MR spectroscopy in relapsing remitting MS

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Hold fastere omkring mig
Med dine runde Arme;
Hold fast, imens dit hjerte
Endnu har Blod og Varme.

Om lidt, saa er vi skilt ad,
Som Bærrene paa Hækken;
Om lidt, er vi forsvundne,
Som Boblerne i Bækken..

(Emil Aarestrup 1838)

Preface

This thesis is submitted to obtain the PhD degree in medicine at the Faculty of Health Sciences, University of Copenhagen. The work was carried out at the Danish Research Centre for Magnetic Resonance, Copenhagen University Hospital Hvidovre (DRCMR) in collaboration with my colleagues at the Neurological Department, Copenhagen University Hospital Rigshospitalet (RH), the Neurological Department, Copenhagen University Hospital Glostrup (Glostrup), and the Centre for Magnetic Resonance, Trondheim University Hospital, Norway (CMR). The PhD thesis is based on the following papers:

> *Mathiesen HK, Tscherning T, Sorensen PS, Larsson HB, Rostrup E, Paulson OB, Hanson LG. Multi-slice echo planar spectroscopic MR imaging provides both global and local metabolite measures in multiple sclerosis (accepted for publication in Magnetic Resonance in Medicine, Appendix 1).*
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> *Mathiesen HK, Jonsson A, Tscherning T, Hanson LG, Andresen J, Blinkenberg M, Paulson OB, Sorensen PS. Global N-acetyl aspartate correlates with cognitive dysfunction in multiple sclerosis (prepared for submission to Archives of Neurology, Appendix 2).*

Danish readers will find more information on the backgrounds in these papers:

> *Mathiesen HK, Langkilde AR, Larsson HB. [Magnetic resonance and multiple sclerosis I. Conventional diagnostic techniques]. Ugeskr Laeger 2002; 164: 1026-1031 (Danish review, Appendix 3).*
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> *Mathiesen HK, Langkilde AR, Larsson HB. [Magnetic resonance and multiple sclerosis II. New diagnostic techniques]. Ugeskr Laeger 2002; 164: 1031-1036. (Danish review, Appendix 4).*

I wish to thank all my patients who, despite their disease, had the patience and strength to participate in this study of repeated two and a half hour MRI scans, and I wish them all the best in the future. I would also like to thank all my collaborators, including the technical and clinical staff at our department, for their help initiating the project, their continuous support and fruitful discussions and suggestions throughout the study period. In particular I wish to express my gratitude to all my supervisors:

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Dansk resumé

Formålene med dette phd-studium var at udvikle, validere og implementere nye MR-metoder med højere specificitet for de patologiske forandringer, der ses ved attakvis MS. Dels patologiske forandringer i MS-læsionerne (ødem, demyelinisering, remyelinisering, gliose samt neuronal dysfunktion eller tab), og dels mere diskrete diffuse patologiske forandringer i tilsyneladende normalt hjernevæv, herunder cortex. De overordnede mål er at opnå en bedre sammenhæng mellem MR-fundene og patienternes kliniske tilstand og dermed en højere prognostisk værdi af MR og bedre muligheder for at afgøre hvilke patienter, der skal behandles samt hvornår i sygdomsforløbet og hvordan.

MR-spektroskopi er en metode, der kan belyse graden af neurotab eller dysfunktion ved at måle metabolitten N-acetyl aspartat (NAA). I dette arbejde benyttede vi en metode udviklet på MR-afdelingen, Hvidovre Hospital (*multi-slice echo planar spectroscopic imaging (EPSI)*). Metoden er videreudviklet og valideret på raske forsøgspersoner og er vist at have en række fordele i forhold til eksisterende spektroskopiske metoder. Traditionelle metoder måler enten metabolitter i hele hjernen inklusive områder, der kan ødelægge kvaliteten af undersøgelserne eller måler i større eller mindre kasseformede udsnit af hjernen, der kan indeholde både patologisk og rask væv foruden CSF. Multi-slice EPSI kan måle globale forandringer i store hjerneområder, hvor problematiske områder er ekskluderet, hvilket fører til højere kvalitet samt måle i mindre irregulære områder (f.eks. læsioner, cortex eller andre specifikke hjerneområder) ved samme undersøgelse.

Kognitive forstyrrelser ses hos op mod 50% af MS-patienterne, selv i de tidligste faser af sygdommen. Sammenhængen med konventionelle MR-mål som f.eks. det totale læsionsvolumen har kun været moderat, hvorfor metoder, der kan belyse sygdomsgraden mere præcist er ønskede. Vi benyttede multi-slice EPSI til at måle mere diffuse patologiske forandringer. 20 patienter med attakvis MS blev fulgt med både konventionelle T_2- and T_1-vægtede målinger samt MR-spektroskopi. Patienterne blev tillige evalueret klinisk og neuropsykologisk med et batteri bestående af 18 neuropsykologiske tests med 29 forskellige delmål. Vi fandt en klar sammenhæng mellem globale MR-spektroskopiske mål og kognitive dysfunktionsmål. Dette tyder på, at multi-slice EPSI har potentiale som markør for udviklingen af kognitive deficits tidligt i forløbet af MS. Hvis metoden blev implementeret i kliniske undersøgelser, synes den således at kunne afgøre hvorvidt den testede behandling beskytter mod udviklingen af kognitive forstyrrelser. De fremtidige udfordringer ligger i at standardisere og automatisere metoden, således at den kan anvendes udenfor MR-afdelingen for herefter at blive implementeret i større undersøgelser for at vurdere dens sande værdi.

Summary

The aims of this PhD thesis were to develop, validate and implement new MR (magnetic resonance) techniques with higher specificity for pathological changes seen in lesions and in normal appearing brain tissues in early relapsing remitting multiple sclerosis (RRMS). Another aim was to perform image acquisition and data-analysis of the non-conventional techniques in a relatively simple way to make these techniques usable in daily practice or clinical trials thus limiting the technical demands and the time consumption. The ultimate goal is to provide better correlations between MRI (MR imaging) and disability measures and to obtain new information on disease evolution and hence faster and more precise decisions on who, when and how to treat the patients.

MR spectroscopy (MRS) can provide information about neuronal loss or dysfunction measuring decreases in N-acetyl aspartate (NAA), a metabolite widely believed to assess neuronal viability. In this thesis we have used multi-slice echo planar spectroscopic imaging (EPSI). This method was proven reproducible and to have some advantages compared to conventional non-localised or single-voxel spectroscopy. It is possible to obtain global metabolite measures and in the same session measurements of metabolites in specific brain areas, including normal appearing white matter (NAWM) and cortical grey matter (GM), which might be of great importance in MS.

Cognitive dysfunction can be seen in 50% of patients with multiple sclerosis (MS), with somewhat lower incidences in the early stages of the disease. The correlations between cognitive dysfunction and conventional T_2- and T_1-weighted MRI measures (e.g. lesion load) have been moderate, and a substantial number of studies suggest that cognitive dysfunction is related to the overall disease burden of the brain. To assess global metabolic changes we used multi-slice EPSI.

20 patients with early RRMS were scanned with conventional MRI and spectroscopic imaging and evaluated with a battery of 29 neuropsychological measures. We found high correlations between spectroscopic measures and neuropsychological measures suggesting that multi-slice EPSI can be used as a surrogate marker for cognitive impairment in early MS. If implemented in clinical trials this method might help to assess whether new treatments can prevent the development of cognitive impairment in MS. Future challenges will be to evaluate the true value of multi-slice EPSI in a larger scale and to implement this technique outside our department in clinical settings.

Abbreviations

B_0	Amplitude of static (polarizing) magnetic field
B_{eff}	The total effective field at the proton
BBB	Blood brain barrier
BPF	Brain parenchymal fraction
Cho	Choline
CIS	Clinically isolated syndrome
CNS	Central nervous system
Cr	Creatine
CSE	Conventional spin echo
CSF	Cerebrospinal fluid
CSI	Chemical shift imaging
DTPA	Gadolinium diethylene triamine pentaacetic acid
DWI	Diffusion weighted imaging
EDSS	Expanded disability status scale
EPSI	Echo planar spectroscopic imaging
FLAIR	Fluid attenuated inversion recovery
fMRI	Functional magnetic resonance imaging
FOV	Field of view
γ	Gyromagnetic ratio
GM	Grey matter
MPRAGE	Magnetization prepared rapid acquisition gradient echo
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MS	Multiple sclerosis
MTI	Magnetization transfer imaging
NAA	N-acetyl aspartate
NAWM	Normal appearing white matter
ppm	Parts per million
PPMS	Primary progressive multiple sclerosis
RF	Radiofrequency
ROI's	Regions of interest
RRMS	Relapsing remitting multiple sclerosis
σ	Secondary field generated by electrons
SPM	Statistical parametric mapping
SPMS	Secondary progressive multiple sclerosis
T	Tesla
T_1	Longitudinal relaxation time
T_2	Transverse relaxation time
TBI	Traumatic brain injury
TE	Echo time
TI	Inversion time
TR	Repetition time
WBNAA	Whole-brain NAA
WM	White matter
WMSG	White Matter Study Group

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Introduction

MS was first described clinically in the 19th century. In 1868 Jean-Martin Charcot, described a young woman with tremor, abnormal eye movements and slurred speech. When she died despite all the efforts he made to treat her, he examined her brain and found the characteristic lesions or "plaques" of MS. The first pathological descriptions of MS lesions were given thirty years earlier, presumably by Robert Carswell in 1838 (Compston 1988).

Today, MS is one of the most common neurological diseases around the world, with a preference for young people, especially women, and for those who grew up in the northern latitudes. More than 2.5 million patients are affected worldwide (Compston & Coles 2002).

The aetiology of this disease is still unknown despite more than a century of research, but it is believed to involve genetic susceptibility, although not directly inherited. An auto-immunological response plays an important part of the pathophysiology, but what triggers it is not yet discovered although there has been focus on many different agents, including viruses, toxins and impaired blood flow, in the last decades. Antibodies against myelin can be detected, leading to destruction of the myelin and hence the neurons, but direct destruction or dysfunction of the neurons also seem to take part in the evolution of the disease.

The discovery of MS patients having altered antibodies against different viruses brought back the attention of virus, not as a direct infection, but more as an agent altering the immune system and hence triggering the destruction of myelin. When the immune system reacts against myelin basic protein in MS, it may very well combine features of both autoimmune and infectious diseases.

MS can cause any neurological symptom since it can involve any part of the central nervous system (CNS), including the cerebral cortex, and the evolution of the disease may vary from very benign cases with few clinical attacks to critical cases with severe neurological and cognitive deficits and a rapid evolution leading to death. MS might be a multi-factorial disease - and the existing of different diseases with different aetiology and evolution is still a possibility.

Since the evolution cannot be foreseen at the time of the diagnosis, MS leads to great fear and anxiety among the patients and their relatives and a lot of efforts have been done trying to improve the prognostic value of clinical and paraclinical tests, including MRI.

