WBNAA as a surrogate marker of multiple sclerosis neurodegeneration*

D. J. Rigotti BSc, M. Inglese MD PhD, O. Gonen PhD
Department of Radiology, New York University School of Medicine. New York. USA

ABSTRACT. Multiple Sclerosis (MS) is the most common demyelinating disease of the central nervous system. Although it is of unknown etiology and currently incurable, several disease-modifying treatments have been available for over a decade. The heterogeneous nature of MS, however, makes for an uncertain prognosis and difficult clinical assessment of its progression. Within the past two decades MR metrics have proven effective in the diagnosis of MS and the monitoring of its evolution and response to treatment and experimental therapies. Amongst these MR modalities, proton spectroscopy (^{1}H-MRS) is often used in tandem with the MRI to provide biochemical specificity via the level of several brain metabolites. Key amongst them is the N-acetylaspartate (NAA) which is considered a marker for axo-neuronal health and viability. Unfortunately, most MRS techniques to date employ small volumes of interest that often miss more than 80-99% of the brain volume. Serial studies using ^{1}H-MRS are also often confounded by voxel misregistration and partial volume errors, and often are limited by the time required (many minutes) to achieve sufficient sensitivity. The quantification of the whole-brain NAA concentration (WBNAA) addresses all these concerns by (quickly and non-invasively) providing a global measure of neuronal damage, i.e., the total load of the disease. This paper reviews the method, its applications to MS so far and its potential to monitor the degenerative processes associated with MS through the use of the global NAA concentration as their marker.
Key words: Brain, N-acetylaspartate (NAA), Multiple Sclerosis (MS), MR spectroscopy (MRS), whole brain NAA (WBNAA).

RESUMEN. La esclerosis múltiple (EM) es la enfermedad desmielinizante más frecuente del sistema nervioso central. Aunque su etiología es desconocida, y por ahora es una enfermedad incurable, en la última década se ha dispuesto de distintos tratamientos modificadores de la enfermedad. La naturaleza heterogénea de la EM, sin embargo, hace que sea incierto su pronóstico y difícil evaluar clínicamente su progresión. En las últimas dos décadas, las medidas de RM se han mostrado eficaces en el diagnóstico de la EM y en la monitorización de su evolución y respuesta al tratamiento y a las terapias experimentales. Entre estas modalidades de RM , la espectroscopía de protones por resonancia magnética (^{1}H-MRS) se usa conjuntamente con la RM para ofrecer una especificidad bioquímica a través de varios metabolitos cerebrales. Entre todos ellos es fundamental el N-acetilaspartato (NAA) que está considerado como un marcador de la salud y viabilidad de neuronas y axones. Desafortunadamente, en la actualidad la mayoría de las técnicas de resonancia magnética, emplean pequeños volúmenes de interés que a menudo pierden más del 80-99% del volumen cerebral. Estudios seraidos utilizando ^{1}H-MRS en ocasiones se han confundido por falta de registros de voxels y errores de volúmen parcial, y con frecuencia están limitados por el tiempo que precisan (muchos minutos) para adquirir suficiente sensibilidad. La cuantificación del NAA cerebral total (WBNAA) obvia todos estos aspectos (de una manera rápida y no invasiva) dando una medida global del daño neuronal, esto es., la carga total de la enfermedad. Este artículo revisa el método, su aplicación a la EM y su potencial para monitorizar el proceso degenerativo asociado a la EM a través del uso de la concentración global del NAA como su marcador.
Palabras clave: cerebro, N-acetilaspartato (NAA), esclerosis múltiple (EM), espectroscopía por RM (1H-MRS), NAA cerebral total (WBNAA)

Correspondencia: Oded Gonen, PhD – Department of Radiology – New York University School of Medicine – 650 First Avenue, 6th Floor New York, New York 10016 – Telephone/FAX: (212) 263-3532/(212) 263-7541 – E-mail: oded.gonen@med.nyu.edu

Multiple sclerosis (MS), the most common demyelinating disease of the central nervous system (CNS), affects over 2 million worldwide and is the leading cause of non-traumatic neurological disability in young and middle-aged adults[1]. Roughly 85% of MS patients, two thirds of whom are young women [average age of onset in the US is 27 years[2]] undergo acute episodes lasting days to weeks followed by partial or complete remission for months to years, entering the relapsing-remitting (RR) stage[3]. These cycles continue and accumulate disability from incomplete remissions. This chronic progression entails increasing motor, sensory and cognitive deficits, yet no significant decrease in life expectancy[4]. As a result, a newly diagnosed MS patient can expect decades of progressively deteriorating quality of life[5, 6].

It has been suggested that axonal damage followed by neuronal cell death from Wallerian degeneration is the probable cause of permanent neurological deficits in MS[7-10]. This can be assessed directly and non invasively by proton MR spectroscopy (^{1}H-MRS) quantification of *N*-acetylaspartate (NAA), shown in Fig. 1, the second most common amino acid-derivative in the CNS[11, 12]. Since its 10-14 mM concentration in neurons exceeds the 80-100 μM in the interstitium[13], the mature brain NAA is considered the neuronal MR marker[14-16].

The presence of NAA in the brain was first

**Supported by NIH Grants EB01015, NS51623, NS050520 and NS29029.*

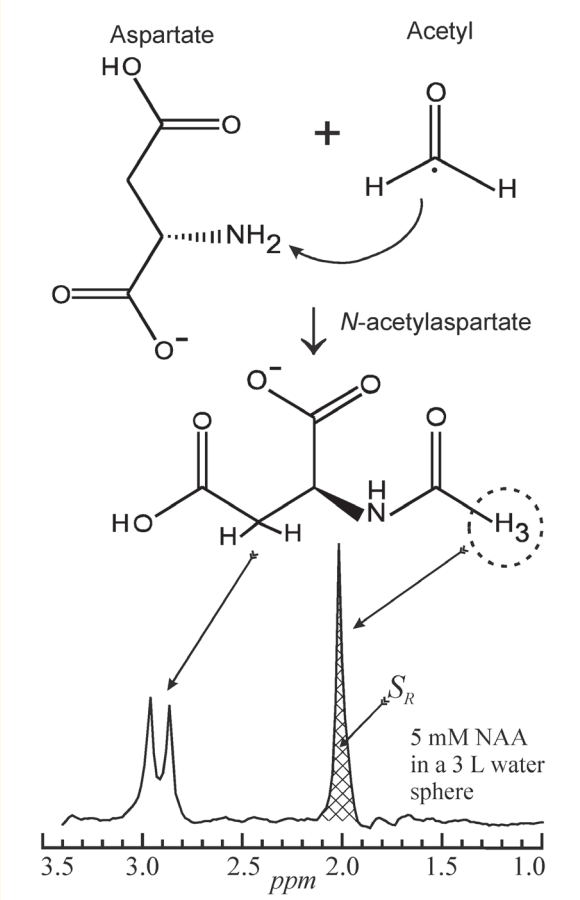

Figura 1 *Top: Schematic description of the structures and acetylization of aspartate into N-acetyl aspartate in the mitochondria. Bottom: The ^{1}H MR spectrum of 10.5 mM NAA in 3 liters of water at 1.5 T. Note the three protons of the CH_3 yield the most prominent peak.*

