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Global *N*-acetylaspartate Concentration in Benign and Non-Benign Multiple Sclerosis Patients of Long Disease Duration

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Abstract

BACKGROUND and OBJECTIVE—To examine whether clinically benign multiple sclerosis patients (BMS) show similar losses of their global *N*-acetylaspartate (NAA) neuronal marker relative to more clinically disabled patients of similar disease duration.

METHODS—The whole-brain NAA concentration (WBNAA) was acquired with whole-head non-localizing proton MR spectroscopy. Fractional brain parenchymal volume (fBPV), T_2 and T_1 lesion loads, were obtained from the MRI in: *(i)* 24 BMS patients: 23.1±7.2 years disease duration, median Expanded Disability Status Scale (EDSS) score of 2.0 (range: 0–3); *(ii)* 26 non-benign MS patients (non-BMS), 24.5±7.4 years disease duration, median EDSS of 4.0 (range: 3.5–6.5); *(iii)* 15 healthy controls.

RESULTS—Controls' 12.4±2.3 mM WBNAA was significantly higher than the BMS's and non-BMS's 10.5±2.4 and 9.9±2.1mM (both $p<0.02$), but the difference between the patients' groups was not ($p>0.4$). Likewise, the controls' 81.2±4.5% fBPV exceeded the BMS and non-BMS's 77.0±5.8% and 76.3±8.6% ($p<0.03$), which were also not different from one another ($p>0.7$). BMS patients' T_1 hypointense lesion load, 2.1±2.2 cm^3, was not significantly different than the non-BMS's 4.1±5.4 cm^3 ($p>0.08$) and T_2 hyperintense loads: 6.0±5.7cm^3 and 8.7±7.8 cm^3, were also not different ($p>0.1$).

CONCLUSIONS—WBNAA differentiates normal controls from MS patients but does not distinguish BMS from more disabled MS patients of similar disease duration. Nevertheless, all MS patients who remain RR for 15+ years suffered WBNAA loss similar to the average RR MS population at fourfold shorter disease duration suggesting relative global neuronal sparing or leveling-off of the neurodegeneration rate.

Keywords

MR spectroscopy; WBNAA; multiple sclerosis; benign

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INTRODUCTION

Multiple sclerosis (MS) is the most common demyelinating disorder of the central nervous system, affecting over 2 million people worldwide.[1] Over 80% of new MS patients, typically young adults in their third decade,[2] first enter into the "relapsing remitting" (RR) phase characterized by cycles of acute symptoms lasting weeks to months, followed by partial or complete remissions for months to years.[3] As these cycles continue, patients accumulate disability from incomplete remissions, leading 50% into the secondary-progressive (SP) phase of the disease within 15 years.[3, 4] This phase is characterized by increasing permanent disability, fewer relapses, less inflammation and more pronounced neurodegeneration entailing chronic increase in motor, sensory and cognitive deficits and 5–10 year reduction in life expectancy.[5]

There is however, a subgroup estimated from 5 to 60% of all RR MS patients,[6] that seems to "do well," *i.e.,* suffer minimal disease-related deficits, reflected by their Expanded Disability Status Scale (EDSS)[7] score, for many years. They are retrospectively described as having benign MS (BMS).[8] There are various definitions how long disease duration has to be (10 to 15 years) and how low the disability (EDSS ≤2 or 3) to qualify as BMS.[8, 9] Moreover, since the definition relies on the mostly locomotor weighted EDSS as the metric of disability, it largely ignores cognitive dysfunction that affects more than half of all patients.[10] As a result, not only is the percentage of BMS debated with even a range of 10–20% considered by some an overestimate,[6] but also whether truly begin MS even exists has become controversial.[11]