No curative treatment exists, and the existing treatments which can reduce the severity and the number of new attacks are very expensive and the long term effects are not known. Methods are needed to decide which patients to treat and how early. In this the importance of antibodies against interferons which can hamper the effect of the treatment also has to be considered (Sorensen et al. 2003).

Since the first MRI was performed on MS patients in 1981 (Young et al. 1981), MRI have changed our picture of MS. Sequential MRI has shown that MS is an ongoing disease even though clinical symptoms may only appear sporadically. MRI showed how the lesions emerge and develop, even in clinical silent periods of the disease. In fact new MS lesions can be seen

on conventional T_1- and T_2-weighted MRI 5-10 times more frequent than new clinical attacks in RRMS and secondary progressive MS (SPMS) (Miller et al. 1993). Measuring decreases in NAA, a marker of neuronal viability, MRS has brought back the attention to neuronal loss or dysfunction, known to exist from the very first pathological descriptions of the disease, however gaining much less focus than demyelination in the past.

In the 1970s it could take more than 5 years from the first symptom of MS until the final diagnosis was made. This often meant many years of anxiety and uncertainty and missed opportunities. Now MS lesions can often be demonstrated immediately and conventional MRI has become essential in the diagnosis of the disease (McDonald et al. 2001).

MRI also changed MS treatment. The discovery that MS is not a disease that flares up only intermittently with periodic attacks as once believed, but rather an ongoing disease that causes silent damage within CNS added urgency to the need for effective treatment to reduce the damages as early as possible. MRI gave the opportunity to faster testing of new treatments, because the effects of new drugs can be seen on MRI activity before they can be demonstrated clinically. Research on the treatment of MS was thus greatly accelerated leading the way for the disease modifying interferons in the 1990s.

Despite the advantages of MRI in the diagnosis and follow-up in clinical trials, conventional MRI with T_1- and T_2-weighted sequences cannot predict the prognosis, although many lesions and early debut might be related to a more severe evolution of the disease (Brex et al. 2002). The risk of developing MS is very high in patients with clinically isolated syndromes (CIS), such as optic neuritis, brain stem or spinal cord syndromes if all the MR criteria for MS are fulfilled (Barkhof et al. 1997). Numerous studies have shown that the risk of progression to confirmed MS is much greater for people with abnormal T_2-weighted MRI (not necessary fulfilling the Barkhof criteria) than for those with normal scans (Frederiksen et al. 1991, Martinelli et al. 1991, Lee et al. 1991, Jacobs et al. 1991, Ford et al. 1992, Beck et al. 1993, Campi et al. 1995, Tas et al. 1995). In a 10 year follow-up it was shown that 83% of patients with CIS and abnormal MRI progressed to (probable or definite) MS compared with only 11% of patients with normal MRI (O'Riordan et al. 1998). A significant relationship between the number of lesions at presentation and the score on the expanded disability status scale (EDSS) at follow-up was also shown. These data were supported after 14 years of follow-up (Brex et al. 2002). However, the correlation between conventional MRI and the clinical status of the patients measured by the EDSS (Kurtzke 1983) remains modest (Thompson et al. 1990, Filippi et al. 1995 C, IFN(beta) Multiple Sclerosis Study Group 1995). Several explanations have been given including limitations in the MRI measurements as well as limitations in assessing the clinical status using EDSS. This scale is heavily weighted toward motor dysfunction, and it does not fully account for fatigue or cognitive dysfunction. Motor dysfunction may be due to spinal lesions (approximately 10% of the lesions) which are seldom visualised. Much of the disability revealed by the EDSS can be explained by periventricular lesions in the internal capsule, whereas lesions in white matter (WM) tracts in the grey-white junction linking associative areas leads to cognitive

impairment (Charil et al. 2003). Assessing lesion location can hence lead to better understanding.

Although widespread decreases in T_1 intensity (Matthews & Arnold 2001) and widespread increases in T_2 intensity (Gasperini et al. 1996) has been described, conventional MRI is unable to visualise subtle pathological changes in cerebral cortex and in NAWM. Changes which have been shown with a number of techniques, i.e. MTI (magnetization transfer imaging) (Filippi et al. 1995 B, Pike et al. 1999), diffusion weighted imaging (DWI) (Werring et al. 1999) and MRS (Fu et al. 1998), and might be very important for the clinical status of the patients. Furthermore, conventional MRI does not account for the pathological changes in the lesions, and it might be of great importance whether oedema, demyelination, remyelination gliosis or neuronal dysfunction or loss dominates in each lesion.

A lot of efforts have been done in the last decades to develop new MR methods with higher pathological specificity and better correlation to disability to reduce the uncertainty about the prognosis and to lead to faster and more precise decisions of when and how to treat the patients. Methods which might be able to shorten clinical trials, determine the ability of new and existing treatments to prevent demyelination and axonal loss, and facilitate remyelination. This thesis is such a work concentrating on the use of multi-slice EPSI in early RRMS.

Methods and backgrounds for the choices

Patients and controls

Twenty patients (16 women, 4 men) with newly diagnosed RRMS fulfilling the Poser criteria (Poser et al. 1983) were included. The patients were selected from a larger cohort of patients followed at the Neurological Department at the Copenhagen University Hospital Rigshospitalet. Inclusion criteria: disease duration less than 5 years, no recent steroid treatment, no known allergy to gadolinium-DTPA (diethylene triamine pentaacetic acid), no pregnancy, and no participation in other clinical trials. The mean age was 36 years (range 22-48), and the mean EDSS score was 2.5 (range 0-4.5). The patients were followed with MRI every 6 months for 2 years. Eighteen healthy age and sex matched control persons (15 women, 3 men) were selected among the staff and their relatives at the paediatric department at the Copenhagen University Hospital Hvidovre. The mean age was 34 years (range 20-51). They were followed with MRI at baseline and after one year. All subjects studied gave their written informed consent and the study was approved by the local Scientific Ethics Committee (KF 01-055/01).

The MRI scanner

All included patients and healthy control persons were scanned according to the same protocol of conventional and non-conventional MRI techniques on a Siemens Vision 1.5 T (tesla) whole-

body MRI scanner using a standard circular-polarized head coil (Siemens AG, Erlangen, Germany).

Positioning

In MS studies internal landmarks, e.g. the HYFA-line (a line from the inferior border of the hypophysis (*HY*) to the fastigium (*FA*) of the 4th ventricle moved to the inferior border of the splenium of corpus callosum) is usually used to assure that axial slices are obtained in the same way between patients and within patients in follow-up scans. In this study DWI (not reported here) prohibited angulations during image acquisition. Therefore, in follow-up scans the patients preferably had to be positioned in the scanner as they were placed in the scanner at baseline as corrections for changed directionality otherwise had to be employed. Keeping the orientation between scans also minimizes other potential sources of signal variations. This was obtained using the same vacuum fixation pillow (Med-Tec, Inc. USA) and placing the head of the patients in the same position in the head coil. The patients were positioned in the coil with no or minimal rotation in the coronal and axial planes. The head was further fixated using the pads on the head coil. The position of the head in the coil was controlled using external landmarks, and the vertical beam of the positioning light in the scanner were chosen to touch the upper limit of the ear and go through the eyebrow to add consistency in the positioning of the patients. Then three orthogonal localizer images were performed to control the rotation in the coronal and axial planes, and a midsagittal localizer image was acquired to assess the rotation in the sagittal plane. In follow-up scans the position and the rotation of the head was controlled using the localizer images and the positioning was corrected if necessary to assure minimal rotation in the coronal and axial planes and the same rotation in the sagittal plane as in the first scan.

Angulations of the imaging plane before image acquisition using internal landmarks is possible in all the used sequences except for DWI. To compensate for slight movement of the patients during image acquisition and minor differences in positioning between scans, the images were aligned using SPM2 (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London, UK; for more details see Mathiesen et al. 2004 A, Appendix 1).

MRI protocol

The total time used with the patient in the scanner room including preparation of the injections system, injection catheter, positioning and scanning far exceeded 2 hours. This is close to the limit of what is possible concerning patient compliance and occupation of the MRI scanner. The main goal was to implement non-conventional techniques and in the same time get conventional data such as the volumes of T_2-weighted hyperintense lesions, T_1-weighted hypointense lesions (black holes), gadolinium enhancing lesions, as well as atrophy measures

which lead to some choices concerning the MRI protocol. A second goal was to perform image acquisition and data-analysis of the non-conventional techniques in a relatively simple way to make these techniques usable in daily practice or in clinical trials thus limiting the technical demands and the time consumption.

The MRI protocol consisted of FLAIR (fluid attenuated inversion recovery) imaging to measure the number and volume of T_2-weighted hyperintense lesions, DWI, spectroscopic imaging, T_1-weighted imaging before and after injection of gadolinium-DTPA to assess the volume of black holes and enhancing lesions, ultra-fast T_1 measurements during bolus injection and finally MPRAGE (magnetization prepared rapid acquisition gradient echo) to assess the brain parenchymal fraction (BPF) which is a measure of atrophy. This protocol was strictly followed in all scan performed, with very few exceptions due to rare lacks of patient compliance or technical difficulties.

The DWI and the ultra-fast T_1 measurements during bolus injection are not analysed yet and will not be discussed further.

Regions of interest

The regions of interest (ROI's) were MS lesions, NAWM, cerebral cortex and a large brain volume for evaluation of global metabolic changes. To assess atrophy, the volume of GM and WM was calculated using SPM2 segmentation, and to measure the total intracranial volume the margins of the brain were drawn on the FLAIR images. These measures were used to calculate the BPF (Mathiesen et al. 2004 B, Appendix 2).

T_2-weighted MS lesions and NAWM were drawn on the FLAIR images and unenhanced and gadolinium enhanced T_1-weighted MS lesions were drawn on the T_1-weighted images using programs developed in our department (by MD, Egill Rostrup) programmed in Matlab© (The Mathworks, Inc, USA). NAWM was selected from six central slices containing subcortical and periventricular WM for spectroscopic evaluation. Approximately 35 ml (range 18-63 ml) was selected.

The brain parenchyma suitable for global spectroscopy was selected semi-automatically. Areas not suitable for evaluation because of poor shim or cerebrospinal fluid (CSF) were excluded automatically. In the automatically calculated brain mask the following areas of the brain were excluded; the inferior frontal lobes and inferior parts of the temporal lobes, particularly near the frontal and sphenoid sinuses, the nasal cavity and the inner ear. All these areas are known to cause problems because of considerable magnetic field inhomogeneities due to susceptibility differences (Gonen & Grossman 2000, Truong et al. 2002). The ventricles were excluded in all cases. Manual editing added consistency in the choice of regions between patients and normal controls before the metabolite spectra and ratios were calculated (see **Figure 1** in Mathiesen et al. 2004 A, Appendix 1 for the brain mask and a corresponding spectrum). The adapted brain mask did not cover the whole brain, but approximately 60% of the brain parenchyma. However,

the excluded areas would have degraded the spectra, and the method is still able to provide information on diffuse metabolic changes (Mathiesen et al. 2004 A, Appendix 1).