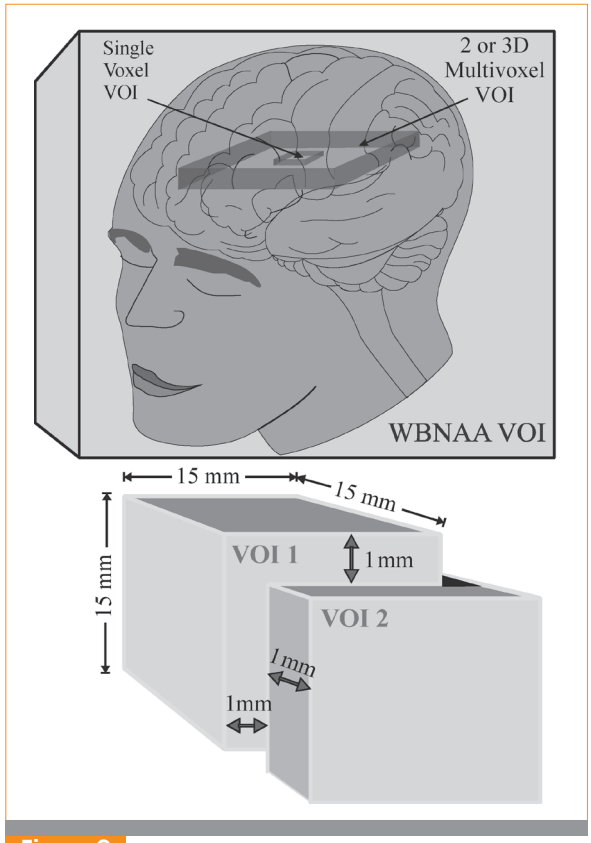

Figura 2 *Top: Schematic comparison of relative VOI size and coverage between single-voxel, 2 or 3D multi-voxel localized ^{1}H-MRS and WBNAA in the human brain. Note that the single and multi-voxel VOIs cover only fractions of the brain and must be kept away from the skull, missing most of the cortex, whereas WBNAA accounts for the entire head. Bottom: The consequence of a 1 mm (1 guiding-image pixel) placemat error in each direction, X, Y, Z, on a 1 cm voxel in a serial study. This 7% error leads to only 80% of the VOI common to both measurements. Note that while this error is technically unavoidable in localized spectroscopy it is not an issue for WBNAA, as shown above.*

described by Tallan *et al.* in 1956[17]. Although trace amounts of NAA are found in other tissues, *e.g.,* liver, kidney, muscle, oligodendrocyte progenitor cells, it is almost exclusive to CNS neurons and their processes and is consequently, regarded as a marker for their viability and density[14, 15, 18]. Its two key attributes are that its ^{1}H-MRS signal at 2.02 *ppm* is the most prominent *in vivo*; and with the exception of Canavan disease[19], its concentration is reported to decline in all CNS disorders[20, 21]. In addition to ^{1}H-MRS evidence[11, 22, 23], NAA loss has also been described in transected rat optic nerve axons[24], biopsies of MS lesions[25] and postmortem spinal cords in humans[26].

NAA is synthesized through acetylization by acetyl CoA of free aspartate by the L-aspartate *N*-acetyltransferase (Asp-NAT), as shown in Fig. 1[27] and catabolized by the enzyme aspartoacylase (ASPA) whose deficiency is believed to be the cause of Canavan disease. Despite over fifty years of research, however, the function(s) of NAA remain(s) controversial. Several hypotheses proposed for its role including, *i*) an organic neuronal osmolyte[28], *ii*) an acetate source in myelin synthesis[29], *iii*) mitochondrial energy source[30], *iv*) precursor for *N*-acetylaspartylglutamate, and *v*) a ligand for some metabotropic glutamate receptors[31].

Unfortunately, most of the numerous ^{1}H-MRS studies to date used either small (3.5-8 cm^3) single-voxels, or larger two-or three-dimensional volumes-of-interest (VOI), as shown in Fig. 2[32-34]. These VOIs must be placed away from the skull to avoid contamination from bone marrow and subcutaneous lipids' signals, missing most of the cortex[35] and due to their size must be image-guided to MRI-visible pathologies. Since most CNS disorders are diffuse, missing 80–99% of the brain subjects estimates of their load

to extrapolation errors[36]. Localized MRS also suffers three additional limitations: *(i)* susceptibility to VOI repositioning errors, *e.g.,* for the (1.5 cm)^3 common in single-voxel studies, a 1 mm relative offset (one guiding MRI pixel) leads to ~80% common volume (worse for smaller voxels), as shown in Fig. 2. *(ii)* Long acquisition, ~7 min, is needed for 3 – 8 cm^3 voxels for sufficient signal-to-noise-ratio (SNR). Finally, *(iii)* the T_1 and T_2 relaxation times are needed in patients and controls for accurate quantification[37].

These concerns can be addressed by a non-echo, non-T_1-weighted, non-localizing ^{1}H-MRS sequence acquiring the signal from the entire head, as shown in Fig. 2[38]. While the NAA signal is implicitly restricted to the brain, the lack of explicit localization removes issues of VOI guidance, serial misregistration or SNR. Since MS pathology is intrinsically diffuse, whereas its MRI lesions are focal and rarely exceed 5% of the brain volume[39], global NAA assessment is a better estimate of the full load of the disease. This paper reviews a ^{1}H-MRS method to quantify the whole-brain NAA (WBNAA) level and its applications to the study of MS pathogenesis.

❑ The WBNAA Method

I. The ^{1}H-MRS sequence

The amount of NAA in the whole-brain, Q_{NAA}, is obtainable with a non-localizing TE/TI/TR= 0/940/10,000 ms ^{1}H-MRS sequence shown in Fig. 3a[38]. Unlike the previous lipid-nulling inversion-recovery used[40], this sequence relies on the much shorter $T_1 \approx 220$ ms of the lipids compared with the $T_1 \approx 1.4$ s of the NAA to null the latter every second acquisition[38]. The long TI=$ln(2)\times T_1$=940 ms NAA nulling delay is greater than $4\times T_1$ of the lipids ensuring that they are at thermal equilibrium each acquisition, as shown in Fig. 3b, whereas the NAA is thermal only in odd ones (no inversion) and null every even. Subtracting even from odd signals, therefore, destructively interferes the lipids but not the long-T_1 metabolites' signals, as shown in Fig. 3c. Since all other metabolites, *e.g.,* creatine, choline, *myo*-inositol, glutamate, *etc.,* are present in all tissue types, however, only the NAA signal is uniquely attributable to the brain with this method.

Quantification is done against a reference 3 L sphere of 1.5×10^{-2} mole NAA in water. Subject and reference NAA peaks, S_S and S_R, are integrated, (*cf.* Figs. 1 and 3c) and Q_{NAA} obtained as,

$$Q_{NAA} = 1.5\times10^{-2}\cdot\frac{S_S}{S_R}\cdot\frac{V_S^{180^\circ}}{V_R^{180^\circ}}\ \text{moles.}\ [1]$$

$V_R^{180^\circ}$ and $V_S^{180^\circ}$ are the transmitter voltages for a non-selective 1 ms 180^0 inversion pulse on the reference and subject reflecting their relative coil loading and by reciprocity, sensitivity.

