# Global N-acetyl aspartate correlates with cognitive dysfunction in multiple sclerosis

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**Background:** Whole-brain N-acetyl aspartate (NAA), a measure of neuronal viability, can be assessed by multi-slice echo planar spectroscopic imaging (EPSI).

**Objective:** To test the hypothesis that global brain NAA measures correlates with a general measure of cognitive dysfunction in multiple sclerosis (MS).

**Design:** Survey.

**Setting:** Research-oriented hospitals.

**Patients:** Twenty patients, 16 females and 4 males, with early relapsing remitting MS (RRMS). Disease duration less than 5 years from diagnosis. Mean age 36 years (range 22-48). Mean Expanded Disability Status Scale (EDSS) score 2.5 (range 0-4.5).

**Main Outcome Measures:** Correlation between global NAA/creatine (Cr) measures and a Cognitive Dysfunction Factor (CDF) including 16 measures (out of 29 test scores from 18 neuropsychological tests) which best distinguished MS patients from normal controls.

**Results:** Fourteen out of 15 non-impaired patients (CDF > 40) had a global NAA/Cr ≥ 1.5 compared to only one out of 5 impaired patients (CDF ≤40) (*P*=0.005). A strong correlation was found between global NAA/Cr and CDF (r=0.70, *P*=0.0006). Forward stepwise multiple regression analysis ruled out systematic effects of atrophy, age, EDSS, treatment and education.

**Conclusion:** Multi-slice EPSI can provide global metabolic measures which distinguish patients with and without cognitive dysfunction and correlate with global cognitive measures in MS. Additional validation, and standardisation of the technique are needed, and analysis in larger scale studies including healthy controls is suggested.

Cognitive impairment occurs in approximately 50% of MS patients,[1-3] with lower incidences in the early stages of the disease. Some cognitive functions are more frequently impaired than others, such as memory, information processing, attention, executive functions and verbal fluency.[1,4] The pathophysiology of the cognitive deficits is not clear. The presence of MS lesions affecting the inter- and intra-hemispheric white matter tracts connecting cortical areas seem to be important factors, but undetected pathological changes in normal appearing brain, might also play a relevant role. It is hypothesised that the overall cognitive dysfunction in MS is related to the overall disease burden of the brain. To assess pathology in grey and white matter which appears normal on conventional magnetic resonance imaging (MRI), techniques with higher pathological specificity such as MR spectroscopy (MRS) are needed. Multi-slice EPSI is a flexible and fast spectroscopic imaging method able to cover most of the brain rapidly and provide reproducible global as well as local metabolite measures.[5] Measurements of NAA provide information of neuronal loss or dysfunction. Few previous studies have dealt with MRS and cognitive dysfunction in MS. Gadea and colleagues demonstrated that axonal damage of the right locus coeruleus relates to selective attention impairment in early RRMS.[6] Other studies have suggested that focal NAA levels may relate to cognitive variables,[7,8] and Christodoulou and colleagues found correlations between metabolic measures and cognitive dysfunction in a single-slice multi-voxels study of a two cm thick slice through corpus callosum.[3] In the present study, we tested the hypothesis that global NAA/Cr correlates with a general measure of cognitive dysfunction in MS.

## Methods

Twenty patients, 16 females and 4 males, with newly diagnosed clinically definite RRMS were included (**Table 1**). The mean age was 36 years (range 22-48, SD=8) and the mean EDSS score was 2.5 (range 0-4.5, SD=1.1). All patients had disease durations since diagnosis less than 5 years. None of the patients had recent steroid treatment or relapse, upper limb impairment or visual deficits interfering with neuropsychological test performance, or contraindications to MRI. Fifteen patients received immuno-modulatory therapy. The study was approved by the local Ethics Committee (KF01-055/01).

Brain scans were obtained using a Siemens Vision 1.5 tesla whole-body scanner with a standard circular-polarized head coil. To assess the total lesion volume and the total intracranial volume, $T_2$-weighted images were obtained using fluid attenuated inversion recovery (FLAIR). Thirty 5 mm axial slices centred 10 mm above a transversal tangent plane at the top of the mesenchephalon covered the brain. Repetition time (TR)/inversion time (TI)/echo time (TE)=9000/2500/110 ms, 2 acquisitions, echo train length=11, and pixel size 0.9x0.9 $mm^2$. Scan time: 13 minutes. The contour of the brain including the subarachnoid space was outlined manually by an experienced observer with an intra-observer variance less than 1%, as well as the total $T_2$-weighted lesion volume (LV).