To assess metabolic changes in the cerebral cortex a border of approximately 1 cm was automatically selected at the surface of the brain based on FLAIR images, and again areas known to degrade spectral quality were manually excluded before the spectra and metabolite ratios were calculated. The spectroscopic images were registered to the anatomical images using SPM2 (more details in Mathiesen et al. 2004 A, Appendix 1), and the pooled spectra and metabolites were calculated for the chosen regions including metabolic changes in NAWM and the lesions (see **Figure 2** in Mathiesen et al. 2004 A, Appendix 1 for the regional spectra and examples of the ROI's).

T_2-weighted imaging (FLAIR)

T_2-weighted imaging is the most widely used MRI technique to determine disease activity and load in MS. It is routinely used for MS diagnosis because of high sensitivity in detection of MS lesions - about 95% of MS patients have hyperintense lesions on T_2-weighted MRI (Paty el al. 1988). It is used to monitor short-term MS activity (by counting new and enlarged lesions on serial scans) and to monitor long-term MS evolution (by assessing the changes of the total lesion load (Rovaris & Filippi 2000).

A MS lesion varies from a few millimetres to more than a centimetre in size and appears round or ovoid. Larger areas of hyperintensity resulting from confluence of lesions are also commonly seen. Lesions are typically located in the periventricular WM including corpus callosum, subcortical regions and in infratentorial regions (Fazekas et al. 1999) leading to the MR criteria for MS of Barkhof and co-workers (Barkhof et al. 1997).

At 5 mm slices the smallest lesions are missed, since it has been shown that slice thickness reduction to 3 mm lead to a 9% increase in lesion load (Filippi et al. 1995 A). Thus, 3 mm slices are often used in clinical trials. However, the results in longitudinal studies are not significantly affected and gains of using 3 mm slices does not seem justified compared to the prolonged acquisition time (also reviewed in Mathiesen et al. 2002 A, Appendix 3). There is no further increase in total lesion load going from 3 to 1 mm slices (Molyneux et al. 1998).

In this study 5 mm slices were selected to reduce the acquisition time and to facilitate the alignment of the anatomical slices to the 10 mm slices of the spectroscopic measures, and T_2-weighted lesion load was assessed with FLAIR. No conventional double spin echo T_2-weighted and proton density weighted scans were performed. This was mainly because the total time consumption did not allow for additional sequences. Furthermore, FLAIR yields heavily T_2-weighted images in which CSF is nulled using inversion recovery (De Coene et al. 1992). Using FLAIR instead of conventional T_2-weighted imaging the number of MS lesions detected can be increased (White et al. 1992). Because CSF appears dark, this is especially the case in lesions, which are at the interface between brain and CSF, such as cortical and subcortical lesions. These lesions are generally difficult to visualise with conventional techniques. Several studies

have reported a high sensitivity of FLAIR for detecting MS lesions in the brain (Filippi et al. 1996 C, Bastianello et al. 1997, Gawne-Cain et al. 1997, Yousry et al. 1997). FLAIR performed better than CSE (conventional spin echo) in cortical/subcortical areas, comparably for periventricular lesions but worse in the posterior fossa and the spinal cord (Filippi et al. 1996 D, Gawne-Cain et al. 1997). Fast FLAIR detects a 15-20% higher lesion load than CSE on brain MRI and the reproducibility on serial scans is better (Rovaris et al. 1997, Filippi et al. 1998 C, Gawne-Cain et al. 1998, Warach et al. 1998).

The count of new MS lesions on serial scans is also maximized by using FLAIR (Filippi et al. 1998 B); however the reduced sensitivity in detecting infratentorial lesions limits the use in the diagnosis of MS. There have been difficulties in standardization of the acquisition parameters across centres and a high interscanner variability of MS lesion volumes assessed using FLAIR limiting the use in clinical trials (Rovaris & Filippi 1999). However, since the spectroscopic measurements did not fully cover the infratentorial regions (see **Figure 1** in Mathiesen et al. 2004 A, Appendix 1) the concerns about the lower sensitivity of FLAIR in infratentorial regions were neglected in this study.

Thirty 5 mm interleaved axial slices centred 10 mm above a transversal tangent plane at the top of the mesencephalon covered the brain. No coronal or sagittal slices were obtained which might be standard in some clinical settings to define and evaluate the lesion morphology. However, this is usually not done in clinical trials and the total time consumption did not allow further sequences. The acquisition parameters were: TE/TI/TR = 110/2500/9000 ms, 2 acquisitions, matrix 220 x 256, field of view (FOV) 230 mm, and pixel size 0.9 mm x 0.9 mm. The total scan time was 13 minutes.

Unenhanced T_1-weighted imaging and black holes

Unenhanced T_1-weighted imaging is not used to monitor disease burden and activity in routine clinical management of MS or in clinical trials, but can reveal hypointense lesions - black holes - which may provide information on lesion pathology and means to link MRI parameters with clinical disability. Black holes are common in MS (Uhlenbrock & Sehlen 1989). About 10-20% of the MS lesions seen on T_2-weighted MRI are seen as hypointensities on T_1-weighted sequences (Fazekas et al. 1999). Up to 80% of new enhancing lesions are initially hypointense (Barkhof et al. 1998 A), and they can remain hypointense or return to isointensity during a follow-up of one year (van Waesberghe et al. 1997 B).

Severe tissue loss has been shown histopathologically in a post mortem MR study of hypointense lesions and the degree of hypointensity of the black holes correlated with tissue destruction and loss of axons (van Walderveen et al. 1998). The data, however, where based on the examination of only 5 unfixed whole brains from MS patients which were scanned within 24 hours from death. The lesions showed hypocellularity, absence of or thinly myelinated axons, astrocytosis and gliosis, and the degree of hypointensity on MRI correlated significantly with widening of the extracellular space and loss of axons (van Walderveen et al. 1998).

However, oedema might also explain the hypointensity and contributes to the reversible hypointensity in acute lesions in vivo. Hypointense lesions load on T_1-weighted images correlates better to EDSS than T_2-weighted lesion load (van Walderveen et al. 1995, Truyen et al. 1996). Despite that, there has been no shift from the hyperintense lesions on T_2-weighted images to black holes on T_1-weighted images. DWI and MRS have shown large structural and metabolic variations in these lesions why they are not used to monitor disease activity (Filippi et al. 2002) and are not a part of the diagnostic criteria of MS.

Twenty-one 5 mm slices with no gaps positioned and centred the same way as the FLAIR images were obtained. Each slice corresponded exactly to one slice of the FLAIR images. Acquisition parameters were: TE/TR = 14/650 ms, 2 acquisitions, matrix 192 x 256, FOV 230 mm, and pixel size 0.9 mm x 0.9 mm. The total scan time was 4 minutes.

T_1-weighted gadolinium enhanced imaging

Gadolinium is a paramagnetic contrast agent. It is chelated to DTPA because the free ion is very toxic (Bronen & Sze 1990). This complex is stable, and there is no evidence of dissociation of the gadolinium ion from the DTPA ligand in vivo (Weinmann et al. 1984). Chelated to DTPA it is not metabolized or bound to proteins. Gadolinium-DTPA is distributed in the intravascular and extracellular fluid compartments, and it does not cross the intact blood-brain barrier (BBB) or cell membranes because of its charge, high molecular-weight, and hydrophilic nature (Weinmann et al. 1984, Bronen & Sze 1990). Gadolinium-DTPA effectively shortens the relaxation processes of adjacent protons, and in case of increased BBB permeability in the lesions associated with ongoing inflammation the effect is seen as signal enhancement on T_1-weighted images (Bronen & Sze 1990, Kermode et al. 1990).

The standard dose (single-dose) of gadolinium-DTPA is 0.1 mmol/kg body weight. In this dosage adverse reactions are rare. Less than 2% in total has been reported (Niendorf et al. 1998). Nausea/vomiting, local warmth/pain at the site of injection and headache are seen most often. More rare adverse reactions such as parasthesia, dizziness, focal convulsion, urticaria, flush, cardiovascular reactions, tachycardia and arrhythmia has been described, and more serious adverse events, such as oedema of the larynx, lung oedema, visual disturbance, convulsion, anaphylactic shock and death are extremely rare (less than one out of a million).

In this study the contrast agent was injected through a Venflon™ (Becton Dickinson Vialon™ Biomaterial, Sweden) in a cubital vein using a power injector (Medrad® Spectris™ MR Injection System SSM 200 2, Medrad, Inc., USA). The injection rate was 2.5 ml/s and the same type of contrast was used in all patients and control persons (Magnevist®, 469 mg/ml, 0.5 mmol/ml, Schering AG, Berlin). The contrast was given in double-dose (0.2 mmol/kg) to increase the sensitivity to enhancing lesions compared to single-dose, and in the same time reduce the costs and the number of flow artefacts and false positive lesions (small vessels) compared to triple-dose contrast injection (Filippi et al. 1996 A, Gasperini et al. 2000, Filippi et al. 2002). This administration led to mild (mostly warmth and taste of metal) adverse reaction reported at the

baseline scan in more than 25% of the cases in contrast to the report of no or very few adverse reaction reported by others (Niendorf et al. 1998, Gasperini et al. 2000, Filippi et al. 2002). In a study on the effect of antibodies against interferon on the MRI activity in RRMS (Sorensen et al. 2004) the contrast was given in double dose manually with an injection rate much lower than 2.5 ml/s leading to adverse reaction only in a few percent of the cases (data not published). The chosen way to administrate the contrast agent was considered safe. However, the injection rate seems to play a role in the development of mild adverse reactions.

Other ways to increase the sensitivity of T_1-weighted gadolinium enhanced imaging, other than higher contrast doses, are weekly scanning (Lai et al. 1996), spinal imaging (Thorpe et al. 1996), MTI (Silver et al. 1997, van Waesberghe et al. 1997 A), delayed scanning (Silver et al. 1997) and thinner slices (Filippi et al. 1996 B). These techniques interfere with patient throughput and some of the gains are minor compared to the time consumption or costs. In this study we selected a delay of 10 minutes mainly because of ultra-fast T_1 measurements during bolus injection (data not yet analysed).

T_1-weighted gadolinium enhanced images are more sensitive in detecting disease activity than clinical examination or T_2-weighted images. Most new lesions in patients with RRMS and SPMS begin with disruption of the BBB, and the phase of enhancement may persist for 2-8 weeks (Lai et al. 1996, Barkhof & van Walderveen 1999). New enhancing lesions can bee seen twice as often as new T_2-weighted lesions on monthly brain MRI in RRMS and SPMS (Harris et al. 1991, Barkhof et al. 1992, Miller et al. 1993). Thus counting the number of enhancing lesions is a very sensitive and reproducible way to assess MS activity (Rovaris & Filippi 2000). Although a majority of enhancing lesions occur in clinically stable patients, it has been shown that periods of increased lesion activity increases the risk of exacerbation, probably by increasing the risk of lesions occurring in areas of the CNS that will become clinically apparent (Miller et al. 1998).