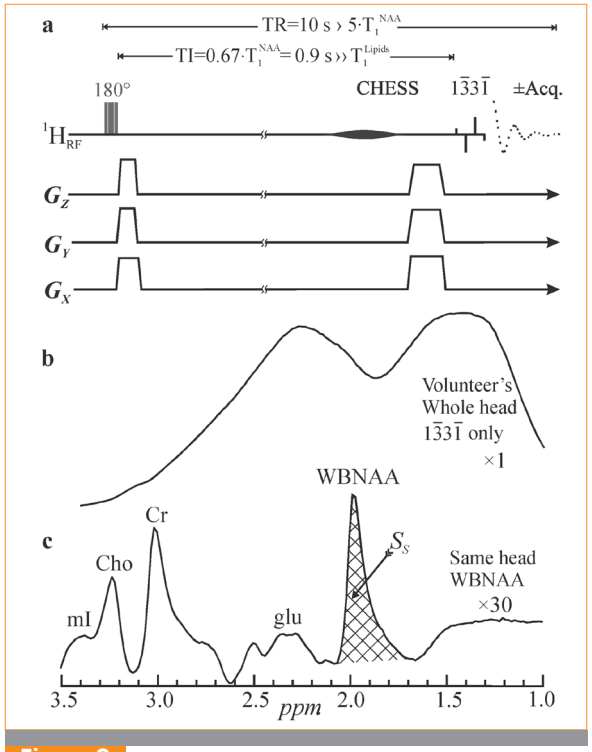

Figura 3 *Top, a: Schematic representation of the two-step WBNAA sequence. It comprises an alternating inversion 180° pulse (applied every odd acquisition) followed by an inversion time, TI, designed to null the NAA signal. It ends with a CHESS and a 133I water suppression pulses (4 ms interpulse delay at 1.5 T, 2 ms at 3.0 T). The latter also serves as the 90° readout pulse. Acquisition commences immediately (TE=0) and every even acquisition is subtracted from every odd one. Note that since the TR is long, 10 s, no T_1 or T_2 weighting are incurred. Center, b: The result of a single 133I 90° on a human head demonstrating the problem of the immense lipid signal from the bone marrow and adipose tissue. Bottom, c: The resulting whole-head ^{1}H-MR spectrum from the WBNAA sequence on the same head as b, demonstrating almost complete lipid suppression. Note that the NAA is implicitly localized to the brain, whereas with the other metabolites' peaks are non localized making it impossible to ascertain where their signals come from. Also note the excellent SNR from this 2.5 min acquisition.*

II. Normalization of WBNAA concentration

To normalize for normal variations in human head sizes, Q_{NAA} is divided by total brain parenchyma volume, V_B, to yield the whole-brain NAA concentration WBNAA:

$$\text{WBNAA} = \frac{Q_{NAA}}{V_B}\ \text{mM},\ [2]$$

a specific, size-independent metric. V_B can be obtained with any MRI segmentation software and several, *e.g.,* MIDAS, 3DVIEWNIX, SPM, SIENA and SIENAX were already used[41-46].

III. Accuracy. How much is "whole" brain?

Due to severe magnetic field inhomogeneities at air-tissue interfaces, *e.g.,* at the sinuses and auditory canals[47], some of the NAA signal may be shifted outside the integration window shown in Fig. 3**c**. The extent of the signal (lost) outside this interval was estimated using three dimensional, high-spatial resolution, chemical shift imaging of the entire head water signal[48]. Due to the extremely high SNR of water, it is simple to quantify the fraction of its signal outside a frequency interval of the same width at under 10%. By analogy, the integrated S_S in Fig. 3c, captures over 90% of brains NAA signal[48].

IV. Intra- and inter individual reproducibility - The precision

The serial *intra*-individual WBNAA variations first reported were small, ±5% and the *inter*-individual ones were 6% in a group of five healthy women[38]. The inter-individual variations in (much) larger cohorts of several scores of healthy subjects, each, reported since then, have been consistent with coefficients of variations in the 6-8% range[35, 49, 50].

V. Multi-site and multi-hardware reproducibility

MR hardware differences in multi-center trials can potentially confound the results at their final data-consolidation stage. To assess the sensitivity of WBNAA to the hardware, its values were compared in over 150 controls across six scanners from two manufacturers (GE, Siemens) at three magnetic field strengths (1.5, 3.0 and 4.0 T) in five sites. The results, shown in Fig. 4a, demonstrate that: *(i)* Their WBNAA distribution is statistically indistinguishable (p>0.237)[50]; *(ii)* the WBNAA concentration is 12.2±1.2 mM. No age or sex WBNAA differences were found in this cohort of nearly equal number of men and women ranging in age from late-teens to late-fifties. These indicate that absolute quantification against a reference standard combined with sequence simplicity render the technique hardware-insensitive in multicenter trials[50].

VI. Longitudinal performance in healthy young adults

Serial WBNAA measurements were done in 14 healthy adults over a 2 – 3 year period, common in most treatment clinical trials. No significant change (adjusted for both sex and age) was found either *inter*- or *intra*- persons over the entire 3+ year duration in the 25-50 year old studied, as shown in Fig. 4b[51].

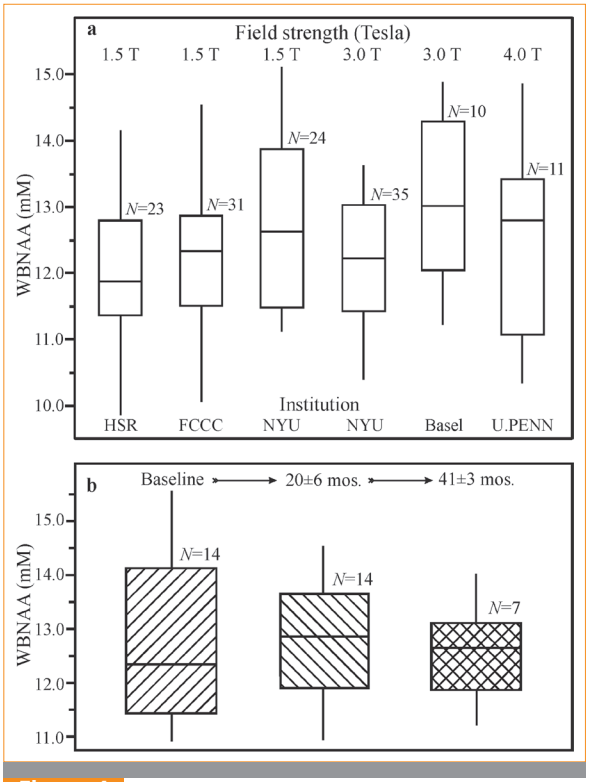

Figura 4 *Top, a: Box plot showing the 25%, 50% (median) and 75% quartiles (box) and ±95% (whiskers), of the distributions of the subjects' WBNAA concentrations in each of the five institutions (HSR – Hospital San Rafael - Italy, FCCC – Fox Chase Cancer Center - USA, NYU – New York University - USA, Basel - Switzerland and U.PENN – University of Pennsylvania -USA) six scanners, three field strengths and two manufacturers (GE – 4 T, Siemens - the rest) used. Note that the distributions differences from 12.2±1.2 mM (means and SDs) for over 150 healthy individuals are insignificant, independent of these subjects' age or sex, indicating robustness to instrumentation. Bottom, b: Box plots of the WBNAA distributions at baseline, first and second follow-up time points of 14 healthy young adults studied longitudinally over 2 – 3 years (the seven subjects at the third time point are a subset of the original 14).*

Since the annual rate of brain parenchymal loss due to normal aging is reported to be ~0.25% over the healthy human lifespan[52] and given that WBNAA is normalized to the brain volume (Eq. [2]), it is insensitive to this atrophy, *i.e.,* it change(s) reflect only the health of the neurons in the remaining tissue.