Since clinical features alone appear to be insufficient to define BMS, imaging metrics are looked to for additional metrics that may better characterize this phenotype.[12] Specifically, since it is postulated that the low clinical impact in BMS reflects sparing of lesion-surrounding tissue as well as more effective compensatory mechanisms than in more disabling subtypes,[13] and that neurodegeneration is mainly responsible for its accumulating disability,[14]; then a marker sensitive to diffuse neuronal damage could better determine the true load of the disease.[15, 16] Indeed, since the first report by Arnold *et al.*[17] the amino acid-derivative *N*-acetyl-aspartate (NAA) that is almost exclusive to neurons, hence, considered to reflect their health,[18] has been used in this role, since it is detectable with proton MR spectroscopy (^{1}H-MRS) using the MRI equipment and its concentration correlates better with disability than other MR metrics.[19, 20]

Since MS pathology is diffuse throughout the central nervous system, it is not surprising that the whole-brain NAA concentration (WBNAA) has shown substantial deficits in RR MS patients relative to matched controls,[21] correlating with disease duration,[22] even for BMS.[23] Therefore, to examine whether BMS merely reflects clinically silent lesion loads and better compensatory mechanisms,[12] we compared the MRI and MRS (WBNAA) metrics in two RR MS patient cohorts of ≥15 years disease duration: One with ≤3.0 EDSS scores (BMS); the other with EDSS >3.0 (non-BMS), (despite a previous finding in similar cohorts that BMS and more progressive patients were similar radiologically *and* that, at least in a small group, progressive patients paradoxically fared better[24]). Our aim, therefore, was to test the hypotheses that the global neuronal injury (that they did not measure) accumulated in BMS, reflected by lower WBNAA than matched controls, is less than more disabled MS patients of similar disease duration.

MATERIALS AND METHODS

Subjects

All patients participated in an ongoing study on the phenotypic-genotypic characterization of MS. They were all treated with best individually selected disease modifying treatments. On the day of their MRI each patient underwent a comprehensive clinical assessment including standardized neurological examination by certified physicians (http://www.neurostatus.net) (18). All patients were clinically stable. Patients with an acute relapse were not examined and the MRI scan was postponed at least 30 days after the last dose of steroid treatment. From this cohort 50 patients with *i)* clinically definite MS;[8] and *ii)* a long disease duration (minimum 15 years) were enrolled prospectively between January 2009 to December 2010. Of these patients, 24 exhibited a benign RR course and 26 (16 RR and 10 SP MS) presented a non-benign course. Fifteen healthy controls were also enrolled. Their "healthy" status was based on self-reported negative answers to a questionaire listing 19 neurological conditions before the scan, and an unremarkable MRI afterwards. All the subjects' demographics and disease summary statistics are compiled in Table 1. Informed consent was obtained in writing from all participants, in accordance with the local ethics committee approval.

MRI and Volumetry

Experiments were done in a 3 T head-only MRI system (Allegra, Siemens AG, Erlangen, Germany) using a transmit-receive head-coil. After placing each subject into the scanner a sagittal T_1-weighted Magnetization Prepared RApid Gradient Echo (MP-RAGE) [TE/TR/TI: 3.49/2150/1000 ms, 7° flip angle, 144 slices 1.1 mm thick, 256×224 matrix and 256×256 mm^2 field-of-view] MRI for tissue volumetry. This was followed by standardized axial 2D PD and T2-weighted sequences for lesion segmentation.

Subjects' brain parenchymal volume, V_B, was segmented from the MP-RAGE MRI using our FireVoxel package.[25] It starts by placing a "seed" region in periventricular WM to obtain its average signal intensity, I_{WM}. Following selection of all pixels at or above 55% of I_{WM} (but below 135% to exclude fat) a brain mask is constructed for each slice in three steps: *(i)* morphological erosion; *(ii)* recursive region growth keeping pixels connected to the seed and *(iii)* morphological inflation to reverse the effect of erosion. Pixels of intensity below 0.55 of I_{WM} were defined as cerebrospinal fluid (CSF). The masks were truncated at the foramen magnum to include the brain stem and cerebellum but not the cord. Finally, V_B was the pixel volume × their number in the masks. The precision of this approach was recently established at 3.4%.[25]

The intracranial volumes, V_{IC}, that do not change with disease or age, were obtained from the MP-RAGE images with MRIcro, a free downloadable software package: www.mricro.com. It uses a brain extraction tool to skull-strip the intracranial surface using a deformable model. The process estimates the threshold between the brain and CSF, determines the head center of gravity C, constructs a small tessellated surface (initially a sphere) centered on C incrementally adjusting its vertices to balance its smoothness and the desired signal intensity criteria. A volume-of-interest masking the extracted brain is then used to calculate V_{IC}. The fractional brain parenchyma volume, fBPV, an atrophy index, was expressed as $V_B / V_{IC} \times 100\%$. Brain lesion were outlined on the PD and post –contrast T1-weighted MRI and their volume estimated using a semi-automatic thresholding contour software AMIRA 3.1.1 (Mercury Computer Systems Inc).

