**Introduction**

MRI has been accepted as a form of paraclinical evidence in the Diagnostic Criteria for MS set up by Poser et al. [Poser, 1983]. These criteria are widely used by specialists and not only for research purposes to define clinically definite MS (laboratory-supported definite or CDMS) and probable MS (laboratory-supported probable or clinically probable MS). Multifocal white matter lesions are not specific for MS, and it has been necessary to develop criteria for the interpretation of MRI to increase diagnostic accuracy.

Our interest in MRI is as a selection basis for MRS investigations. A study comparing immunopathologic findings to MRI showed a decrease of NAA which corresponded to a reduction of axonal density [Bitsch, 1999]. This study also showed increases of Cho and mlns which corresponded to glial proliferation. This suggests that MRS might be a key to pathologic features of demyelinating lesions.

Other metabolites which have been investigated are Cr and Lac. The results in measurement of these metabolites have earlier been given as ratios, but like Helms we found it natural to use absolute quantification which makes intraindividual and intersubject comparisons easier and less likely to contain error [Helms, 1999, 2000; DeBeer, 1998].

In this study we have investigated NAWM in patients, who have CDMS and normal MRI scans of the brain. We have also investigated patients with syndromes suggestive of MS and normal MRI scans. The intention has been to assess if MRS is useful in detecting changes in the NAWM of these patients.

**Materials and Methods**

Patients were recruited from the Clinic of Neurology at the University Hospital in Linköping, Sweden. They were selected from those with the diagnosis of MS or clinically isolated syndromes suggestive of MS. They were reviewed regarding MRI and 21 with normal or near normal MRI scans of the brain were found. Eleven patients (mean age 38) were then included in the study. Our control group has largely been made up of medical staff from the University Hospital in Linköping. The 13 healthy volunteers had a mean age of 39 years.

MRI and MRS examinations were performed on a GE Signa Horizon Echospeed 1.5 T (v 5.8) MR scanner (General Electric, Milwaukee, Wisconsin, USA) using a standard quadrature head coil. Localization images were obtained to locate the position of four voxels (two frontal and two parietal) in NAWM of interest for the MRS examination. The MRI scans were also used for the evaluation of MS plaques, as the intention of this study was to include only those patients with normal or near normal MRI scans of the brain [Filipp, 1999]. Calibration of the Transmitter Gain optimal (TG) was performed using an external CuSO4 (2 mM) reference phantom [Helms, 1999]. The partial volume effects were estimated using a T2 based analysis.

PRESS (Probe-p) was used to obtain 1H-MRS spectra at 63.87 MHz, using TR = 6.0 s, TE = 35 ms, with voxel dimensions =17 x 17 x 17 mm3 (4.91 mL), collecting 64 transients. Water suppression was obtained using CHESS LCModel [Provencher, 1993] was used to obtain concentrations of individual metabolites (using a recently corrected NAA to NAAG relation), and the spectra were also corrected for eddy current artifacts. Data were re-calculated to reflect the tissue concentrations based on the water content in average white matter; 71% [Whittall, 1997]. Fourteen metabolites were analysed using the linear combination of model spectra approach [Provencher, 1993], and six of these were extracted for a more detailed analysis.

**Results**

The results for the patient group were divided into two groups; one with all the included patients and one with the patients who had CDMS. Of the eleven patients presently entered into the study one was excluded, (no longer fulfilling the criteria of normal white matter).

**GROUP COMPARISON** - There was a significantly lower concentration of Cho in the patients NAWM when compared to control WM (p = 0.0002, Student’s t-test / Mann-Whitney U-test). This result was consistent when running analysis on the patients with CDMS with or without the four patients with syndromes suggestive of MS.

An increase in mlns in patient NAWM was also statistically significant (p=0.01). The difference was increased when only CDMS patients were included.

Comparison of the other studied metabolites (Cr, Lac, NAA, and NAAG) did not show any significant differences between the patient NAWM and the control WM, except for Cr which was higher in the group containing all patients.

**TABLE** - The observed 95% range (t-test) of absolute tissue concentrations in NAWM (mM in aqueous fraction). Significance of difference compared to controls (if better than p=0.05) within brackets. Controls (13), patients (10), CDMS patients (6): Cho 2.06–2.22; 1.81–2.00 (<0.01); 1.82–2.09 (<0.01); Cr; 6.17–6.49; 6.38–6.78 (0.02); 6.21–6.61; mlns: 4.29–4.77; 4.69–5.18 (0.01); 4.77–5.45 (<0.01); Lac; 0.56–0.88; 0.61–1.12; 0.58–1.30; NAA: 9.21–9.75; 9.20–9.92; 8.80–9.88; NAA+NAAG; 12.73–13.55; 13.07–13.97; 12.53–13.65.

**Discussion**

myo-INOSITOL. This metabolite is considered to be a glial marker that is not present in neurons [Pouwels, 1998]. It is associated with pathological processes in lesions and NAWM in MS patients. Several studies have shown a significant increase in mlns in MS lesions [Bitsch, 1999]. One recent study has shown an increase of mlns in NAWM in patients with CDMS, but all had lesions on MRI [Pouwels, 1998]. Longitudinal studies have shown an increase in mlns in lesions, prior to a decrease in NAA; thus suggesting that membrane disruption occurs before neuronal impairment [Husted, 1994]. The increase of mlns that was found in our group of patients compared to controls could be an indication of pathological processes in the NAWM which would be expected, also in the absence of lesions.

CHOLINE. When studying MS lesions there is a described increase in the concentration of Cho. This has been found to correspond to glial proliferation and may reflect the breakdown of myelin membranes.

Previous studies of NAWM in MS patients has not shown any difference in the relative concentration of Cho compared with controls. Thus, our finding of decreased choline absolute concentrations in NAWM is unique, and it may reflect a redistribution of choline in this particular category of MS patients.

N-ACETYL-ASPARTATE. This is a marker used when neuronal loss is a major aspect of pathology, as it is considered unique to neurons and their processes in the mature human brain [Simmons, 1991]. Our study showed no significant difference in NAA when comparing the patients NAWM with the controls white matter. There was however a significant difference in the distribution of NAA+NAAG with higher values in parietal compared to frontal voxels in the controls. This has been shown in another study of healthy volunteers and was there related to an increase in NAAG as the distribution of NAA was rather homogeneous [Pouwels, 1997].

**Conclusions** - Significantly lower absolute choline concentrations, and higher myo-inositol concentrations were observed in MRI-negative CDMS patients. No significant change was observed in NAA or NAAG concentrations. In addition, a trend suggested increased lactate concentration in CDMS.

**References**