Measuring enhancing lesions correlates better to EDSS than T_2-weighted imaging (Stone et al. 1995). A number of studies have shown that the volume and the number of lesions may predict the onset and severity of relapses (Smith et al. 1993, Khoury et al. 1994). However, the method is not able to predict the cause of the disease. The main reason for using gadolinium is to increase the certainty of the diagnosis (Barkhof et al. 1997, McDonald et al. 2001), and to assess activity in clinical trials. The value of contrast enhancement in clinical trials was recently challenged. The fact that a new treatment decreases enhancement in MS does not necessary indicates that this treatment is effective. Indeed it is possible for treatments to be effective, especially longitudinal, even thought it has little or no effect on BBB (Filippi et al. 2002).

The T_1-weighted sequence used before contrast injection was repeated unchanged after the 10 minutes delay after contrast injection.

Atrophy

The presence of both cerebral atrophy and spinal cord atrophy in excess of what might be expected for age are seen in patients with MS (Losseff & Miller 1998, Stevenson et al. 1998).

Baseline atrophy of corpus callosum and infra- and supratentorial atrophy has been demonstrated in patients with RRMS and SPMS (Barkhof et al. 1998 B, Liu et al. 1999, Rudick et al. 1999, Paolillo et al. 2000, Fox et al. 2000), and ventricular enlargement can be detected in the very earliest stages of the disease. In a longitudinal study of patients with CIS ventricular enlargements could be demonstrated in the patients who had developed MS at follow-up 1 year from onset (Brex et al. 2000). It has been demonstrated in several longitudinal studies that cerebral atrophy worsens during time in MS patients, often without clinical manifestations (Losseff et al. 1996, Rudick et al. 1999, Fox et al. 2000). Atrophy measured with BPF calculated as the ratio between the brain parenchymal tissue volume and the total volume contained within the brain surface contour (Rudick et al. 1999) is detectable within 3 months in untreated RRMS patients (Hardmeier et al. 2003). As it is the case in the cerebrum spinal cord atrophy can be seen at baseline, however more common in later stages of the disease. And the spinal cord area measured at several vertebral levels decreases with time and correlates to clinical disability (Kidd et al. 1993, Stevenson et al. 1998, Liu et al. 1999).

Correlation between cerebral atrophy and neurological disability (EDSS) has also been shown in a number of studies (Losseff et al. 1996, Liu et al. 1999, Dastidar et al. 1999, Fisher et al. 2002). This association between atrophy and disability lead to suggestions to use atrophy as a measure of progressive neurological deterioration and to monitor therapeutic efficacy in treatment trials, however atrophy measures are non-specific and measure the end result of many pathological processes. Atrophy represents global tissue loss; however it is difficult to establish the exact contribution of demyelination compared to axonal loss.

Other factors may influence cerebral volume such as alcohol, anorexia, corticosteroid administration and acute dehydration and it is important to be aware of this in the study of cerebral atrophy. With this in mind atrophy can be measured reliably and reproducibly (Losseff & Miller 1998, Rudick et al. 1999), but the practical value of cerebral atrophy measurements is yet to be established. Atrophy measures do not reflect disease activity, nor does it provide information on specific pathology. However, it can provide a global assessment of the neurodegenerative process (Filippi et al. 2002), and might still be important in treatment trials (Fisher et al. 2002, Hardmeier et al. 2003).

The BPF was derived from MPRAGE images providing the total volume of GM + WM, which was divided with the total intracranial volume drawn on FLAIR images (Mathiesen et al. 2004 B, Appendix 2). The acquisition parameters of the MPRAGE sequence were: TR/TE = 11.4/4.4 ms, 1 acquisition, flip angle 8 degrees, matrix 224 X 256, rectangular FOV 250 mm, pixel size 0.98 x 0.98. Number of slabs = 1, slab thickness 250 mm, effective thickness 1 mm, number of partitions 250. The total scan time of the MPRAGE sequence was 15 minutes.

MRS

Spectroscopy is a term covering a wide range of techniques based on measuring frequencies, and we ourselves perform frequency analysis every day, e.g. in hearing and seeing colours.

The spatial information in conventional MRI is based on different frequencies of the signal from different positions in the tissue. This became possible with the introduction of gradients in the magnetic field in the 1970s (e.g. Lauterbur and Mansfield), and further development of signal location techniques which made the clinical use of MRI possible. Paul Lauterbur and Peter Mansfield were awarded with the Nobel Price in medicine and physiology in 2003 for their discoveries.

MRS is a technique for non-invasive chemical analysis that provides information on the nature and quantities of chemical compounds in the brain. MRS has provided insight into the biochemical pathology and evolution of MS and it offers pathological specificity when used to monitor specific metabolites. In the magnetic field of the MR scanner protons precess at a frequency *f* determined by the strength of the external magnetic field B_0 and the gyromagnetic ratio $\gamma = 42.5$ MHz/T:

$$f = B_0 \gamma \qquad \text{[Equation 1]}$$

This equation is often termed the Larmor equation, and the frequency, the Larmor frequency. Radiofrequency (RF) pulses with this frequency are able to bring the protons to a higher energy level, and when the RF pulses are turned off, the protons return to equilibrium, and the result is radio-wave signal emission from the tissue at the same frequency. The magnetic field around a proton depends not only on the external magnetic field, but also on a very small secondary field generated by the electrons ($10^{-6} < \sigma < 10^{-3}$), i.e. the total effective field B_{eff} at the proton is also dependent upon the electronic environment or the chemical environment, expressed in σ:

$$B_{eff} = B_0 (1 - \sigma) \qquad \text{[Equation 2]}$$

From equation 1 and 2 we can see that protons in different chemical environments give rise to signals at different frequencies, since σ differs:

$$f = B_0 (1 - \sigma) \gamma \qquad \text{[Equation 3]}$$

So, it is possible to separate various metabolites using MRS because of their signals having slightly different frequencies. To avoid difficulties in comparing Δ*f* values (because magnets do not all have the same field strengths) a field-independent scale of parts per million (ppm) was introduced to separate frequencies from an arbitrary chosen reference (modified from Bolinger & Insko 1996):

$$\text{ppm} = \Delta f / f + R \qquad \text{[Equation 4]}$$

Δ*f* is the difference in hertz between the metabolite and the reference, *f* is the frequency of the metabolite and R is the ppm value of the reference (tetramethylsilane). The result is a spectrum

(see **Figure 1** in Mathiesen et al. 2004 A, Appendix 1) where the peaks represent different frequencies or metabolites and the areas under the peaks are proportional to the number of nuclei, i.e. the amount of a particular metabolite in the tissue. The peak area also depends on the concentration of the tissue in the voxel (CSF, oedema), coil sensitivity and the relaxation times T_1 and T_2 for the metabolite. The relaxation times are commonly not known (measurements are very time consuming). Therefore, concentrations in "quantitative" studies are mostly based on assumptions and are often not true concentration measurements.

MRS requires small, mobile molecules in high concentrations (> 0.1 mM), which is limiting the number of metabolites that can be examined. However, studies of a few metabolites have provided new information about MS, including evidence for pathological changes in normal appearing brain tissue and have drawn the attention to axonal loss or dysfunction in the pathogenesis of MS.

At long echo times (e.g. 144 ms as used in this context) 3 peaks stands out in the proton spectrum: choline (Cho) at 3.22 ppm, creatine (Cr) at 3.04 ppm and NAA at 2.01 ppm (Ross & Sachdev 2004) (see **Figure 1** in Mathiesen et al. 2004 A, Appendix 1). Lactate is also visible (at 1.33 ppm) if present, e.g. in acute lesions. The functions of these metabolites are not fully understood, and little is known about normal changes or variations between individuals and various brain regions. However, it is known that the amount of NAA and Cr are higher in GM compared to WM and the opposite is the case for Cho. The GM/WM proportions of each metabolite differ, and may vary between brain areas (Kreis et al. 1993), and the relative volume of GM to WM varies with age and atrophy. Also, different proportions of several metabolites may contribute to each peak in the spectrum, e.g. other compounds with N-acetyl groups may contribute to the NAA signal (i.e. N-acetyl-aspartyl-glutamate). So it has to be emphasised that interpretations of the metabolic changes observed in MS patients and any conclusions must be drawn with caution. However, Cho is a component of phospholipids present predominantly in cell membranes, and thought to be a marker of membrane-turnover (Arnold et al. 1998). Cr is a molecule associated with cellular energy metabolism, which has been widely used as an internal standard to correct for oedema and artifactual variations in signal intensity over space due to magnetic field and RF inhomogeneities. The use of Cr as a reference and the pitfalls has been discussed in detail elsewhere (Mathiesen et al. 2004 A, Appendix 1). In the mature brain, NAA is found exclusively in neurons and neuronal processes (axons and dendrites) (Birken & Oldendorf 1989) and the level of NAA has been used as a marker of neuronal integrity. Decreases of NAA may reflect axonal injury, which may be either reversible or irreversible (Arnold 1999). However, reversible or persistent oedema might confuse the image. NAA may be involved in the metabolism of neurotransmitters, or in the regulation of neuronal protein synthesis and myelin production (Birken & Oldendorf 1989).

In MS, the MR spectroscopic methods used have been dominated by proton spectroscopy using single-voxel spectroscopy (the majority of the studies), whole-brain (e.g. Gonen et al. 2000), and chemical shift imaging (CSI) (e.g. Adalsteinsson et al. 2003) including multi-slice

EPSI used in this thesis. In single-voxel spectroscopy a box of tissue (ranging from a few ml to more than 100) is selected before signal acquisition. The advantages are that local adjustment is possible, it is fast and easy to perform and it is well suited for short TE spectroscopy where more metabolites can be detected. However, it can be hard to separate the peaks at short echo times, and the baseline might be poorly defined. Also the spatial information is limited and may be compromised by motion or inaccuracy in voxel positioning. The major drawbacks are that the ROI's have to be selected during scanning, and different tissues (lesion, normal tissue, WM, GM) and CSF might be included in the selected volumes. Non-localized whole-brain spectroscopy is relatively simple to perform, however, regions known to degrade spectral quality are included, and any information on local metabolic changes is lost to the global average. With CSI or multi-slice EPSI *both* local metabolite ratios in irregular volumes (such as large MS lesions) selected after image acquisition *and* global measures of metabolites without areas degrading spectral quality can be obtained in the same session (Mathiesen et al. 2004 A, Appendix 1).

The MR spectroscopic sequence used in this study has been designed by Hanson and co-workers in our department and described in detail elsewhere (Hanson 1999, Hanson et al. 2000). In this study, however, lipid signal nulling was provided by inversion recovery and water suppression was obtained using a 32 ms chemical shift selective RF pulse. Eight 10 mm axial slices covered most of the cerebrum with 1 ml isotropic voxels (see **Figure 1** in Mathiesen et al. 2004 A, Appendix 1).

Due to longer acquisition time and expected significant regional magnetic field inhomogeneities degrading the spectral quality in the lower infratentorial regions we concentrated our measurements to 8 slices although there are no technical obstacles in selecting 9, 10 or more slices. Relatively little extra brain tissue suitable for spectroscopy would be covered and the duration would increase in proportion to the number of slices. Furthermore, as already mentioned, the sensitivity of the FLAIR technique to detect MS pathology is lower in the infratentorial regions. The slice position was chosen so that each slice exactly corresponded to two 5 mm slices of the conventional imaging.