❑ WBNAA as a surrogate of multiple sclerosis neurodegeneration

I. Overview of the disease and its current treatments

While several phenotypes of MS are described, the most common in 85-90% of patients is the RR-MS subtype[5]. It is characterized by relapses of variable severity followed by remissions of various

length[3]. Nearly 20% of them will remain clinically stable for at least 2 decades, a phenotype referred to as benign MS[53]. Within 25 years, however, most untreated RR-MS patients will evolve into a secondary progressive (SP) phase characterized by a steady increase in chronic disability. The remaining 10-15% of patients, however, never go through a relapse-remitting course, but rather progress slowly towards disability[54]. This primary-progressive (PP) subtype of MS usually strikes men in their middle age - fifties and sixties[55, 56].

Although (still) without cure, six drugs are currently FDA approved for MS in the US as disease-modifying agents that alter the natural history of the disease: The intramuscular beta-interferon-la (Avonex), subcutaneous beta-interferon-1a (Rebif), subcutaneous beta-interferon-1b (Betaseron), glatiramer acetate (Copaxone), and Natalizumab (Tysabri) a laboratory-produced monoclonal antibody given by IV infusion. For SP-MS the most convincing data favors mitoxantrone (Novantrone) as most likely to retard progression and delay disability[57-59].

II. WBNAA versus age and brain atrophy in MS

Extensive ^{1}H-MRS studies to characterize the metabolic profiles of MS lesions as well as the normal appearing white and gray matter (NAWM, NAGM) revealed loss of NAA in all tissue types, confirming the diffuse nature of the pathological processes underlying the disease[60]. This was confirmed by the first WBNAA study which demonstrated age dependent deficits in patients compared with matched controls in both RR[61] and PP-MS[62]. This age-dependence is probably due to the similar age of onset, transforming patients' "age" to "disease-duration."

Although lesions and atrophy are the hallmarks of MS, they only represent end points of its complex pathological processes, and unfortunately do not identify them. Furthermore, their predictive value for the clinical status of the disease or its course, is tenuous at best[63-65]. Comparing WBNAA levels with atrophy (reflected by the fraction of parenchymal brain volume) as functions of disease duration in a cohort in of 42 RR-MS patients has shown the former to decrease nearly 3.5 times faster than the latter[66], as depicted in Fig. 5. This suggests that neuronal cell injury precedes atrophy and that degenerating axons may leave behind their empty myelin sheaths, suggesting that WBNAA is a more sensitive indicator of disease progression than either lesion load or atrophy[66].

III. Is MS neurodegeneration purely in the white matter?

For over a century MS was considered a white matter (WM) disease. Although evidence that gray

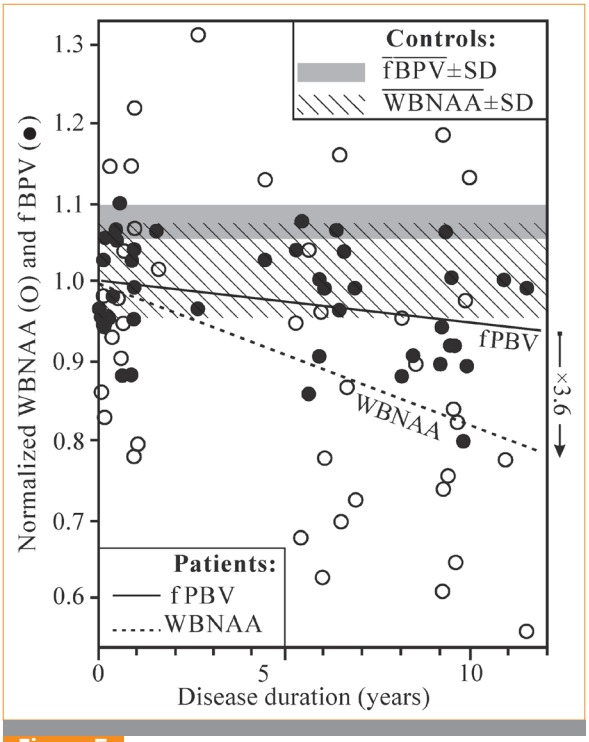

Figura 5 *Normalized (unitless) for comparison, by dividing measured values (fractional brain volume – fBPV and WBNAA) with their values at estimated disease onset versus disease duration for each of 42 RR MS patients. The 41 controls' average±SD for these normalized metrics WBNAA=1.12±0.06 and fBPV=1.08±0.02, are indicated by the hatched and gray zones, respectively. The solid (_) and dashed (---) lines are the normalized prediction for fBPV and WBNAA, respectively. Note that (a) while both metrics exhibit (statistically significant) decline with disease duration, the WBNAA's is ×3.6 fold faster (steeper slope) than the fBPV; and (b) 85% of the patients' fBPVs (35/42) and 60% of the WBNAA values (25/42) are below the controls'.*

matter (GM) is also affected has been available for over 30 years, only recently has post mortem pathology shown many cortical and subcortical lesions that are usually missed by T_2-weighted MRI[67]. In addition, quantitative MRI techniques, *e.g.,* magnetization transfer (MTI) and diffusion tensor imaging (DTI) revealed abnormal physical properties of otherwise NAGM. Since the brain's WM:GM volume fractions are approximately 40:60[68], and the concentration of NAA in the former is approximately 2/3 of the latter[69], WBNAA deficits over 20% must reflect GM involvement. Indeed, out of 71 RR MS patients, thirteen had 40% less WBNAA than the controls, (*cf.* Fig. 6a) a loss that cannot be explained in terms of WM alone, leading to a conclusion of extensive GM involvement even in a relatively early, RR, stage of the disease[35].

It is noteworthy that although axonal damage

has been reported in classical neuropathology for over a century, only recently have *post mortem* studies shown that neurons are also targets of the MS process[9, 26, 70-72] and that their dysfunction may contribute to clinical disability[70, 73]. Indeed, Peterson *at al.* reported that cerebral cortex MS lesions are characterized by demyelination, axonal and dendritic transection and apoptotic loss of neurons[72]. In addition, subcortical lesions which result in axonal transection, might be accompanied by retrograde neuronal degeneration. Unfortunately, such lesions are frequently missed by both conventional T2WI, due to their small size, poor contrast with surroundings GM and partial volume effects with adjacent WM[73]; and by pathology which inspects only few small tissue samples, usually obtained *post mortem*. Consequently, several important parameters pertaining to GM involvement, *e.g.*, time of onset; location and the extent of neuronal damage, are uncertain.

Since all the patients in that study were in their initial, only mildly-disabled RR stage, the finding that nearly 20% of them already display sufficient NAA loss to implicate their GM, may indicate that neuronal damage may be an early consequence of the MS process. As for the conjugate issues of its location(s) and extent: A 10% and higher GM involvement indicates that lesion density similar to the white matter's (<3%) is insufficient to explain such profound NAA decline. This finding is consistent with a recent report of ~20% average WBNAA decrease in patients at the earliest clinical stage of MS, who typically show negligible, <1%, lesion volumes[49]. Therefore, in analogy with NAWM axonal pathology, substantial diffuse neuronal dysfunction in the GM must also be assumed. This concept is also corroborated by MTI and DTI studies showing differences between the magnetization transfer ratio and mean diffusivity histograms from the GM of MS patients compared with matched controls[74, 75], suggesting subtle diffuse tissue damage. However, since MTI and DTI reflect structural abnormalities, increased extra-cellular water and loss of barriers to its motion, neither are as specific to neuronal (NAA) loss nor cover as extensive a brain fraction as WBNAA.