^{1}H-MRS and WBNAA quantification

Following the MRI we adjusted the magnetic field homogeneity over the whole head, by adjusting the system first and second order shims, using our own 3D chemical-shift-imaging

based approach,[26] to yield consistent 27±4 Hz water linewidths, in under 5 minutes. The brain's NAA signal was obtained with non-localizing *TE/TI/TR*=0/940/10^4 ms ^{1}H-MRS.[21] The long *TR* and short *TE* ensure insensitivityto typically unknown T_1 and T_2 variations. Because the signal from the whole-head is acquired, the signal to noise ratio is high, which is exploited for a short, 2 minute and 40 second long sequence acquisition (16 add-subtract averages at TR=10 s, each). The absolute amount, Q_{NAA}, was scaled against a 3 L sphere of 1.5×10^{-2} mole NAA in water. Subject and reference NAA peak areas, S_{S} and S_R, were obtained by manual phasing, selection of peak limits and integration, using in-house IDL software (Research Systems Inc. Boulder CO), as shown in Fig. 1, by four blinded readers. A result more than two standard deviations (from the average for all readers and all subjects, ~8%) from that subject's mean, $\overline{S_S}$, was discarded. If more than one was rejected the data set was excluded as "poor quality." Q_{NAA} was estimated as,[21]

$$Q_{\mathrm{NAA}}=1.5\times10^{-2}\cdot\frac{\overline{S_{\mathrm{S}}}}{S_R}\cdot\frac{V_{\mathrm{S}}^{180^\circ}}{V_{\mathrm{R}}^{180^\circ}}\mathrm{moles}, \quad [1]$$

where $V_{\mathrm{R}}^{180^\circ}$ and $V_{\mathrm{S}}^{180^\circ}$ are the transmitter voltages for non-selective 1 ms 180° inversion pulses on the reference and subject, reflecting by reciprocity the relative coil sensitivity. Note that although other metabolites (*cf.* Fig. 1), especially macromolecules and other *N*-acetyl bearing species also resonate around 2 ppm, their contribution to the peak is estimated at less than 10%.[27]

To normalize for brain size differences, each subjects Q_{NAA} was divided by their brain parenchyma volume, V_B, to yield the whole-brain NAA concentration:

$$\mathrm{WBNAA}=Q_{NAA}/V_B\ \mathrm{mM}. \quad [2]$$

This is a specific, brain size independent metric and its *inter*- and *intra*-subject variability in younger healthy individuals has been shown to be better than ±8%.[22]

Statistical analyses

Analysis of variance (ANOVA) was used to compare groups in terms of age, using the ranks of the ages as the dependent variable to conduct a non-parametric analog of the ANOVA based on the actual ages. Reported *p* values are two-sided and results defined as significant if associated with a $p<0.05$. SAS version 9.0 (SAS Institute, Cary, NC) was used.

RESULTS

Representative axial MP-RAGE images from age-matched control, BMS and non-BMS patients, are shown in Fig. 1. They illustrate the relative atrophy of the MS brain as well as smimilarities between the phenotypes in tissue loss and T_1-hypointense lesion loads. Indeed, while controls' fBPV 81.2±4.5%, was higher than the BMSs' 77.0±5.8% and the non-BMS' 76.3±8.6% (both: $p<0.03$), the patient groups were not different ($p>0.7$), as shown in Fig. 2*a*

The BMS patients' T_1-hypointense lesion load, 2.1±2.3 cm^3, was not significantly different than the non-BMS' 4.1±5.4 cm^3 ($p>0.08$), as shown in Fig. 2*b*. T_2-hyperintense lesion loads: 6.1±5.8 versus 8.7±7.8 cm^3, were also not different ($p>0.1$) between patient groups.