To determine the amount of grey and white matter a magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence was performed. TR/TE=11.4/4.4 ms, 1 acquisition, pixel size 0.98x0.98 $mm^2$. 250 axial slices of 1 mm. Scan time: 15 minutes. SPM2 segmentation (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London, UK) was used to assess the total volume of grey and white matter. The brain parenchymal fraction (BPF) was calculated dividing this volume with the total intracranial volume.

The multi-slice EPSI sequence and analysis used in this study is described in detail elsewhere.[5] TR/TE=4300/144 ms, matrix 32x32. Eight 10 mm axial slices covered most of the cerebrum with 1 ml isotropic voxels. Scan time: 20 minutes. Global NAA was calculated as a ratio relative to Cr to correct for cerebrospinal fluid (CSF) content, coil sensitivity variations and oedema. Brain parenchyma not suitable for evaluation because of poor shim or CSF, were excluded automatically.[5] Typically areas near the frontal and sphenoid sinuses, the nasal cavity and the inner ear were excluded, areas causing problems because of considerable magnetic field inhomogeneities (**Figure 1**). Manual editing added consistency in the choice of regions. The adapted brain mask covers approximately 60% of the brain parenchyma. However, exclusion of areas degrading spectra, improves quality and reproducibility.[5] The images were all evaluated by the same observer unaware of clinical and neuropsychological findings.

All patients completed a battery of 18 neuropsychological tests resulting in 29 neuropsychological measures covering a broad range of cognitive functions.[9-23] All

neuropsychological measure were normalized and converted to T-scores (mean=50, SD=10) based on a normal Danish control group (n=75) with age, sex and education as independent variables in a regression analysis. Educational levels: 1) primary education ($9^{th}$ grade), 2) semi-skilled worker, 3) skilled worker, 4) non-university higher education, 5) academic degree. CDF was constructed from the 16 measures which best distinguished MS patients from normal controls (**Table 2**). Patients with CDF ≤ 40 were classified as patients with cognitive dysfunction.

Statistical analysis of the main outcome measure was evaluated with a linear regression analysis and a two-tailed significance t-test. Categorical data were tested in a frequency table with Fisher's Exact Test. Correlations were considered significant at $P < 0.05$.

## Results

Patient data are shown (**Table 1**). As a group the patients performed significantly below the normal control group in eight of 29 neuropsychological measures (**Table 2**). The mean CDF was significantly decreased in the MS group (*P*=0.002). Cognitive impaired patients had significantly lower global NAA/Cr than non-impaired patients (**Table 3**). Fourteen out of 15 non-impaired patients had a global NAA/Cr ≥ 1.5, compared to one out of five patients with cognitive dysfunction (*P*=0.005). The mean global NAA/Cr was 1.55 (range 1.35-1.69, SD=0.10). A significant correlation was found between CDF and global NAA/Cr (r=0.70, *P*=0.0006) (**Figure 2**). To control for independent effects of BPF, age, EDSS, treatment and education, 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 CDF individually or improved the significance of the simplest model. The mean BPF was 0.89 (range 0.83-0.95, SD=0.03). No significant correlations were found between BPF and global NAA/Cr (r=0.04), LV (r=0.22) or EDSS (r=0.09), or between EDSS and global NAA/Cr (r=0.20) or LV (r=0.36).

