RRMS and ON. Within 2 years, 33% of patients with ON developed CDMS, 50% of these, with initial spectroscopic abnormalities of the NAWM, and 25% of those with normal MRS parameters. In group 3, 12/17 patients (70%) developed CDMS within 2 years, five of whom had abnormal MRS in the NAWM. This confirms once again the role of MRI lesions as the best predictive factor of developing CDMS in isolated syndromes. No significant change in myoinositol, an indicator of gliosis [24], was found in NAWM.

At short echo times, isolated abnormal resonances at 0.89 and/or 1.3 ppm were detected in three patients with ON, two with RRMS and two with SPMS. These peaks reflect the presence of free lipids, consistent with lipid breakdown in demyelination [13, 25]. In two other patients with RRMS and one with SPMS these free lipid resonances were associated with a significant increase in choline-containing compounds, which may indicate associated inflammation. Thus, we demonstrated, in the NAWM of patients with ON and MS, changes consistent with inflammatory and/or demyelinating processes. The combination of long and short echoes may allow separation of predominant inflammation (10 patients with increased Cho/Cr without resonances for free lipids), predominant demyelination (seven with free lipids), and the association of inflammation and demyelination (three with increased Cho/Cr and free lipid resonances). Abnormal lipid resonances have recently been reported in the NAWM on MRS imaging [26]. Thus, MRS examinations might have implications for the improvement of the predictive value of magnetic resonance techniques, and for therapeutic approaches to patients with ON and MS.

Even if the spectroscopic changes observed in the NAWM of patients with ON and MS are not specific, and some at least may be observed in almost all neurodegenerative diseases, proton MRS may provide insight in vivo into changes in cellular metabolites that correlate with histopathological findings in cerebral lesions [25]; this has been demonstrated by other techniques [27, 28].

Acknowledgements This work was supported by l'Association pour la Recherche contre la Sclérose en Plaques (ARSEP). We would like to thank GEMS for providing spectroscopy software and David Payet for technical assistance.

References

1. Kurtzke JF (1985) Optic neuritis or multiple sclerosis. Arch Neurol 42: 704–710
2. Sandberg-Wolheim M, Bynke H, Cronqvist S. Holtås G, Platz G, Ryder LP (1990) A long term prospective study of optic neuritis: evaluation of risk factors. Ann Neurol 27: 286–393
3. Optic Neuritis Study Group (1997) The 5-year risk of MS after optic neuritis: experience of the Optic Neuritis Treatment Trial. Neurology 49: 1404–1413
4. Richards TL (1991) Proton MR spectroscopy in multiple sclerosis: value in establishing diagnosis, monitoring progression and evaluating therapy. AJR 157: 1073–1078
5. 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: 23–31
6. 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: 235–241
7. Grossman R, Lenkinski RE, Ramer KN, Gonzales-Scarano F, Cohen JA (1992). MR proton spectroscopy in multiple sclerosis. AJNR 13: 1535–1543
8. Koopmans RA, Li DKB, Zhu G, Allen PS, Penn A, Paty DW (1993) Magnetic resonance spectroscopy of multiple sclerosis: in-vivo detection of myelin breakdown products. Lancet 341: 631–632
9. 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 31P and 1H magnetic spectroscopic imaging. Ann Neurol 36: 157–165
10. 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 Neuradiol 23: 49–55
11. Frahm J, Michaelis T, Merboldt KD, Bruhn H, Gyngell ML, Hänicke W (1990) Improvement in localized proton NMR spectroscopy of human brain, water suppression, short echo times and 1 ml resolutions. J Magn Reson 90: 464–473
12. 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: 49–58
13. Arnold DL, Ries GT, Matthews PM, Francis GS, Collins DL, Wolfson C, Antel JP (1994) Use of magnetic resonance spectroscopy for monitoring disease progression in multiple sclerosis. Ann Neurol 36: 76–82
14. Lee KH, Hashimoto SA, Hooge JP, Kastrukoff LF, Oger JJ, Li DK, Paty DW (1991) Magnetic resonance imaging of the head in the diagnosis of multiple sclerosis: a prospective 2-year follow-up with comparison of the clinical evaluation, evoked potentials, oligoclonal banding, and CT. Neurology 41: 657–660

15. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GA, Johnson KA, Sibly WA, Silberberg DH, Tourtelotte WW (1983) New diagnostic criteria for multiple sclerosis: guidlines for research protocols. Ann Neurol 13: 227–231
16. Frahm J, Bruhn H, Gynegell ML, Merbolt KD, Hänicke KD, Sauter R (1989) Localized high-resolution proton NMR spectroscopy using stimulated echoes: initial applications to human brain in vivo. Magn Reson Med 9: 79–93
17. Birken DL, Oldendorf DH (1989) N-acetyl-L-aspartic acid: a literature review of a compound prominent in 1H-NMR spectroscopic studies of brain. Neurosci Biobehav Rev 1: 23–31
18. Koller KJ, Zaczek R, Coyle JT (1984) N-acetyl-aspartate-glutamate: regional levels in rat brain and the effect of brain lesions as determined by a new HPLC method. J Neurochem. 43: 1136–1142
19. Miyake M, Kakimoto Y, Soromachi M (1981) A gas chromatographic method for the determination of N-acetyl-L-aspartic acid, N-acetyl-alpha-aspartylglutamic acid and beta-citryl-L-glutamic acid and their distribution in the brain and other organs of various species of animals. J Neurochem 36: 804–810
20. Patel TB, Clark JB (1979) Synthesis of N-acetyl-L-aspartate by rat brain mitochondria and its involvment in mitochondrial: cytostolic carbon transport. J Biochem 184: 539–546
21. Simmons ML, Frondoza CG, Coyle JT (1991) Immunocytochemical localization of N-acetyl-aspartate with monoclonal antibodies. Neuroscience 45: 37–45
22. Zaczek R, Koller K, Cotter R, Heller D, Coyle JT (1983) N-acetylaspartylglutamate: an endogenous peptide with high affinity for a brain "glutamate receptor". Proc Natl Acad Sci USA 80: 1116–1119
23. Tourbah A, Stievenart JL, Iba-Zizen MT, Lubetzki C, Baumann N, Eymard B, Moser H, Lyon-Caen O, Cabanis EA (1997) Localized proton magnetic resonance spectroscopy in patients with adult adrenoleukodystrohpy: increase of choline compounds in normal appearing white matter. Arch Neurol 54: 586–592
24. Brenner ME, Munro PMG, Williams SCR, Bell JD, Barker GJ, Hawkins CP, Landon DN, McDonald WI (1993) The proton NMR spectrum in acute EAE: the significance of the change in the cho: Cr ratio. Magn Reson Med 29: 737–745
25. Allen IV, McKeown SR (1979) A histological, histochemical and biochemical study of macroscopically normal white matter in multiple sclerosis. J Neurol Sci 41: 81–91
26. Narayana PA, Doyle TJ, Lai D, Wolinsky JS (1998) Serial magnetic resonance spectroscopic imaging, contrast-enhanced magnetic resonance imaging, and quantitative lesion volumetry in multiple sclerosis. Ann Neurol 43: 56–71
27. Barbosa S, Blumhardt LD, Roberts N, Lock T, Edwards RHT (1994) Magnetic resonance relaxation time mapping in multiple sclerosis: normal appearing white matter and the "invisible" lesion load. Magn Reson Imaging 12: 33–42
28. Filippi M, Campi A, Dousset V, et al (1995) A magnetization transfer imaging study of normal-appearing white matter in multiple sclerosis. Neurology 45: 478–482