The measurements were preceded by positioning and fully automated global high order shimming and a non-suppressed single water reference signal acquisition for alignment and spectral processing. To increase the signal to noise ratio in the measurements of the metabolites, the sequence was repeated four times with two phase cycled acquisitions in each (Mathiesen et al. 2004 A, Appendix 1). The acquisition parameters were: TE/TR = 144/4300 ms, matrix 32 x 32. The total scan time was 20 minutes.

MRS findings in MS

In acute lesions at both long and short echo times Cho (and lactate) resonances increases early in the demyelinating process and this is followed by a decrease in NAA (Arnold et al. 1990, Larsson et al. 1991, Matthews et al. 1991). Spectra at short echo times give evidence for

transient increases in visible lipids (Larsson et al. 1991). Over a period of days or weeks the raised lactate declines to normal concentrations. Cho and lipids slowly return to baseline over months. NAA may remain decreased or show subsequent partial recovery thought to reflect reversible axonal dysfunction (Arnold et al. 1992, De Stefano et al. 1995 A).

The observation that the decrease in NAA in acute lesions was, to a variable extent, reversible was unanticipated. It was originally thought that the decrease in NAA represented wallerian degeneration only, and therefore irreversible changes were expected. However, transient decreases in NAA have been found in various conditions (Arnold 1999). Several reasons for NAA recovery have been proposed, including resolution of interstitial and intracellular oedema, increases in relative axonal volume in lesion voxels and finally, it may reflect reversible metabolic changes in neurons (De Stefano et al. 1995, Arnold et al. 1998).

MRS using NAA as a neuronal marker has emphasized the fact that substantial axonal damage may occurs in MS, in addition to demyelination. Although axonal injury and loss in and around MS lesions has been recognized since the first descriptions in the 19th century, the focus has been on demyelination explaining the functional impairments in MS. This is partly due to the fact that acute demyelination leads to conduction block (Arnold 1999). However, since sodium channels along the axon increase in density following demyelination (Waxman 1998) and demyelinated axons may function adequately after adaptation (Rivera-Quinones et al. 1998), demyelination alone cannot adequately explain the functional impairments in MS. Furthermore, partial remyelination might occur (Prineas et al. 1993).

NAA reductions outside the MS lesions (Larsson et al. 1991) have been shown in NAWM (Fu et al. 1998), cortical GM (Kapeller et al. 2001) and the whole brain (Gonen et al. 2000). The initial MRS studies of MS patients suggested a substantial damage to axons in WM (Arnold et al. 1990, Arnold et al. 1992, Arnold et al. 1994, Matthews et al. 1991, Davie et al. 1994, Husted et al. 1994). NAA in NAWM can decrease to 50% and in lesions to less than 20%, presumably reflecting different degrees of axonal damage or loss. These observations are in agreement of histopathologically studies in which loss of up to 50% of axons or more has been reported with considerably variations between lesions (Prineas & Connell 1978, Barnes et al. 1991).

Early in the course of MS the changes in cerebral NAA are usually relatively focal and most of the brain has apparently normal levels of NAA (De Stefano et al 1995 A + B). However, NAA in NAWM can be abnormally low in the very earliest stages of MS, even before significant clinical disability is evident (De Stefano et al. 2001) and before the final diagnosis is made (Tourbah et al. 1999), and whole-brain NAA (WBNAA) reductions has also been demonstrated in patients with CIS (Filippi et al. 2003), However, early NAA decreases in NAWM is not always seen (Brex et al. 1999), and Tourbah and co-workers only found NAA decreases in 17% of their patients (Tourbah et al. 1999).

A progressive decrease in NAA/Cr in NAWM over a period of 18 months has been demonstrated in MS patients (Arnold et al. 1994). This was confirmed in a larger group of patients, although the rate of decrease of NAA in the larger group was slower (De Stefano et al.

1998, Fu et al. 1998). It has also been shown that patients with higher EDSS had significantly lower brain NAA concentrations than normal controls and patients with lower EDSS scores showed smaller decreases of NAA or no differences from the controls (Arnold et al. 1990, Matthews et al. 1991, Larsson et al. 1991). NAA loss in MS lesions correlates to clinical disability (De Stefano et al. 1995 B), and changes in NAA in NAWM correlated better with disability than NAA changes in MS lesions (Fu et al. 1998). Furthermore, correlation between changes in regional NAA/Cr ratios and changes in the EDSS over time has been shown in a study of large central single-voxels (De Stefano et al. 1998). WBNAA was found to be lower in MS patients compared to healthy volunteers, especially with increasing age (Gonen et al. 2000), but no correlation was found between WBNAA and clinical disability measured by EDSS in a recent study (Bonneville et al. 2002). This could be explained by limitations in the EDSS score rather than lack of correlation between WBNAA and the true clinical status of the patients including cognitive deficits and fatigue which are poorly evaluated with EDSS.

WBNAA has been suggested as a marker of disease progression and treatment efficacy in MS and attempts to use WBNAA to differentiate between different evolutions of MS has been made using cross-sectional data (Gonen et al. 2002). However, true longitudinal studies using this technique are still missing.

Even though axonal loss or dysfunction are less severe in NAWM than in individual lesions, axonal loss or damage in NAWM may be proportionally more significant for clinical disability since NAWM constitutes the far greatest bulk of WM. Furthermore, cortical lesions known to exist from post-mortem studies but not visualised with conventional MRI, may play an important role in the development of cognitive and other deficits in MS. Thus, accounting for diffuse occult pathology in normal appearing brain may be very important to improve the correlation between disability and MRI. The focus of the work presented in this thesis has been to further develop and validate a spectroscopic method capable of providing information on global metabolic changes as well as localised spectroscopy from cortex, lesions and NAWM, and to implement this technique in a clinical setting of RRMS patients. We wanted to correlate the spectroscopic finding with clinical measures (EDSS, neuropsychological testing) and conventional MRI measures such as lesion volume, enhancing lesion volume, and atrophy.

MRS in non-MS cognitive research

Metabolic changes measured by MRS have been shown to correlate with the cognitive status or cognitive dysfunction in a range of neurological and psychiatric disorders as well as in normal subjects (reviewed in Ross & Sachdev 2004).

The basis for individual variations in cognitive ability is not well understood. The correlation between event-related potentials, brain size, cerebral metabolic rate or blood flow and cognition has not been strong (Vion-Dury et al. 1995). However, metabolites measured by MRS vary in direct relationship to cognitive performance. In healthy subjects correlations between metabolic

changes (e.g. NAA or NAA/Cr decreases) and cognition have been demonstrated in a number of studies. These studies were with few exceptions single-voxel studies measuring metabolites in GM, WM or both in various brain areas (i.e. cerebellar, frontal, temporal, and occipito-parietal areas) or the whole brain correlating to different neuropsychological measures such as working memory (Yeo et al. 2000), intelligence (Jung et al. 1999), visual memory (Buckley et al. 1994), executive-attentional tasks (Valenzuela et al. 2000) and recognition (Pfefferbaum et al. 1999 B).
Correlation between metabolic changes measured with MRS and cognitive deficits have also been described in a number of diseases. In traumatic brain injury (TBI) reductions in NAA/Cr correlated with the clinical outcome assessed by the Glasgow Outcome Scale and the overall performance on neuropsychological tests, especially verbal memory, attention and frontal processing (Friedman et al. 1999). In temporal lobe epilepsy NAA/Cr reductions have been shown to correlate with the frequency of seizures, and successful lobectomy lead to decreased seizure frequency as well as recovery of NAA/Cr and increases in cognitive performance (Jokeit & Ebner 1999). Patients with schizophrenia have reduced NAA/Cr especially in the prefrontal cortex. These changes are associated with cognitive deficits in working memory and attention (Bertolino et al. 1998). In AIDS Dementia Complex reduced NAA/Cr is detected in most brain regions (Barker et al. 1995) compared to normal subjects and AIDS patients without cognitive impairment. In Alzheimer's disease reductions in NAA are also seen (Adalsteinsson et al. 2000).

Cognitive dysfunction in MS and the correlation to MRI

Cognitive impairment occurs in approximately 50% of MS patients (Peyser et al. 1980, Heaton et al. 1985, Ron et al. 1991, Fischer et al. 2000), with somewhat lower incidences in the early stages of the disease. The disability is able to profoundly disrupt social and occupational functioning (Rao et al. 1991, Amato et al. 2001, Schultheis et al. 2001).
There is no unambiguous pattern of cognitive deficits, but some cognitive functions are more frequently impaired than others, such as memory, information processing, attention, executive functions and verbal fluency (Amato et al. 1995), and the pathophysiology of neuropsychological deficits is not clear. The extend and the presence of MS lesions affecting the inter- and intra-hemispheric WM tracts connecting subcortical and cortical areas seem to be important factors, but other mechanisms, such as the involvement of normal appearing GM and WM, might also play a role. The overall relationship between cognitive test performance and MRI measures such as lesion load, atrophy, black holes, MTI, DWI, and MRS has been assessed. Results from clinical studies (Peyser et al. 1980, Heaton et al. 1985, Amato et al. 1995, Kujala et al. 1997) as well as MRI studies (Rao et al. 1989, Ron et al. 1991, Swirsky-Sacchetti et al. 1992, Arnett et al. 1994, Rovaris et al. 1998 + 1999, Comi et al. 1999, Foong et al. 1999, Fulton et al. 1999, Pan et al. 2001) support the concept that MS cognitive dysfunction is related to the overall disease burden of the brain, although the correlation between T_2-weighted lesion load and cognitive dysfunction in MS is moderate most likely due to the poor pathological specificity of

conventional MRI. The extent of GM pathology is likely to be correlated with cognitive dysfunction, and an association has been found in some (Damian et al. 1994, Miki et al. 1998, Rovaris et al. 2000), but not in all studies (Catalaa et al. 1999). Given the difficulties assessing cortical lesions with conventional MRI this discrepancy is not unexpected.

The relations between lesion location and specific cognitive changes have also been assessed. However, the association between the dysfunction of specific cognitive domains and the corresponding regional lesion load remains controversial (Swirsky-Sacchetti et al. 1992, Arnett et al. 1994, Comi et al. 1999, Rovaris et al. 2002). The results have also been contradictory in short term serial studies assessing the relations between cognitive function and conventional MRI. Some studies have found increases in cognitive impairment following increases in lesion load; others did not (Miller et al. 1998). In a 4-5 year follow-up of patients with optic neuritis, an increase in impairment of attention was observed in patients with increased lesion burden progressing to MS (Feinstein et al. 1992).