## Comment

Cognitive impairment is common in MS, and able to profoundly disrupt social and occupational functioning.[4,24-26] Neuropsychological testing is costly and time consuming,

and simple ways to screen for and assess cognitive dysfunction are therefore wanted. Although the subject has been addressed in numerous projects, no unambiguous relationship exists between MRI and cognitive impairment. In previous studies, $T_2$-weighted lesion load have shown only rather weak correlations to neuropsychological measures, probably due to the lack of pathological specificity, and studies correlating lesion location or lesion volume in specific brain areas to specific cognitive deficits have been contradictory.[27] Brain plasticity and redundancy in the neural functional systems might confuse the interpretations and though lesion location is taken into account, it is not without significance whether oedema, demyelination, gliosis, or axonal dysfunction and loss dominate in each lesion. MRI techniques with known higher correlation to physical disability (EDSS), such as atrophy measures, magnetization transfer imaging, and $T_1$-weighted hypointense lesions have shown slightly better correlations with cognitive dysfunction.[28-30] Although the correlation between cognitive impairment and metabolic changes has been described in various neurological and neuropsychiatric disorders as well as in normal subjects,[31] the topic has gained relatively little attention in MS. However, MRS has the potential to improve the pathological specificity of MRI. With multi-slice EPSI, a novel MR spectroscopic method in the field of MS, information on metabolic changes, and hence neuronal loss or dysfunction, can be obtained from both local and global measurements.[5] Measurements of global or diffuse pathology might be important assessing the overall cognitive function of the patients as suggested by the high correlation found in this study. Assessment of relationships between the metabolism in specific brain areas and specific cognitive domains would require larger patient materials and rigorous definitions of which neuropsychological measures belong to specific cognitive domains.

No significant correlations were found between CDF and BPF, age, EDSS, treatment or education, possible due to the small sample size. Furthermore, the role of Cr or metabolite relaxation times in the metabolite ratio is unknown. The data support, that global NAA/Cr measures aspects of MS pathology, exemplified by cognitive dysfunction, independent of atrophy, age and clinical disability. The correlations found in this work does not account for causality. It also remains unknown in which degrees a low CDF

represents true decreased cognitive functioning due to MS or low native values, as well as a high CDF could conceal cognitive deterioration. However, our working hypothesis remains that global NAA/Cr measured by multi-slice EPSI does represent a measure of the neuron capacity of the brain, including possible neuronal death, decreased neuronal metabolism or reduced volume of dendrite arborisation, and therefore should be a rational choice when selecting a parameter for screening potentially cognitively impaired MS patients. The presented data needs confirmation in larger scale studies, including neuropsychological tests on healthy controls and in longitudinal studies evaluating the ability of multi-slice EPSI to assess true changes in the cognitive status of the patients. In conclusion, multi-slice EPSI using a standard brain template with a corresponding standard volume of interest might become a simple yet important tool in clinical trials of treatments for prevention of cognitive deterioration in MS patients.

## References


1. Rao SM, Leo GJ, Bernardin L, Unverzagt F. Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction. *Neurology.* 1991;41:685-691
2. Fischer JS, Priore RL, Jacobs LD, et al. Neuropsychological effects of interferon beta-1a in relapsing multiple sclerosis. Multiple Sclerosis Collaborative Research Group. *Ann Neurol.* 2000;48:885-892.
3. Christodoulou C, Krupp LB, Liang Z, et al. Cognitive performance and MR markers of cerebral injury in cognitively impaired MS patients. *Neurology.* 2003;60:1793-1798.
4. Amato MP, Ponziani G, Pracucci G, Bracco L, Siracusa G, Amaducci L. Cognitive impairment in early-onset multiple sclerosis. Pattern, predictors, and impact on everyday life in a 4-year follow-up. *Arch Neurol*. 1995;52:168-172.
5. Mathiesen HK, Tscherning T, Sorensen PS, et al. Multi-slice echo planar spectroscopic imaging provides both global and local metabolite measures in multiple sclerosis. *Magn Reson Med.* In press.
6. Gadea M, Martinez-Bisbal MC, Marti-Bonmati L, et al. Spectroscopic axonal damage of the right locus coeruleus relates to selective attention impairment in early stage relapsing-remitting multiple sclerosis. *Brain.* 2004;127:89-98.
7. Foong J, Rozewicz L, Davie CA, Thompson AJ, Miller DH, Ron MA. Correlates of executive function in multiple sclerosis: the use of magnetic resonance spectroscopy as an index of focal pathology. *J Neuropsychiatry Clin Neurosci.* 1999;11:45-50.
8. Pan JW, Krupp LB, Elkins LE, Coyle PK. Cognitive dysfunction lateralizes with NAA in multiple sclerosis. *Appl Neuropsychol.* 2001;8:155-160.
9. Wechsler D. Wechsler adult intelligence scale. Manual. New York: Psychological Corporation; 1955.
10. Raven JC. Guide to the standard progressive matrices. London: H. K. Lewis; 1960.
11. Mortensen EL, Gade A. On the relation between demographic variables and neuropsychological test performance. *Scand J Psychol.* 1993;34:305-317.