Whole-head ^{1}H-MR spectra, from which the WBNAA is estimated from a representative of both subject cohorts, are shown in Fig. 1. No data had to be rejected due to "poor quality," as described in the Methods. Overall, the controls' 12.4±2.3 mM WBNAA was 18% higher

than the BMS 10.5±2.4 mM and 25% than the non-BMS patients' 9.9±2.1 mM ($p<0.01$ for all), as shown in Fig. 3 However, the patient groups were not different ($p>0.4$).

DISCUSSION

Despite efforts to establish prospective criteria for a BMS course, there is still no consensus.[6] Consequently, (retrospective) "benign" diagnosis is still a controversial predictor of future course with regards to actual prevalence over time and long term outcomes.[28, 29] Addition of imaging metrics in order to better define BMS in terms of atrophy, lesion loads magnetization transfer or diffusion characteristics, have also yielded inconclusive results.[9, 28] Given the notion that permanent disability in MS reflects accumulating neuronal pathology,[14] motivated us to apply a spectroscopicmarker of their global integrity that would pick up both the locomotor damage, to which EDSS is sensitive and other diffuse gray matter damage that may be responsible for cognitive decline, to which EDSS is not.[30] This prompted our hypothesis that compared with matched controls, less NAA loss is suffered by BMS than more disabled MS patients.

Surprisingly, the data does not support the hypothesis. There were no statistically significant differences between the patient groups in any MR-related metric: fBPV, WBNAA or either lesion load, similar to Strasser-Fuchs *et al.*[24] Moreover, there was no difference between the BMS group and non-BMS group in cognitive impairment as well. Consequently, neural sparing in focal lesions or normal appearing white and gray matter does *not* seem to be a central feature distinguishing BMS from non-BMS patients of long (15+ years) disease duration.

Interestingly, both RR subgroups' mean WBNAA values: 10.5 and 9.9 mM, are similar to the 10.0 and 10.5 mM reported for RR MS patients, with similar EDSS, but of much shorter (means of 4 and 6 years) disease duration in two separate studies, while their fBPV is lower,[22, 31] suggesting that, neuronal damage accrues more slowly than atrophy. Although this WBNAA similarity patients of vastly dissimilar disease durations can be explained by assuming all the NAA loss occurred very early on, it is unlikely. This is because a recent serial study has shown still a continual ~5%/year WBNAA decline around 4 years from clinically definite diagnosis of RR MS.[22] Therefore, the data here supports the assertion that the relatively low EDSS-s of these cohorts is reflected by their relative neuronal sparing regardless of the clinical phenotype.

Interestingly, there was no difference between the patient groups in cognitive impairment. The clinical differences between the two is solely in EDSS-sensitive functional areas. This is likely a consequence of the sharp transition from BMS to RR MS at EDSS=3.0. Thus, for example, two patients with 20 years disease duration, one with an EDSS score of 3.0 is "benign," the other with 3.5 is *not,* despite minimal 0.5 unit difference that distinguishes only between "mild disability in three or four functional systems" and "mild disability in five functional systems".[7] Nevertheless, distinct clinical disparity obviously exists at the opposite ends of our 0 – 6.5 EDSS range. There are several possible explanations for this. First, that a BMS phenotype likely reflects more effective compensatory mechanisms. Indeed, Filippi *et al.* have recently shown that, contrary to what happens in secondary progressive MS the movement-associated pattern of activations seen in benign MS resembled that of healthy individuals, suggesting that the long-term preservation of brain functional adaptive mechanisms may contribute to their favorable clinical course.[32] Second, that in BMS disease pathology fortuitously missed brain regions that influence their EDSS scores.