Since a large degree of pathological changes seem to go undetected using conventional MRI, methods with higher pathological specificity are expected to provide better correlations between neuropsychological test performance and MRI. However, correlations between cognitive deficits and black holes and MTI are similar to that of T_2-weighted lesion load (Rovaris et al. 1998, Comi et al. 1999). In a DWI study, no significant difference between cognitively impaired and unimpaired patients were found in terms of diffusion tensor derived measures, and only moderate correlations between individual neuropsychological test scores and MRI and diffusion tensor quantities were found (Rovaris et al. 2002). Gadea and co-workers used MRS to demonstrate that axonal damage of the right locus coeruleus relates to selective attention impairment in early RRMS (Gadea et al. 2004). Other studies have suggested that NAA levels may be related to cognitive variables (Foong et al. 1999, Pan et al. 2001), and Christodoulou and colleagues found correlations between MRS measures and cognitive dysfunction in a single-slice multi-voxels study in a 2 cm thick slice through the posterior and anterior aspects of corpus callosum (Christodoulou et al. 2003).

No studies correlating global metabolic measures and cognitive impairment existed, so this was done in this thesis with the hypothesis that diffuse global axonal loss or dysfunction measured with multi-slice EPSI would correlate with cognitive impairment (Mathiesen et al. 2004 B, Appendix 2).

Cognitive tests and the Cognitive Dysfunction Factor

All patients completed a battery of 18 neuropsychological tests at baseline, and after approximately two years of follow-up. These test resulted in 29 neuropsychological measures (see **Table 2** in Mathiesen et al. 2004 B, Appendix 2) covering a broad range of cognitive functions, such as abstract thinking, mental processing/speed, working memory, attention,

general memory, visuospatial memory, naming and verbal retrieval. The following tests were performed:

1. Wechsler Adult Intelligence Scale Similarities (Wechsler 1955).
2. Raven Progressive Matrices (Raven 1960).
3. Digits Forward (Spreen & Benton 1969, Mortensen & Gade 1993).
4. Digits Backward (Spreen & Benton 1969, Mortensen & Gade 1993).
5. Arithmetic (Levin 1979).
6. Serial Sevens Subtraction Test (Smith 1967).
7. Stroop (MacLeod 1991).
8. List Learning, 10 words (Buschke & Fuld 1974).
9. Spatial Recall Test (Rao et al. 1984).
10. Boston Naming Test (Kaplan et al. 1983).
11. Naming of Famous Faces, Danish version (Rune 1998).
12. Controlled Oral Word Association Test (Benton & de Hamsher 1976).
13. Symbol Digit Modalities Test (Smith 1973).
14. Tower of London (Shallice 1982).
15. Design Fluency (Regard et al. 1982).
16. Rey Complex Figure (Lezak 1995).
17. Mesulam Cancellation Test (Mesulam 1985).
18. Street Gestalt Completion Test (Gade et al. 1988).

Based on a normal Danish control group (n = 75) and with age, sex and education as independent variables in a regression analysis, all neuropsychological test scores were normalized and converted to T-scores (mean = 50, SD = 10). The 16 measures which best distinguished between MS patients and normal controls (see **Table 2** in Mathiesen et al. 2004 B, Appendix 2) were used to construct a general Cognitive Dysfunction Factor.

Results placed in context

A significant part of this PhD study was dedicated to the validation and implementation of a new MR spectroscopic method developed in our department by Hanson and colleagues (Hanson 1999, Hanson et al. 2000). Using this technique it has been shown that global metabolite ratios can be obtained and in the same session measurements of metabolites in specific brain areas (e.g. NAWM, lesions, cortical GM or other specific areas) chosen after image acquisition (Mathiesen et al. 2004 A, Appendix 1). A small reproducibility study of seven healthy control persons demonstrated a low within-subject variance which was promising for longitudinal studies of MS. This is wanted in the MS research since whole-brain measurements of NAA has been suggested as a measure of global neuronal cell loss and a marker of disease progression

and treatment efficacy in MS. However, no longitudinal studies using Gonen and colleagues (Gonen et al. 2000) more conventional measures of WBNAA exist. The evaluation of metabolic changes and hence neuronal viability in specific regions of the brain might be very important in assessing evolution and prognosis of MS and needs further investigation, especially measuring possible changes in cerebral cortex known to contain lesions which goes undetected with conventional T_1- and T_2-weighted imaging. However, this possibility is lost to the global average using non-localized spectroscopy.

The ability to select large (global) as well as small (less than 1 ml) irregular areas of interest after image acquisition is an advantage compared to traditional single-voxel spectroscopy where the ROI's are selected during the scan. Furthermore these volumes are more likely to include different types of tissue and CSF due to their boxlike shape. Inversion recovery used to get rid of lipid in multi-slice EPSI reduces the signal-to-noise ratio 20-30% (Hanson 1999), resulting in a more noisy spectrum if only one 1 ml voxel is analysed, as shown earlier by Hanson and colleagues (Hanson et al. 2000). However, the analysis was not performed on single-voxel spectra, and the reduced signal-to-noise ratio can be compensated for by pooling many voxels (or increasing the acquisition time).

In this study we were able to get pooled spectra from all lesions as well as irregular volumes of approximately 35 ml of juxtacortical and periventricular NAWM. A border of 1 cm at the surface of the brain to assess cortical metabolic changes and a large "whole-brain" volume of approximately 60% of the brain parenchyma was selected. Areas not suitable for spectroscopic evaluation because of poor shim and CSF (e.g. frontal areas and areas near the basis of the skull) were excluded leading to improvement of quality and reproducibility. The spectra obtained with this technique are of high quality (global, NAWM, lesion, cortex). The main metabolites NAA, Cho and Cr are clearly visible, and the peaks are well separated and with narrow line widths (see **Figure 1** + **Figure 2** in Mathiesen et al. 2004 A, Appendix 1). The metabolite images are also shown (see **Figure 3** in Mathiesen et al. 2004 A, Appendix 1). Artefacts seen in front of the brain in the metabolite images result from residual water combined with movement (e.g. tongue and eyes). Artefacts due to residual lipids appear subcutaneously. In poorly shimmed regions, e.g. near the basis of the cranium (bone and air in the ear canal) more noise is to be expected. The exact origin of this has not been identified. It is likely to be a consequence of strong inhomogeneity or flow known to cause artefacts in the same regions of other imaging sequences. However, it only appears outside of the brain mask, and might be neglected in this context.

In agreement with earlier findings lesion NAA/Cr was significant lower than NAWM NAA/Cr (e.g. Larsson et al. 1991). However, no significant differences in NAWM NAA/Cr were found between healthy control persons and the patients. Cortex Cho/Cr was decreased in MS versus controls, but no significant differences in NAA/Cr were found. In global spectroscopy we found large variations in NAA/Cr and Cho/Cr within the healthy controls and the MS group (see **Figure 4 A** in Mathiesen et al. 2004 A, Appendix 1). We found no differences in global NAA/Cr between

healthy controls persons and the patients, and global Cho/Cr was decreased in the MS group. This is not in agreement with the literature. NAA is expected to be decreased, even in the earliest stages of MS (Fu et al. 1998, De Stefano et al. 1998, Tourbah et al. 1999, Filippi et al. 2003). Differences in the patient populations might at least to some extend explain this. In our study (Mathiesen et al. 2004 A, Appendix 1) only relatively mildly disabled RRMS patients were included (disease duration < 5 years, mean EDSS = 2.5). Gonen and co-workers included patients with disease duration of at least 5 years in their study (Gonen et al. 2000), and perhaps more importantly, only approximately 40% of their patients received immunomodulatory treatment. In our study 75% of the patients received treatment, and it has been shown that treatment with interferons can lead to increases in NAA/Cr (Narayanan et al. 2001), although it has been shown that this is not always the case in a more recent study (Parry et al. 2003). However, immunomodulatory treatment might explain some of the discrepancies also seen in other studies concerning metabolite measures. Other explanations could be related to differences in the techniques used and regions selected, or expected variations in the metabolites and their relaxation times between brain areas, and variations depending on atrophy and age (Christiansen et al. 1993, Kreis et al. 1993, Pfefferbaum et al. 1999 A). Our exclusion of infratentorial, frontal and temporal areas may influence the results. To minimize the effect of regional differences on the comparison between healthy controls and MS patients, the same areas were carefully selected for evaluation in the global measurements. And in the follow-up the exact same brain masks were reused after alignment. To facilitate the use of multi-slice EPSI in clinical settings, the data analysis can be fully automated aligning the data to standard brain templates with standard brain volumes of interest before calculating the metabolites. If this was done, this technique could become a simple tool to evaluate global metabolic changes.

Multi-slice EPSI was used in a cross-sectional study of 20 RRMS patients to test the hypothesis that global NAA/Cr correlates with the global measure of cognitive dysfunction, the Cognitive Dysfunction Factor (Mathiesen et al. 2004 B, Appendix 2). All neuropsychological tests and primary analysis of the data, i.e. calculations of T-scores and comparisons with a healthy Danish control group (N = 75), as well as the design of the Cognitive Dysfunction Factor were performed by neuropsychologist Agnete Jonsson and Jente Andresen before further analysis and calculations of correlations to MRI data. On only one test score, the Rey Complex Figure (time to copy), the patient group performed significantly better ($p = 0.01$) than the normal controls indicating that they work fast, and unconcerned about the number of errors made. The mean of the Cognitive Dysfunction Factor were significantly decreased in the MS group ($p = 0.002$) compared to the normal control group.

When correlating the Cognitive Dysfunction Factor with global NAA/Cr we found a significant correlation between the two measures ($r = 0.70$, $p = 0.0006$; see **Figure 2** in Mathiesen et al. 2004 B, Appendix 2). To rule out systematic effects of atrophy (BPF), age, disability (EDSS), treatment, and educational level, inclusion of these parameters in the model was tested in a

multiple regression analysis using forward stepwise regression. None of these parameters correlated to the Cognitive Dysfunction Factor individually or improved the significance of the simplest model (Mathiesen et al. 2004 B, Appendix 2). We also correlated the data from the Cognitive Dysfunction Factor with conventional MRI measures. No significant correlations were found to T_2-weighted (FLAIR) lesion volume or contrast enhancing lesion volume. However, the Cognitive Dysfunction Factor correlated with the total volume of T_1-weighted hypointense lesions ($r = -0.473$, $p = 0.35$), which also might be expected to provide the best correlate to the overall disease burden.

Correlating the Cognitive Dysfunction Factor to other MRS measures, we found a significant correlation to cortex NAA/Cr ($r = 0.511$, $p = 0.03$), but no correlations to other local measures (NAWM and lesion metabolite ratios) or to any Cho/Cr ratios. We also analysed the correlation between global NAA/Cr and the Cognitive Dysfunction Factor in a 2x2 frequency table (see **Table 3** in Mathiesen et al. 2004 B, Appendix 2) defining a value of 40 (mean T-score of healthy controls minus 1 SD) to separate cognitively impaired patients from non-impaired patients. A global NAA/Cr value of 1.5 seemed able to distinguish impaired from non-impaired patients (Fisher's Exact Test, one-tailed $p = 0.005$). These data needs to be confirmed in larger scale. Of the 18 healthy control persons included in the MRI part of this study, only one had a global NAA/Cr significantly below 1.5 (see **Figure 4 A** in Mathiesen et al. 2004 A, Appendix 1). However, we do not know the mental capacity for this person, or any of the healthy control persons MRI scanned, since none of them were tested neuropsychologically. The multi-slice EPSI technique was validated on a group of healthy control which was not tested neuropsychologically and the neuropsychological data were normalized and converted to T-scores based on an existing normal Danish control group not MRI scanned. These decisions were mainly forced by lacks of capacity not permitting the MRI scanned control group also to be neuropsychologically evaluated. Although global NAA/Cr seems to be able to screen potentially cognitive impaired MS patients, the data urgently needs confirmation in larger scale including MRI and neuropsychological evaluation in both MS patients and healthy controls.