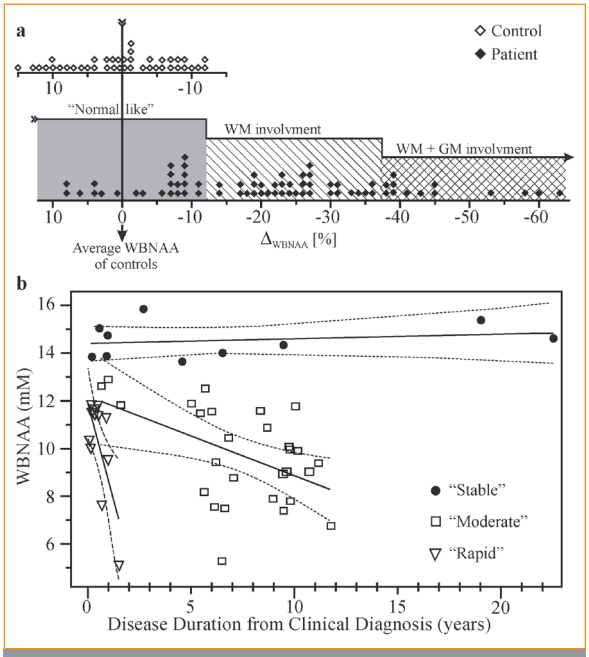

Figura 6 *Top, a: Dot plot of the 41 healthy controls' WBNAA deviations (in %) from their average (vertical arrow). Below: same for the 71 MS patients'. Those inside the shaded zone, deviation of ±2 σ (±12%) from healthy controls, are statistically indistinguishable from the controls. The hatched region, from -12% to -37%, highlights patients whose NAA deficit can be accounted for by lesions and WM involvement. Higher deficits (in the crosshatch, loss of more than -37%) cannot be accounted by WM deficits, therefore, must include GM involvement as well. Bottom, b: Individuals' WBNAA levels of 42 RR MS patients as a function of their Confirmed Diagnosis disease duration. The groupings: "Stable", "Moderate" and "Rapid" were determined according to[91]. Solid lines are the regression for each subgroup, and the dashed lines are their ±95% certainty intervals.*

IV. How early in its course does MS neuronal dysfunction occur?

To ascertain how early does neuroaxonal pathology occur in MS, Filippi *et al.* obtained the WBNAA levels in patients experiencing a clinically isolated syndrome suggestive of MS (CIS), the first presentation of the disease[49]. Their WBNAA was significantly lower (mean 20%) than their controls but not different from patients with or without enhancing lesions at baseline MRI or between patients with and without lesion dissemination in time[49]. Nor did the WBNAA correlate with lesion volumes. To assess whether such axonal damage is transient or persistent, WBNAA scans were repeated at 1 year showing an average 7% decrease in the CIS patients[76]. These preliminary findings suggest that widespread axonal pathology may occur even at the earliest stage of MS, independent of MRI-visible inflammation and too extensive to be reversible. These results also suggest that axonal pathology may not always be the end-stage of repeated inflammatory events, making a strong case for early neuroprotective intervention.

V. WBNAA as MS predictor, monitor and selector

Although disease modifying treatment has been available for nearly two decades, at an annual cost of

about $15,000/patient[77], spending in the US exceeds $3 billion[78]. Consequently, outside the US its cost/benefit is controversial and not universally offered. In the UK for example interferon is administered to only 3% of patients[79]. Considering the (young) age of onset, decades of duration, high cost of treatment, its side-effects and inconvenience (subcutaneous injection), newly diagnosed patients face three questions: *(i)* What will *my* course be? *(ii)* Is *my* disease activity severe enough to need therapy? *(iii)* If so, how effective is it for *me*?

Unfortunately, there are currently no reliable long-term prognostic indices to provide definitive answers to these questions. Clinical and cognitive measures do not predict course[80-82], whereas laboratory markers of progression, *e.g.,* oligoclonal bands, have been only moderately useful and are invasive[83]. MRI methods, while diagnostic for individuals experiencing an unconfirmed clinical event[84-86], provide little prognostic information due to the variable course and pathological heterogeneity of the disease[87-89]. Furthermore, other quantitative MRI metrics such as T_1 lesion loads DTI or global or local atrophy represent end-stages of several pathological processes but do not identify their nature[64, 90].

This paucity of reliable clinical prognostic metrics in MS confounds treatment choices, making laboratory tools to predict and monitor an individual's disease course crucial. To investigate the potential role of WBNAA, its rate of decline was quantified in 49 RR-MS patients by dividing their difference from the average of controls, by their disease duration[91]. Three distinct subgroups of cross-sectional decline rates were found: ~15% of the patients were considered "stable" (annual loss ~0%), 65% were considered "moderate" (annual loss of 2.8%) and 20% were "rapid" with an annual loss of 28%, as shown in Fig. 6b[91].

It is noteworthy that the average clinical expanded disability status scale (EDSS) score in each one of these three subgroups *was the same*, 2.0, representing a very slight impairment in all. These results were recently replicated in a different 20 RR-MS patient cohort[92], indicating that they are probably characteristics of MS and corresponds with its known epidemiology, *e.g.,* as described by Weinshenker as comprising "benign," "intermediate" and "acute"[4], rather than being a particular recruitment pattern. Although they need to be confirmed in a longitudinal study, these findings suggest that WBNAA might *(a)* be a sensitive gauge of clinical course; *(b)* might help monitor treatment; and *(c)* might serve as tool to identify patients for clinical trials based on similarity of metabolic disease activity, rather than clinical status. Specifically:

V.a. For prognosis

A major concern for newly diagnosed patients is the future course of their disease. As yet, no clinical or paraclinal measure provides a definitive forecast. While Scott *et al* isolated six indices to identify patients at 'high risk' for more rapid progression[93], these applied to only 24% of his cohort, underscoring the difficulty of assigning prognosis. WBNAA dynamics in contrast, may provide prognostic measure for *all* patients. Specifically, "Stable" patients may anticipate decades of little accumulation of cerebral pathology, hence, no need for therapeutic intervention. The majority of patients, those exhibiting "Moderate" decline, may expect to follow the established model of MS progression with its 10 and 20 year disability landmarks[4] due perhaps to their clinically recognized course and duration. Finally, those in the "Rapid" subgroup should perhaps be advised, despite a short disease duration, of the likelihood of decline in their quality of life[4, 94, 95] and encouraged to engage in aggressive treatment[96, 97].

V.b. For staging/prioritizing treatment

Criteria for treatment of MS vary from country to country. Enrollment into therapeutic regimens is usually determined by clinical status, age, general health, acceptance of injection regime and, increasingly, funding. WBNAA suggests that clinically similar RR MS patients accumulate axonal pathology at significantly different rates. Therefore, their WBNAA dynamics may provide the sought non-invasive indication for staging treatment. Specifically, it may be beneficial to start medication from the most rapidly declining population and proceed to include more patients with lesser predicted severity as the available resources allow[96].