Admittedly, our study is subject to several limitations. First, we did not account for possible medication effects, given the 15–30+ years disease duration leading to several possible changes in their type, dose and duration over that period. Second, a cross-sectional study cannot ascertain *when* over the disease course the NAA loss occurred. This would require a large cohort, long-term serial study. Third, the WBNAA approach trades localization for sensitivity and acquisition speed, rendering it insensitive to either global changes smaller than the 6–8% sensitivity; and to regional NAA variations.[21] Finally, the relatively small (~25) cohorts of the patient phenotypes may have limited the statistical power do detect WBNAA differences between them.

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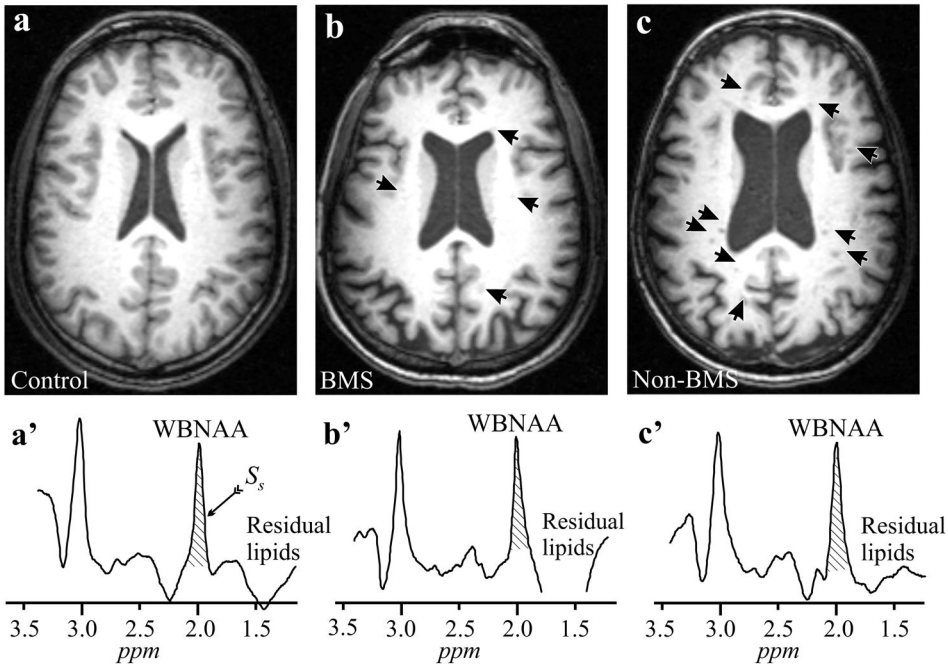

Fig. 1.

Top:Axial T1-weighted MP-RAGE brain slices of a healthy 47 year old control (**a**); a 45 year old BMS (**b**); a 48 year old non-BMS (**c**); all male. Note the relatively similar atrophy of both patients compared with the control and the higher T_1-hypointense lesions load of the non-benign patient (marked by arrows).

Bottom, **a**′ – **d**′: The subjects' corresponding whole-head ^{1}H-MRS on common intensity and frequency scales. Note the lipids suppression performance and that of all the other peaks in the spectrum only NAA at 2 *ppm*, is implicitly localized by its biochemistry to just the brain. Subject NAA peak area, S_S, was obtained by integration for use in Eq. [1].