Multi-slice EPSI may also be a useful tool for detecting relationships between metabolic changes in specific areas of the brain and dysfunction in specific domains. However, more rigid study designs and data analysis as well as larger patient populations are also needed to show such relationships.

The data reported in this thesis suggest implementing multi-slice EPSI in longitudinal studies to evaluate the true value of the technique in predicting and assessing cognitive impairment in MS. If not able to predict the evolution in individual patients the method might still be proven of significant value in treatment trials assessing whether the tested treatment can prevent cognitive deterioration. As mentioned in the method section the patients and the MRI scanned controls were followed with repeated scans, and it was planned to correlate the cognitive changes and the global NAA/Cr as well as other MRI measures after two years of follow-up. During the final neuropsychological follow-ups, preliminary data analysis revealed that the

patients in this population seemed to *improve* their performance in various neuropsychological tests. Even taking potential positive effects of treatment into account, this is unlikely to be a result of genuine normalisation and was interpreted as a result of uncontrolled test-retest effects of some of the included neuropsychological tests. The research group therefore decided not to proceed with the data analysis before the test-retest effects measuring the Cognitive Dysfunction Factor has been evaluated in a healthy control group.

Unfortunately, a very late discovery of these limitations in the study design left no time for other approaches in the data analysis to be accomplished within the time limits of this thesis. One track to be followed is the description of the longitudinal global metabolic changes. Changes in WBNAA have been estimated from cross-sectional data pointing out the need for true information on the longitudinal changes in global brain metabolites.

Discussion

This thesis is yet another contribution among a large number of studies in the search for methods with higher specificity leading to faster diagnosis, more precise measures of activity and better assessments of the prognosis in MS. Despite the development of a number of new techniques with higher specificity to the pathological heterogeneity in MS lesions and the ability to detect pathology in normal appearing brain tissue, the use in clinical settings of techniques such as fMRI (functional MRI), DWI, MTI and MRS are very limited due to standardisation and reproducibility difficulties. New MRI techniques have been reviewed in Danish (Mathiesen et al. 2002 B, Appendix 4) and the use of conventional T_2- and T_1-weighted imaging have also been reviewed (Mathiesen et al. 2002 A, Appendix 3). Conventional T_2- and T_1-weighted imaging is still the golden standard in the diagnosis, and measuring disease activity and treatment efficacy in MS, despite the relatively weak correlations between conventional MRI and the clinical status of the patients. Limitations perhaps due to inaccuracies in measuring lesion load, studying patients with widely different disease duration or treatments, relatively short follow-up periods in longitudinal studies, lack of spinal imaging and not taking lesion location into account as well as limitations in the assessments of disability. The use of conventional MRI and non-conventional techniques in the diagnosis and assessing disease activity and treatment efficacy will be discussed in the following, as well as what is needed to be able to use multi-slice EPSI in clinical settings in the future. There are a need for standardisation even of the conventional techniques both in daily clinical practise and in clinical trials to facilitate assessment of the evolution of the disease and the comparison of the findings between scans and different centres. This is especially the case for the non-conventional techniques.

Since the first MRI scans were performed on MS patients in the early 1980s (Young et al. 1981), it has become the most important paraclinical technique in the diagnosis and monitoring the efficacy of new treatments in clinical trials in MS (Miller et al. 1998). Disease modifying

treatments reducing relapse frequency and increasing the interval between relapses, and their potential to be effective in the very earliest stages of MS (Jacobs et al. 2000, Comi et al. 2001) have made it very important to identify the patients who might benefit from an early treatment.
Revised diagnostic criteria were presented by the International Panel on MS Diagnosis in 2001 (McDonald et al. 2001), still focusing on demonstrating dissemination of the disease in both time and space. MRI was integrated with clinical and other paraclinical measures (positive CSF and abnormal VEP). CSF analysis provides supportive evidence of the immune and inflammatory nature of lesions, but they cannot provide information about dissemination in time or space. They are, however, helpful when MRI criteria are not fulfilled or MRI of the brain lack specificity, e.g. in older patients. A positive CSF is seen when oligoclonale IgG bands can be demonstrated in CSF (and not in serum) or in case of elevated IgG index. An abnormal VEP typical of MS is a VEP with delayed but well-preserved wave form.
The outcomes of the diagnostic criteria are "MS" or "not MS", and "possible MS" where more tests or new relapses are needed to make the diagnosis. These new criteria facilitates the diagnosis of different presentations, such as monosymptomatic presentation in CIS, or presentations with insidious neurological progression suggestive of primary progressive MS (PPMS) or a more typical relapsing remitting course.

MRI in the diagnosis of MS

The International Panel on MS Diagnosis selected the modified Barkhof MRI criteria (Barkhof et al. 1997, Tintoré et al. 2000, McDonald et al. 2001, Barkhof et al. 2003) because the sensitivity was acceptable and the specificity and accuracy was greater than other MRI criteria for MS proposed by Fazekas and Paty and their co-workers (Fazekas et al. 1988, Paty et al. 1988). Positive MRI criteria for brain abnormality showing dissemination in space require 3 out of 4 of the following:

1) One gadolinium enhancing lesion **or** nineT_2 hyperintense lesions.
2) At least one infratentorial lesion.
3) At least one juxtacortical lesion.
4) At least three periventricular lesions.

Dissemination of the disease in time can be demonstrated paraclinically by a gadolinium enhancing lesion in a scan done at least 3 months following onset of the clinical attack, since it is known that lesion enhancement in most cases lasts for 2-8 weeks (Barkhof & van Walderveen 1999). In the absence of gadolinium enhancing lesions at the 3 month follow-up, dissemination in time can be demonstrated by an enhancing lesion or a new T_2-weighted lesion on a new follow-up scan after additional 3 months (McDonald et al. 2001).
In the diagnostic criteria for MS it was not addressed exactly how to perform the MRI. From the MR criteria it is obvious that T_2-weighted imaging and gadolinium enhanced imaging of the brain are needed. However, numerous factors may influence the outcome, such as field strength,

slice thickness, T_2-weighted technique (i.e. FLAIR versus CSE), contrast dose and delay. Choices which all may impact the number of lesions detected. In case of few lesions, this might at least theoretically influence whether MRI and hence the diagnosis is positive or not, potentially leading to delayed diagnosis and missed early treatment or treatment of non-MS cases. No data exist to assess the differences in specific settings of e.g. 1 T, single dose of gadolinium, 5 mm slices compared to 3 T, 3 mm slices conventional T_2-weighted imaging + FLAIR, and double or triple dose of gadolinium. In a comparative study of 30 patients with optic neuritis we found that additional two patients fulfilled the MR criteria of Barkhof and co-workers (Barkhof et al. 1997, McDonald et al. 2001) going from 1.5 to 3 T based on an increased sensitivity in detecting T_2-weighted lesions at 3 T (unpublished work in our department by MD, Kirsten Nielsen and colleagues).

In a consensus report, the White Matter Study Group (WMSG) of the International Society for Magnetic Resonance in Medicine recently addressed how to perform MRI in the diagnosis of MS and how to measure MS activity accurately and reliably, and how to monitor the effect of treatment on disease evolution (Filippi et al. 2002).

MRI of the brain should be performed as soon as possible in all patients suspected as having MS or CIS. In case of possible steroid treatment, it is best to perform the MRI before the treatment, because steroid treatment reduces the number of enhancing lesions (Miller et al. 1992). The WMSG suggested the following MRI acquisition protocol:

Axial dual-echo conventional or fast spin echo.
Injection of gadolinium-DTPA in standard dose (0.1 mmol/kg).
Sagittal fast FLAIR.
Axial T_1-weighted CSE.

The specification of the acquisition parameters was suggested to follow the guidelines proposed by Fazekas and co-workers, including imaging at minimum 1 T (facilitating detection of small lesions), maximal slice thickness of 5 mm with an inter-slice gap of 10% or less. Axial slices should be obtained perpendicular to the interhemispheric fissure and parallel to a well defined line, e.g. a line connecting the lower borders of the genu and splenium of the corpus callusum (Fazekas et al. 1999).

This protocol should be repeated in the exact same fashion in case dissemination in time needs to be demonstrated according to the McDonald criteria and in follow-up in clinical trials. Gadolinium chelates in the standard dose of 0.1 mmol/kg body weight after a minimum time interval of 5 minutes suffice for diagnostic MRI, although it has been demonstrated that more lesions can be detected after double or triple dose (Filippi et al. 1998 A, Gasperini et al. 2000). The cost of this is not justified in clinical settings (Filippi et al. 2002). These recommendations are largely in agreement with the recommendations we reached in our Danish review (Mathiesen et al. 2002 A, Appendix 3).

Some advantages and limitations of the McDonald criteria have been discussed by Miller and colleagues (Miller et al. 2004) who points out that the MRI criteria were derived from relatively small cohorts of CIS patients with limited follow-up periods, not representative for all MS patients at presentation. The specificity of the criteria to distinguish MS from other diseases with clinical or radiological similarities were not addressed, and the use of MRI was suboptimal since the incorporation of spinal cord findings is incomplete and only conventional MRI findings are considered including the potential higher sensitivity of high field strengths as already mentioned.

Measuring MS activity with MRI

Conventional MRI with T_2-weighted images and gadolinium enhanced T_1-weighted images provide good means to determine the disease activity since new lesions are seen up to 10 times as often as clinical attacks (Harris et al. 1991, Barkhof et al. 1992), and these measures are still the golden standard in MS (Filippi et al. 2002). What is measured, however, is whether the water content of a lesion rendered it visible and whether the BBB breakdown is sufficient to be detected. Disease activity involves inflammation, tissue destruction, demyelination, axonal loss or dysfunction, remyelination and astrogliosis, and we get no information on this using conventional MRI. The lack of pathological specificity and the failure to detect activity beyond a given threshold in normal appearing GM and WM might to some extend explain the limited correlation between conventional MRI and clinical disability, measured by the EDSS (Filippi et al. 1995 C). The extend of undetected disease burden may be clinically significant (Filippi et al. 1999), since the total lesion volume often accounts for less than a few percent of the total brain volume (e.g. Mathiesen et al. 2004 A, Appendix 1). Other reasons for the limited correlation between conventional MRI and disability could be the lack of accounting for lesion location and the lack of spinal cord imaging, as well as limitations in the EDSS. EDSS is heavily weighted toward motor dysfunction and does not fully account for cognitive functions and fatigue.