V.c. For patient stratification for clinical trials

It is now ethically unacceptable to conduct a prospective study of MS treatment of unknown efficacy given that proven ones are already available. Since new drug trials must use the least number of patients for the shortest period of time, induction based on pathological rather than clinical disease status could potentially raise their efficiency. Different levels of pathology and dynamics, shown in Fig. 6 to exist in the general RR MS population, could confound phase II and III clinical trials with type I and II statistical errors[98]. Type I errors may be encountered when "Stable" patients favorably bias an ineffective drug as efficacious. The more costly type II errors could be incurred when "Rapid" patients erroneously cause rejection of an effective drug due to inadequate response. Considering the cost of pharmaceutical development, these could be expensive mistakes[98]. Consequently, randomized recruitment based entire-

ly on clinical metrics such as EDSS will necessitate larger sample sizes and longer durations to achieve a given statistical power[97, 99] than homogeneous "Moderate" or "Rapid" cohorts selected based on WBNAA dynamics.

❑ Conclusions

Amongst the new MR techniques introduced for the quantitative assessment of MS, ^{1}H-MRS, due to its unique specificity, seems to hold the greatest promise. Not only does it appear to be a clinically relevant and specific surrogate marker of neuroaxonal injury, but it also provides both an early indicator of disease activity as well as a reliable measure of disease-related tissue changes overtime. WBNAA spectroscopy builds on these advantages by providing a global measure of neuroaxonal burden and an excellent tool for serial assessment of disease progression. The increasing evidence of a neurodegenerative component in the pathology of MS has motivated development of treatments aimed at neuroprotection and consequently the search for and development of the appropriate surrogate markers of therapeutic response. The specificity, precision, multi-hardware reproducibility and insensitivity to repositioning in serial studies of the WBNAA measurements render the techniques well-suited and reliable surrogate marker for monitoring neurodegeneration and neuroprotection in response to current and experimental treatments.

BIBLIOGRAFÍA

1.- Hauser SL. Multiple sclerosis and other demyelinating diseases. In: Isselbacher KJ, Wilson JD, Martin JB, Fauci AS, Kasper DL, eds. Harrison's Principles of Internal Medicine. New York: McGraw-Hill, 1994; 2287-2295.

2.- Weinshenker BG, Rice GP, Noseworthy JH, Carriere W, Baskerville J, Ebers GC. The natural history of multiple sclerosis: a geographically based study. 4. Applications to planning and interpretation of clinical therapeutic trials. *Brain* 1991; 114: 1057-1067.

3.- Compston A, Coles A. Multiple sclerosis. Lancet 2002; 359:1221-1231.

4.- Weinshenker BG. Natural-History of Multiple-Sclerosis. *Annals of Neurology* 1994; 36: S6-S11.

5.- Wingerchuk DM, Weinshenker BG. Multiple sclerosis: epidemiology, genetics, classification, natural history, and clinical outcome measures. Neuroimaging *Clin N Am* 2000; 10: 611-624, vii.

6.- Kantarci OH, Weinshenker BG. Natural history of multiple sclerosis. *Neurol Clin* 2005; 23: 17-38, v.

7.- Ferguson B, Matyszak MK, Esiri MM, Perry VH. Axonal damage in acute multiple sclerosis lesions. *Brain* 1997; 120: 393-399.

8.- Lexa FJ, Grossman RI, Rosenquist AC. MR of wallerian degeneration in the feline visual system: Characterization by magnetization transfer rate with hystopathologic correlation. *Am. J. Neuroradiol* 1994; 15: 201-212.

9.- Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998; 338: 278-285.

10.- Trapp BD, Ransohoff R, Rudick R. Axonal pathology in multiple sclerosis: relationship to neurologic disability. *Curr Opin Neurol* 1999; 12: 295-302.

11.- Narayanan S, Fu L, Pioro E, *et al.* Imaging of axonal damage in multiple sclerosis: Spatial distribution of magnetic resonance imaging lesions. *Annals of Neurology* 1997; 41: 385-391.

12.- De Stefano N, Narayanan S, Matthews PM, Francis GS, Antel JP, Arnold DL. In vivo evidence for axonal dysfunction remote from focal cerebral demyelination of the type seen in multiple sclerosis. *Brain* 1999; 122: 1933-1939.

13.- Sager TN, Thomsen C, Valsborg JS, Laursen H, Hansen AJ. Astroglia contain a specific transport mechanism for N-acetyl-L-aspartate. *J Neurochem* 1999; 73: 807-811.

14.- Moffett JR, Namboodiri MA, Cangro CB, Neale JH. Immunohistochemical localization of N-acetylaspartate in rat brain. *Neuroreport* 1991; 2: 131-134.

15.- Simmons ML, Frondoza CG, Coyle JT. Immunocytochemical localization of N-acetyl-aspartate with monoclonal antibodies. *Neuroscience* 1991; 45: 37-45.

16.- Clark JB. *N*-Acetylaspartate: A marker for neuronal loss or mitochondrial dysfunction. *Dev. Neurosci.* 1998; 20: 271-276.

17.- Tallan HH, Moore S, Stein WH. N-acetyl-L-aspartic acid in brain. *J. Biol. Chem.* 1956; 219: 257-264.

18.- Jenkins BG, Klivenyi P, Kustermann E, *et al.* Nonlinear decrease over time in N-acetyl aspartate levels in the absence of neuronal loss and increases in glutamine and glucose in transgenic Huntington's disease mice. *J Neurochem* 2000; 74: 2108-2119.

19.- Baslow MH, Resnik TR. Canavan disease. Analysis of the nature of the metabolic lesions responsible for development of the observed clinical symptoms. *J Mol Neurosci* 1997; 9: 109-125.