12. Spreen O, Benton AL. Neurosensory center comprehensive examination for aphasia. Victoria: University of Victoria Press; 1969.
13. Levin HS. The acalculias. In: Heilman KM, Valenstein E, editors. Clinical neuropsychology. Oxford: Oxford University Press; 1979.
14. Smith A, The serial sevens subtraction test. *Arch Neurol.* 1967; 17:78-80.
15. Buschke H, Fuld PA. Evaluating storage, retention, and retrieval in disordered memory and learning. *Neurology.* 1974;24:1019-1025.
16. Kaplan EF, Goodglass H, Weintraub S. Boston naming test. 2nd ed. Philadelphia: Lea & Feibiger; 1983.
17. Benton AL, de Hamsher K. Multilingual aphasia examination. Iowa City, IA: University of Iowa; 1976.
18. Smith A. Symbol digit modalities test. Los Angeles; Western Psychological Services; 1973.
19. Shallice T. Specific impairments of planning. *Philos Trans R Soc Lond Biol Sci.* 1982;298:199-209.
20. Lezak MD. Neuropsychological assessment. 3rd ed. Oxford: Oxford University Press; 1995.
21. Mesulam M. Principles of behavioural neurology. Philadelphia: F. A. Davis Company; 1985.
22. Rune K. Cognitive dysfunction in a population based sample of multiple sclerosis patients. Copenhagen: University of Copenhagen; 1998.
23. Gade A, Udesen H, Mortensen EL. Visual closure: street completion test. *Nord Psyk.* 1988;40:194-210.
24. Rao SM, Leo GJ, Ellington L, Nauertz T, Bernardin L, Unverzagt F. Cognitive dysfunction in multiple sclerosis. II. Impact on employment and social functioning. *Neurology.* 1991;41:692-696.
25. Amato MP, Ponziani G, Siracusa G, Sorbi S. Cognitive dysfunction in early-onset multiple sclerosis. A reappraisal after 10 years. *Arch Neurol.* 2001;58:1602-1666.

26. Schultheis MT, Garay E, DeLuca J. The influence of cognitive impairment on driving performance in multiple sclerosis. Neurology. 2001;56:1089-1094.

27. Rovaris M, Iannucci G, Falautano M, et al. Cognitive dysfunction in patients with mildly disabling relapsing-remitting multiple sclerosis: an exploratory study with diffusion tensor MR imaging. *J Neurol Sci.* 2002;195:103-109.

28. Foong J, Rozewicz L, Quaghebeur G, et al. Executive function in multiple sclerosis. The role of frontal lobe pathology. *Brain.* 1997;120:15-26.

29. Rovaris M, Filippi M, Falautano M, et al. Relation between MR abnormalities and patterns of cognitive impairment in multiple sclerosis. *Neurology.* 1998;50:1601-1608.

30. Comi G, Rovaris M, Falautano M, et al. A multiparametric MRI study of frontal lobe dementia in multiple sclerosis. *J Neurol Sci.* 1999;171:135-144.

31. Ross AJ, Sachdev PS. Magnetic resonance spectroscopy in cognitive research. *Brain Res.* 2004;44:83-102.

**Figure 1** Brain mask areas suitable for global NAA/Cr measurements. CSF and areas degrading spectral quality due to considerable magnetic field inhomogeneities are excluded.