Other measures using conventional MRI is brain volume measurements and other atrophy measures (Miller et al. 2001) and T_1-weighted hypointense lesions (Barkhof et al. 1998 A). Brain volume fraction and other atrophy measures are difficult to interpret since they are non-specific. They measure the end result of many pathological processes, and it is unknown whether demyelination or axonal loss plays the most important role. Atrophy measures do not reflect disease activity, nor do they provide information on specific pathology. However, atrophy is worth measuring, like clinical disability, as an endpoint, since progressive atrophy provides a global assessment for the neurodegenerative process (Filippi et al. 2002).

Hypointense lesion load on T_1-weighted images seems to correlate better to EDSS than T_2-weighted lesion load (van Walderveen et al. 1995, Truyen et al. 1996). However, there has been no shift from T_2-weighted lesions to T_1-weighted hypointense lesions in clinical settings, and it was still not recommended by the WMSG (Filippi et al. 2002). It has been argued that these lesions represents tissue loss (van Walderveen et al. 1998), but oedema might also

appear hypointense on T_1-weighted images, and DWI and MRS has demonstrated structural and metabolic variations in black holes of comparable intensity (Filippi et al. 2001).

To get better measures of MS activity, higher sensitivity and specificity are needed, and new techniques need to be highly reproducible and have a low intra- and interobserver variability. Non-conventional MRI such as MTI, DWI and MRS offers increased specificity (Rovaris & Filippi 1999), and these techniques can focus on specific markers of injury, and they have all been able to demonstrate pathology in normal appearing brain (reviewed in Danish in Mathiesen et al. 2002 B, Appendix 4). Despite of the many qualities, severe problems in standardisation and reproducibility has hampered the introduction of these techniques in routine clinical settings, and measuring MS activity still relies on conventional T_2- and T_1-weighted imaging.

MRI in initiating treatment and monitoring the effect

In individual patients when starting treatment, MRI should be performed according to the diagnostic criteria of McDonald (McDonald et al. 2001). MRI seems to have the highest prognostic value in the early stages of the disease in CIS (Barkhof et al. 1997, Sailer et al. 1999, Brex et al. 2002, Barkhof et al. 2003), and gadolinium enhancing lesions is probably the finding that argues most strongly in favour of treatment, since enhancing lesions reflects ongoing inflammation and active disease (Katz et al. 1993, Filippi et al. 2002). Total T_2 lesion load must also be considered, since the total lesion load in the earliest stages of MS has been shown to have prognostic significance (Filippi et al. 1994, Sailer et al. 1999, Brex et al. 2002), however, no specific volume or number of lesions was suggested to point out when to treat by the WMSG (Filippi et al. 2002). Hypointense lesions on T_1-weighted imaging early in the course of MS might be related to worse prognosis, but data are needed to evaluate the value of black holes in selecting patients for treatment (Filippi et al. 2002).

A number of clinical trials dealing with the treatment in the earliest stage of the disease has been done (Jacobs et al. 2000, Comi et al. 2001), and evaluations of new treatment combinations are still going on. However, little is known on how to select patients with CIS who might benefit more from early treatment and whether there are long term advantages in starting treatment when the patients have CIS rather than established RRMS. It has recently been shown that the treatment effect seemed more evident as the number of positive MRI criteria for MS increased (Barkhof et al. 2003).

The cost and effect of the available treatments, as well as the need to avoid treatment of patients who will not develop MS as well as patients with long-term inactive MS, forces us to address this problem further. This is one of the reasons to search for MRI techniques with better prognostic value in MS. In the meantime the WMSG (Filippi et al. 2002) suggests that patients fulfilling the MR criteria of Barkhof and co-workers (Barkhof et al. 1997, McDonald et al. 2001) at the baseline MRI but with no second clinical attack must be followed with repeated MRI after 3 and 6 months to decide whether to treat. In case the patients show dissemination in time (i.e.

convert to MS according to the McDonald criteria, the patients are then considered treatable (Filippi et al. 2002). Considering the costs of 6 months treatment with interferon beta, the costs of an additional MRI is negligible. Furthermore, this advice will prevent treatment of non MS cases. As pointed out in the consensus report, these criteria are empirical and evidence-based data from prolonged follow-up of treated and untreated CIS patients are needed.

Data to support MRI monitoring of MS patients on therapy is also needed, since no MRI criteria for treatment failure exist. We anticipate that new lesions will appear in clinical stable patients and despite effective treatment with interferon beta. So far, follow-up MRI is not recommended unless new symptoms suggestive of other disease are seen (Mathiesen et al. 2002 A, Appendix 3). A more important issue to consider in this context might be the presence of neutralising antibodies against interferon beta (Sorensen et al. 2003). We found a significantly higher MR activity in RRMS patients with high levels of antibodies compared to patients with few or no antibodies (Sorensen et al. 2004).

In clinical trials lesions on T_2-weighted MRI and gadolinium enhancing lesions on T_1-weighted images are the primary outcomes in phase II trials but limited to a secondary outcome in phase III trials, given uncertainty in predicting clinical benefit and the needs for safety data in the longer clinical trials. Conventional MRI must be performed in all clinical subgroups, and in PPMS trials additional atrophy measures are needed. Non-conventional MRI, such as MTI, DWI and MRS, is still regarded experimental. However, these techniques are worth incorporating in patient subgroups in order to learn more about the natural history and treatment effect, but not as primary MR outcomes (Filippi et al. 2002).

Multi-slice EPSI in the diagnosis, or monitoring activity and treatment?

As reviewed above and concluded by the WMSG there is yet no place for MRS in routine clinical settings. The diagnosis and the exclusion of other diagnosis can be reached with conventional T_2-weighted and gadolinium enhanced T_1-weighted imaging. And non-conventional MRI has little relevance in measuring disease activity and monitoring treatment (Filippi et al. 2002). However, very little is known about the ability of existing treatment regimes to prevent neuronal loss (e.g. Narayanan et al. 2001) and increase remyelination. Methods with the ability to assess this might be of great importance in measuring long term efficacy of MS treatment. A number of non-conventional methods have proven potential, but work is still needed to optimise and validate and to standardise across MRI centres and field strengths. Multi-slice EPSI is among the candidates that deserve more attention since this method can provide localised spectroscopy and also reproducible global assessments of metabolic changes in MS as we have shown (Mathiesen et al. 2004 A, Appendix 1).

Early metabolic changes in specific, including not yet studied, areas of the brain might be important predicting the course of the disease as well as global measurements. In our studies global NAA/Cr seems able to screen potentially cognitive impaired MS patients. And our data suggested implementing multi-slice EPSI in a larger scale and in longitudinal studies to evaluate

the true value of the technique in predicting the disease evolution and assess cognitive impairment in MS (Mathiesen et al. 2004 B, Appendix 2). If not able to predict the evolution in individual patients because of large variations of individual baseline metabolic and cognitive status, the method might still be proven of significant value in treatment trials assessing in a simple way whether the tested treatments can prevent cognitive deterioration or preserve NAA.

Metabolic changes including reductions in NAA or NAA/Cr have been shown to correlate with the cognitive status or cognitive dysfunction in normal subjects as well as in a range of neurological and psychiatric disorders, such as TBI, temporal lobe epilepsy, schizophrenia, AIDS dementia complex, Alzheimer's disease and MS (reviewed in Ross & Sachdev 2004). In MS the correlations between cognitive impairment and other MRI measures (lesion load, atrophy, black holes, MTI, and DWI) have been moderate, and only a few MRS studies exist (Foong et al. 1999, Pan et al. 2001, Christodoulou et al. 2003, Gadea et al. 2004). We have reported the first correlations between global metabolic measures and cognitive impairment (Mathiesen et al. 2004 B, Appendix 2).

This, however, does not clearly indicate causality between global diffuse NAA losses and cognitive impairment. Primarily because we measure ratios, and the role of variations in metabolite relaxation times is unknown in these estimates. Measuring relaxation times is too time consuming in clinical settings. The advantages of measuring ratios have been discussed in details elsewhere (Mathiesen et al. 2004 A, Appendix 1). On the other hand, possible mechanisms that could link cognitive performance and global NAA or NAA/Cr measures do exist (Ross & Sachdev 2004):

1) Neuronal death.
2) Decreased neuronal metabolism.
3) Reduced area of dendritic arborisation.
4) Reduced myelin.

Since NAA only exist in neurons once the CNS is fully matured (Simmons et al. 1991), global loss of NAA may represent neuronal death which will lead to cognitive deficits once the limits of brain plasticity are exceeded. Greater numbers of neurons may be associated with higher intelligence or better performance on neuropsychological tests, and larger neurons with greater conduction speed may contain more NAA. NAA losses could also represent decreased neuronal metabolism, since NAA synthesis occurs in the mitochondria and is coupled to mitochondrial ATP production and oxygen consumption (Bates et al. 1996) suggesting an association between NAA concentration and metabolic efficiency (Taylor et al. 1995). Furthermore, reductions in the area of dendritic arborisation may lead to decreases in NAA, and higher NAA levels may indicate greater extend of dendritic arborisation (Yeo et al. 2000) which has been related to information processing capacity (Scheibel et al. 1990). Finally NAA may be

important in myelin synthesis and/or maintenance (Chakraborty et al. 2001), and reduced myelin thickness may lead to decreased transmission speed.

Conclusions

A lot of efforts have been done to invent and develop MR techniques with higher specificity for pathological changes, evolution, and hence the prognosis of MS. Despite this, the diagnosis and follow-up in treatment trials still rely on conventional T_2- and T_1-weighted imaging. To obtain better measures of MS activity and evolution, new techniques with high sensitivity, specificity and reproducibility as well as low intra- and interobserver variability are needed. Techniques such as fMRI, DWI, MTI and MRS offers increased specificity but have remained research tools because of standardisation and reproducibility problems, or lack of availability and validation.

MRS is useful to evaluate where and when axonal loss or dysfunction takes place in the evolution of MS. Measuring metabolic changes in specific brain areas might have a prognostic value and global NAA measures has been suggested as a marker of disease progression. Longitudinal measures of WBNAA using conventional methods are still missing, and ROI's have to be selected during the scan in single-voxel studies.

We have presented a novel MRS technique with some advantages compared to conventional MRS methods. Global assessments can be performed in the same session as localized spectroscopy, and irregular ROI's can be selected after image acquisition.

Cognitive dysfunction can be seen in 50% of patients with MS, even in the early stages. Although the correlations between cognitive dysfunction and MRI measures have been weak to moderate, a substantial number of studies suggest that cognitive dysfunction in MS is related to the overall disease burden of the brain. To assess global pathology, including subtle pathological changes in NAWM and cortical GM, which generally goes undetected with conventional MRI using T_2- and T_1-weighted imaging, non-conventional MRI techniques are needed, such as MRS. In a cross-sectional study using multi-slice EPSI we found high correlations between global NAA/Cr and a general neuropsychological dysfunction measure suggesting that multi-slice EPSI measures can be used as a surrogate marker for cognitive impairment in early MS. If implemented in clinical trials this method might help to assess whether new treatments can prevent the development of cognitive impairment in MS.

To evaluate the true value of multi-slice EPSI, the technique needs to be implemented in a larger scale in rigidly defined patient populations. To be able to do this, work has to be done to standardise across centres, scanners and field strengths in future studies. The simplest first step could be to select centres with the same type of hardware as used in this thesis.

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