20.- Danielsen EA, Ross B. Magnetic Resonance Spectros-

copy Diagnosis of Neurological Diseases. New York: Marcel Dekker, Inc., 1999.
21.- Ross BD, Bluml S. Magnetic resonance Spectroscopy of the human brain. *Anat Rec* 2001; 265: 54-84.
22.- Davie CA, Hawkins CP, Barker GJ, *et al.* Serial proton magnetic resonance spectroscopy in acute multiple sclerosis lesions. *Brain* 1994; 117:49-58.
23.- De Stefano N, Narayanan S, Mortilla M, *et al.* Imaging axonal damage in multiple sclerosis by means of MR spectroscopy. *Neurol Sci* 2000; 21: S883-887.
24.- Bjartmar C, Battistuta J, Terada N, Dupree E, Trapp BD. N-acetylaspartate is an axon-specific marker of mature white matter in vivo: a biochemical and immunohistochemical study on the rat optic nerve. *Ann Neurol* 2002; 51: 51-58.
25.- Bitsch A, Bruhn H, Vougioukas V, *et al.* Inflammatory CNS demyelination: histopathologic correlation with in vivo quantitative proton MR spectroscopy. AJNR *Am J Neuroradiol* 1999; 20: 1619-1627.
26.- Bjartmar C, Kidd G, Mork S, Rudick R, Trapp BD. Neurological disability correlates with spinal cord axonal loss and reduced N-acetyl aspartate in chronic multiple sclerosis patients. A*nnals of Neurology* 2000; 48: 893-901.
27.- Goldstein FB. Biosynthesis of N-acetyl-L-aspartic acid. *Biochim Biophys Acta* 1959; 33: 583-584.
28.- Baslow MH, Guilfoyle DN. Effect of N-acetylaspartic acid on the diffusion coefficient of water: a proton magnetic resonance phantom method for measurement of osmolyte-obligated water. *Anal Biochem* 2002; 311: 133-138.
29.- Chakraborty G, Mekala P, Yahya D, Wu G, Ledeen RW. Intraneuronal N-acetylaspartate supplies acetyl groups for myelin lipid synthesis: evidence for myelin-associated aspartoacylase. *J Neurochem* 2001; 78: 736-745.
30.- Madhavarao CN, Chinopoulos C, Chandrasekaran K, Namboodiri MA. Characterization of the N-acetylaspartate biosynthetic enzyme from rat brain. *J Neurochem* 2003; 86: 824-835.
31.- Yan HD, Ishihara K, Serikawa T, Sasa M. Activation by N-acetyl-L-aspartate of acutely dissociated hippocampal neurons in rats via metabotropic glutamate receptors. *Epilepsia* 2003; 44: 1153-1159.
32.- Brooks WM, Friedman SD, Stidley CA. Reproducibility of 1H-MRS in vivo. *Magn Reson Med* 1999; 41: 193-197.
33.- Marshall I, Wardlaw J, Cannon J, Slattery J, Sellar RJ. Reproducibility of metabolite peak areas in 1H MRS of brain [see comments]. *Magn Reson Imaging* 1996; 14: 281-292.
34.- Bokde AL, Teipel SJ, Zebuhr Y, *et al.* A new rapid landmark-based regional MRI segmentation method of the brain. *J Neurol Sci* 2002; 194: 35-40.
35.- Inglese M, Ge Y, Filippi M, Falini A, Grossman RI, Gonen O. Indirect evidence for early widespread gray matter involvement in relapsing-remitting multiple sclerosis. *Neuroimage* 2004; 21: 1825-1829.
36.- De Stefano N, Narayanan S, Francis SJ, *et al.* Diffuse axonal and tissue injury in patients with multiple sclerosis with low cerebral lesion load and no disability. *Arch Neurol* 2002; 59: 1565-1571.
37.- Zaaraoui W, Fleysher L, Fleysher R, Liu S, Soher BJ, Gonen O. Human brain-structure resolved T(2) relaxation times of proton metabolites at 3 Tesla. *Magn Reson Med* 2007; 57: 983-989.
38.- Gonen O, Viswanathan AK, Catalaa I, Babb J, Udupa J, Grossman RI. Total brain N-acetylaspartate concentration in normal, age-grouped females: quantitation with non-echo proton NMR spectroscopy. *Magn Reson Med* 1998; 40: 684-689.
39.- Miki Y, Grossman RI, Udupa JK, *et al.* Relapsing-remitting multiple sclerosis: longitudinal analysis of MR images--lack of correlation between changes in T2 lesion volume and clinical findings. *Radiology* 1999; 213: 395-399.
40.- Ebel A, Govindaraju V, Maudsley AA. Comparison of inversion recovery preparation schemes for lipid suppression in 1H MRSI of human brain. *Magn Reson Med* 2003; 49: 903-908.
41.- Udupa JK, Odhner D, Samarasekera S. 3DVIEWNIX: an open, transportable, multdimensional, multimodality, multiparametric imging software system. *Proc SPIE* 1994; 2164: 58-73.
42.- De Santi S, de Leon MJ, Rusinek H, *et al.* Hippocampal formation glucose metabolism and volume losses in MCI and AD. *Neurobiol Aging* 2001; 22: 529-539.
43.- Ashburner J, Hutton C, Frackowiak R, Johnsrude I, Price C, Friston K. Identifying global anatomical differences: deformation-based morphometry. *Hum Brain Mapp* 1998; 6: 348-357.
44.- Smith SM, De Stefano N, Jenkinson M, Matthews PM. Normalized accurate measurement of longitudinal brain change. *J Comput Assist Tomogr* 2001; 25: 466-475.
45.- Smith SM, Zhang Y, Jenkinson M, *et al.* Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *Neuroimage* 2002; 17: 479-489.
46.- Smith SM, Rao A, De Stefano N, *et al.* Longitudinal and cross-sectional analysis of atrophy in Alzheimer's disease: Cross-validation of BSI, SIENA and SIENAX. *Neuroimage* 2007.
47.- Li S, Dardzinski BJ, Collins CM, Yang QX, Smith MB. Three-dimensional mapping of the static field inside the human head. *Magn. Reson. Med.* 1996; 36: 705-714.
48.- Gonen O, Grossman RI. The accuracy of whole brain N-acetylaspartate quantification. *Magn Reson Imaging* 2000; 18: 1255-1258.

49.- Filippi M, Bozzali M, Rovaris M, *et al.* Evidence for widespread axonal damage at the earliest clinical stage of multiple sclerosis. *Brain* 2003; 126: 433-437.

50.- Benedetti B, Rigotti DJ, Liu S, Filippi M, Grossman RI, Gonen O. Reproducibility of the Whole-Brain N-Acetylaspartate Level across Institutions, MR Scanners, and Field Strengths. *AJNR Am J Neuroradiol* 2007; 28: 72-75.

51.- Rigotti DJ, Inglese M, Babb JS, *et al.* Serial Whole-Brain N-acetylaspartate Concentration in Healthy Young Adults. *Am J Neuroradiol* 2007; *In Press*.

52.- Scahill RI, Frost C, Jenkins R, Whitwell JL, Rossor MN, Fox NC. A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. *Arch Neurol* 2003; 60: 989-994.

53.- Sayao AL, Devonshire V, Tremlett H. Longitudinal follow-up of "benign" multiple sclerosis at 20 years. *Neurology* 2007; 68: 496-500.

54.- Montalban X. Primary progressive multiple sclerosis. *Curr Opin Neurol* 2005; 18: 261-266.

55.- Weinshenker BG. The natural history of multiple sclerosis. *Neurol Clin* 1995; 13: 119-146.

56.- Weinshenker BG. The natural history of multiple sclerosis: update 1998. *Semin Neurol* 1998; 18: 301-307.

57.- Cree B. Emerging monoclonal antibody therapies for multiple sclerosis. *Neurologist* 2006; 12: 171-178.

58.- Hartung HP, Bar-Or A, Zoukos Y. What do we know about the mechanism of action of disease-modifying treatments in MS? *J Neurol* 2004; 251 Suppl 5:v12-v29.

59.- Fox EJ. Management of worsening multiple sclerosis with mitoxantrone: a review. *Clin Ther* 2006; 28: 461-474.

60.- Miller DH, Thompson AJ, Filippi M. Magnetic resonance studies of abnormalities in the normal appearing white matter and grey matter in multiple sclerosis. *J Neurol* 2003; 250: 1407-1419.

61.- Gonen O, Catalaa I, Babb JS, *et al.* Total brain N-acetylaspartate: a new measure of disease load in MS. *Neurology* 2000; 54: 15-19.

62.- Rovaris M, Gallo A, Falini A, *et al.* Axonal injury and overall tissue loss are not related in primary progressive multiple sclerosis. *Arch Neurol* 2005; 62: 898-902.