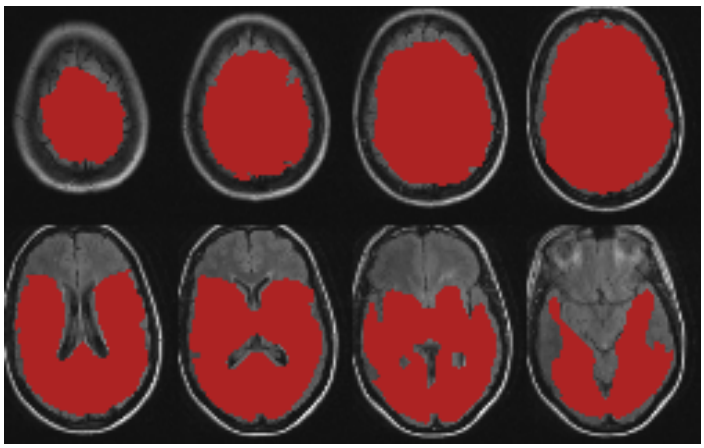

**Figure 2** Relationship between global NAA/Cr and CDF (r=0.70, *P*=0.0006).

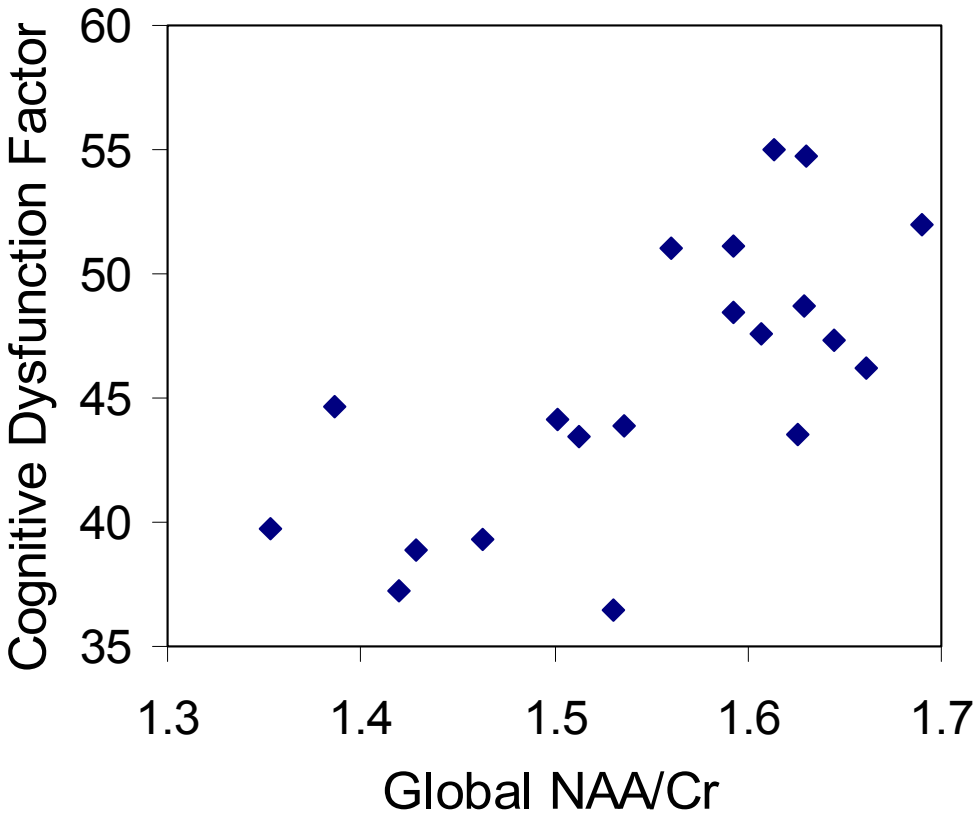

**Table 1** Patient data. See text for further explanations.

| Patient | CDF | Global NAA/Cr | BPF | Age | EDSS | Treatment | Education | LV (ml) |
|---|---|---|---|---|---|---|---|---|
| 1 | 51 | 1.56 | 0.93 | 44 | 3.5 | Yes | 4 | 11.0 |
| 2 | 52 | 1.69 | 0.95 | 43 | 3.5 | No | 5 | 12.0 |
| 3 | 44 | 1.63 | 0.88 | 24 | 0.0 | Yes | 4 | 2.3 |
| 4 | 39 | 1.46 | 0.87 | 37 | 1.0 | Yes | 3 | 13.6 |
| 5 | 51 | 1.59 | 0.89 | 26 | 3.0 | Yes | 5 | 3.0 |
| 6 | 46 | 1.66 | 0.87 | 24 | 1.5 | Yes | 5 | 1.8 |
| 7 | 49 | 1.63 | 0.92 | 40 | 1.5 | Yes | 3 | 2.4 |
| 8 | 48 | 1.61 | 0.90 | 32 | 2.0 | No | 4 | 13.3 |
| 9 | 55 | 1.63 | 0.88 | 32 | 1.5 | Yes | 4 | 7.5 |
| 10 | 36 | 1.53 | 0.88 | 45 | 4.5 | Yes | 1 | 29.0 |
| 11 | 39 | 1.43 | 0.92 | 44 | 3.0 | Yes | 3 | 58.7 |
| 12 | 48 | 1.59 | 0.86 | 22 | 2.5 | Yes | 1 | 2.6 |
| 13 | 37 | 1.42 | 0.83 | 39 | 4.0 | Yes | 4 | 2.1 |
| 14 | 45 | 1.39 | 0.93 | 48 | 2.0 | No | 4 | 0.1 |
| 15 | 40 | 1.35 | 0.90 | 38 | 2.0 | Yes | 3 | 5.0 |
| 16 | 55 | 1.61 | 0.90 | 40 | 2.0 | No | 4 | 1.7 |
| 17 | 47 | 1.65 | 0.87 | 29 | 2.5 | Yes | 5 | 3.6 |
| 18 | 44 | 1.50 | 0.91 | 29 | 4.0 | Yes | 5 | 17.6 |
| 19 | 44 | 1.54 | 0.93 | 43 | 2.5 | Yes | 3 | 1.5 |
| 20 | 43 | 1.51 | 0.87 | 35 | 2.5 | No | 5 | 2.8 |

**Table 2** Cognitive impairment measured by 29 test scores derived from 18 neuropsychological tests. Mean differences in T-scores between MS patients (n=20) and normal controls (n=75).

| Neuropsychological measures | Mean difference in T-scores |