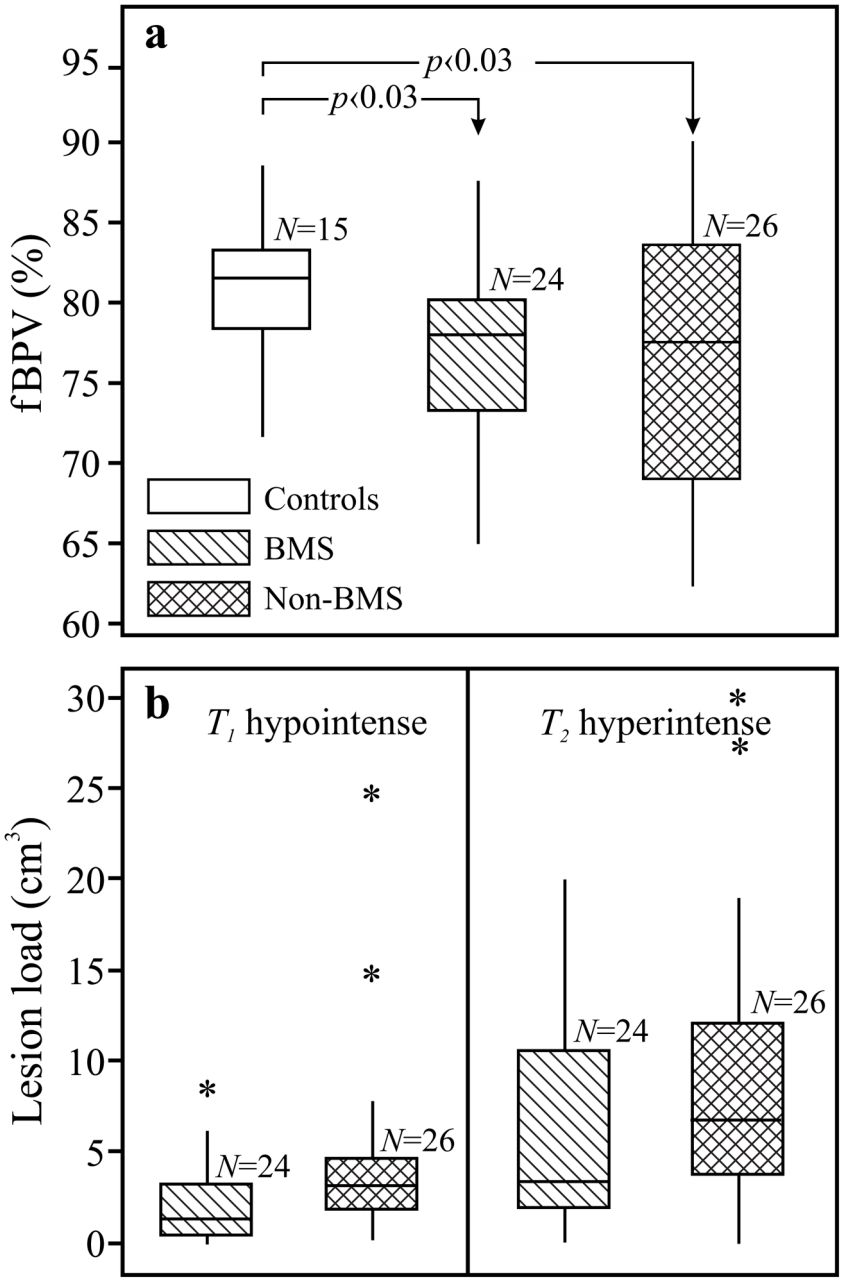

Fig. 2.

Box plots showing the first, second (median) and third quartiles (box), ±95% (whiskers) and outliers (*) of the fBPV (top, **a**); T_1-hypo- and T_2-hyper-intense lesion loads (bottom, **b**)of the controls, BMS and non-BMS patients. Note that the patients' fBPV distributions are significantly lower than the controls but not different from each other; and that the patient groups have similar T_1-hypointense and T_2-hyperintense loads.