63.- Filippi M, Horsfield MA, Hajnal JV, *et al.* Quantitative assessment of magnetic resonance imaging lesion load in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1998; 64 Suppl 1: S88-93.

64.- Li DK, Held U, Petkau J, *et al.* MRI T2 lesion burden in multiple sclerosis: a plateauing relationship with clinical disability. *Neurology* 2006; 66: 1384-1389.

65.- Weinshenker BG. Review: magnetic resonance imaging alone is of limited usefulness in diagnosing multiple sclerosis. *ACP J Club* 2006; 145: 51.

66.- Ge Y, Gonen O, Inglese M, Babb JS, Markowitz CE, Grossman RI. Neuronal cell injury precedes brain atrophy in multiple sclerosis. *Neurology* 2004; 62: 624-627.

67.- Lumsden C. The neuropathology of multiple sclerosis. In: Vinken Pj BG, ed. Handbook of Clinical Neurology. Amsterdam: North-Holland, 1970; 217-309.

68.- Courchesne E, Chisum HJ, Townsend J, *et al.* Normal brain development and aging: quantitative analysis at in vivo MR imaging in healthy volunteers. *Radiology* 2000; 216: 672-682.

69.- Wang Y, Li SJ. Differentiation of metabolic concentrations between gray matter and white matter of human brain by in vivo 1H magnetic resonance spectroscopy. *Magn Reson Med* 1998; 39: 28-33.

70.- Bjartmar C, Kinkel RP, Kidd G, Rudick RA, Trapp BD. Axonal loss in normal-appearing white matter in a patient with acute MS. *Neurology* 2001; 57: 1248-1252.

71.- Cifelli A, Arridge M, Jezzard P, Esiri MM, Palace J, Matthews PM. Thalamic neurodegeneration in multiple sclerosis. *Ann Neurol* 2002; 52: 650-653.

72.- Peterson JW, Bo L, Mork S, Chang A, Trapp BD. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. *Ann Neurol* 2001; 50: 389-400.

73.- Kidd D, Barkhof F, McConnell R, Algra PR, Allen IV, Revesz T. Cortical lesions in multiple sclerosis. *Brain* 1999; 122: 17-26.

74.- Bozzali M, Cercignani M, Sormani MP, Comi G, Filippi M. Quantification of brain gray matter damage in different MS phenotypes by use of diffusion tensor MR imaging. *AJNR Am J Neuroradiol* 2002; 23: 985-988.

75.- Ge Y, Grossman RI, Udupa JK, Babb JS, Kolson DL, McGowan JC. Magnetization transfer ratio histogram analysis of gray matter in relapsing-remitting multiple sclerosis. *AJNR Am J Neuroradiol* 2001; 22: 470-475.

76.- Rovaris M, Gambini A, Gallo A, *et al.* Axonal injury in early multiple sclerosis is irreversible and independent of the short-term disease evolution. *Neurology* 2005; 65: 1626-1630.

77.- Compston A. Interferon beta in multiple sclerosis - Reply. *Lancet* 1999; 354: 512-513.

78.- NIH. Multiple Sclerosis NIH Guide 1993; 22: 8-11.

79.- Hartung HP. NICE and drugs for multiple sclerosis. *Lancet* 2000; 356: 1114-1114.

80.- Willoughby E, Paty D. Scales for rating imparement in multiple sclerosis: A critique. *Neurology* 1998; 38: 1793-1798.

81.- Filippi M, Iannucci G, Tortorella C, *et al.* Comparison of MS clinical phenotypes using conventional and magnetization transfer MRI. *Neurology* 1999; 52: 588-594.
82.- Krupp LB, Elkins LE. Fatigue and declines in cognitive functioning in multiple sclerosis. *Neurology* 2000; 55: 934-939.
83.- Tas MW, Barkhol F, Vanwalderveen MAA, Polman CH, Hommes OR, Valk J. The Effect of Gadolinium on the Sensitivity and Specificity of MR in the Initial Diagnosis of Multiple-Sclerosis. *American Journal of Neuroradiology* 1995; 16: 259-264.
84.- Jacobs LD, Kaba SE, Miller CM, Priore RL, Brownscheidle CM. Correlation of clinical, magnetic resonance imaging, and cerebrospinal fluid findings in optic neuritis. *Annals of Neurology* 1997; 41: 392-398.
85.- Fazekas F, Barkhof F, Filippi M, *et al.* The contribution of magnetic resonance imaging to the diagnosis of multiple sclerosis. *Neurology* 1999; 53: 448-456.
86.- McDonald WI, Compston A, Edan G, *et al.* Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001; 50: 121-127.
87.- Pulizzi A, Rovaris M, Judica E, *et al.* Determinants of disability in multiple sclerosis at various disease stages: a multiparametric magnetic resonance study. *Arch Neurol* 2007; 64: 1163-1168.
88.- Charil A, Yousry TA, Rovaris M, *et al.* MRI and the diagnosis of multiple sclerosis: expanding the concept of "no better explanation". *Lancet Neurol* 2006; 5: 841-852.
89.- Miller DH, Filippi M, Fazekas F, *et al.* Role of magnetic resonance imaging within diagnostic criteria for multiple sclerosis. *Ann Neurol* 2004; 56: 273-278.
90.- Filippi M, Falini A, Arnold DL, *et al.* Magnetic resonance techniques for the in vivo assessment of multiple sclerosis pathology: Consensus report of the white matter study group. *J Magn Reson Imaging* 2005; 21: 669-675.
91.- Gonen O, Moriarty DM, Li BS, *et al.* Relapsing-remitting multiple sclerosis and whole-brain N-acetylaspartate measurement: evidence for different clinical cohorts initial observations. *Radiology* 2002; 225: 261-268.
92.- Gonen O, Oberndorfer TA, Inglese M, Babb JS, Herbert J, Grossman RI. Reproducibility of three whole-brain N-acetylaspartate decline cohorts in relapsing-remitting multiple sclerosis. *AJNR Am J Neuroradiol* 2007; 28: 267-271.
93.- Cott TF, Schramke CJ, Novero J, Chieffe C. Short-term prognosis in early relapsing-remitting multiple sclerosis. *Neurology* 2000; 55: 689-693.
94.- De Stefano N, Matthews PM, Fu LQ, *et al.* Axonal damage correlates with disability in patients with relapsing-remitting multiple sclerosis - Results of a longitudinal magnetic resonance spectroscopy study. *Brain* 1998; 121: 1469-1477.
95.- De Stefano N, Narayanan S, Francis GS, *et al.* Evidence of axonal damage in the early stages of multiple sclerosis and its relevance to disability. *Arch Neurol* 2001; 58: 65-70.
96.- Schwid SR, Bever CT. The cost of delaying treatment in multiple sclerosis. What is lost is not regained. *Neurology* 2001; 56: 1620.
97.- Comi G, Filippi M, Barkhof F, *et al.* Effect of early interferon treatment on conversion to definite multiple sclerosis: A randomised study. *Lancet* 2001; 357: 1576-1582.
98.- Rogatko A, Litwin S. Phase II studies: which is worse, false positive or false negative? *J Natl Cancer Inst* 1996; 88: 462.
99.- Sormani MP, Miller DH, Comi G, *et al.* Clinical trials of multiple sclerosis monitored with enhanced MRI: New sample size calculations based on large data sets. *J Neurol Neurosurg Psychiatry* 2001; 70: 494-499.