|---|---|
| WAIS Similarities | 3.21 |
| Raven Progressive Matrices | |
| Number correct | 1.81 |
| Time to complete the task | -1.00 |
| Digits Forward | 4.07 |
| Digits Backward*† | -5.48 |
| Arithmetic | 2.68 |
| Serial Seven Subtraction Test* | -1.04 |
| Stroop (simplified version)* | -3.00 |
| List Learning* | -1.83 |
| 7/24 Spatial Recall Test | |
| Part A, learning | -0.46 |
| Part B*† | -3.97 |
| Part A, immediate recall | -0.02 |
| Part A, delayed recall* | -1.45 |
| Boston Naming Test*† | -10.99 |
| Naming of Famous Faces (naming %)* | -2.38 |
| Controlled Oral Word Association Test | |
| Animals*† | -4.59 |
| Words with “s”* | -2.90 |
| Symbol Digit Modalities Test*† | -5.39 |
| Tower of London | |
| Number of moves | 1.11 |
| Rule breaks* | -3.61 |
| Time to complete the task | -0.49 |
| Design Fluency | |
| Number correct | -0.24 |
| Errors*† | -6.12 |
| Rey Complex Figure | |
| Copy*† | -10.16 |
| Time to copy | 6.36 |
| Recall* | -0.73 |
| Mesulam Cancellation Test, random shapes | |
| Errors*† | -5.29 |
| Time to complete the task | 1.19 |
| Street Gestalt Completion Test | 2.27 |

*Measures included in CDF.

†Measures in which the patients performed significantly below normal controls ($P < 0.05$).

**Table 3** Frequency table. Fisher's Exact Test, *P*=0.005. A NAA/Cr value below 1.5 indicates cognitive dysfunction defined as CDF≤40.

| | **Global NAA/Cr < 1.5** | **Global NAA/Cr ≥ 1.5** | **Total** |
|---|---|---|---|
| **CDF > 40** | 1 | 14 | 15 |
| **CDF ≤ 40** | 4 | 1 | 5 |
| **Total** | 5 | 15 | 20 |