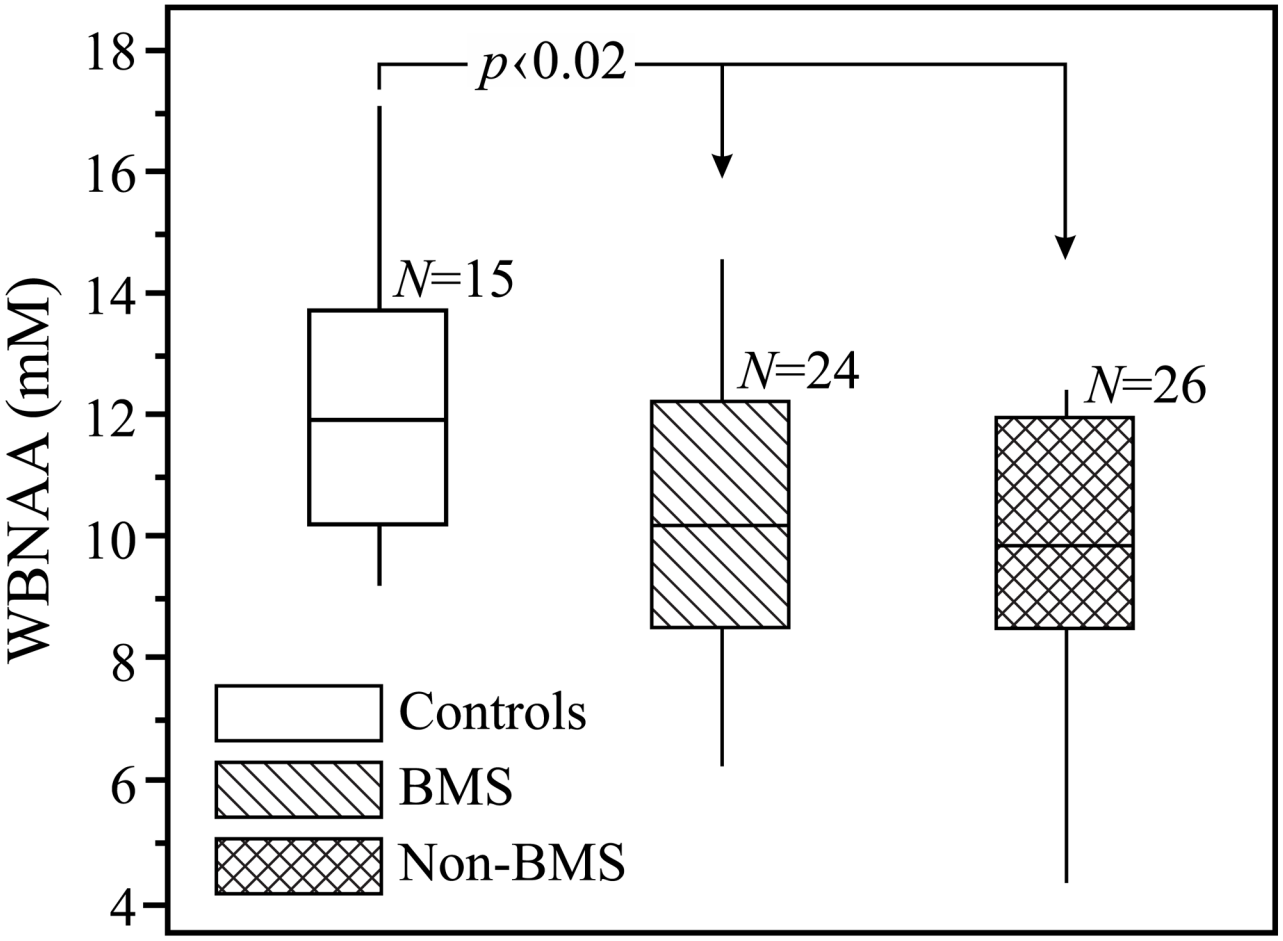

Fig. 3.
Box plots of the WBNAA distributions of the three subject cohorts Note that *both* patients groups' WBNAA are significantly lower than the controls' but not significant from one another (p>0.4).

Table 1

Patient summary demographics, Expanded Disability Status Scale (EDSS) median scores and its range, disease duration in years from first symptom and Symbol-Digit Modalities Test (SDMT) scores (with a z-score below −1.65, defining cognitive impairment according to the 90% confidence interval).

Subject type	[a] Gender	[b] age	[c] Disease duration	[d] EDSS	[e] SDMT
BMS (*N*=24)	20F, 4M	50.9 ± 10.5	23.1±7.2	2.0 (0 – 3.0)	> −1.65
Non-BMS (*N*=26)	12F, 14M	53.3±9.3	24.5±7.4	4.0 (3.5 – 6.5)	< −1.65
Control (*N*=15)	6F, 9M	50.0±6.4	-	-	-

[a] F-female, M-Male;

[b] years: median ± standard deviation,

[c] years from first symptom; median (minimum : maximum);

[d] EDSS mean (minimum – maximum);

[e] Symbol-Digit Modalities Test (SDMT) with a z-score below −1.65, defining cognitive impairment according to the 90% confidence interval.

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