[Frontiers in Bioscience 9, 647-664, January 1, 2004]

ROLE OF MRI IN MULTIPLE SCLEROSIS II: BRAIN AND SPINAL CORD ATROPHY

Robert Zivadinov [1-3], and Rohit Bakshi [1-4]

[1] Buffalo Neuroimaging Analysis Center, [2] The Jacobs Neurological Institute, [3] Department of Neurology, School of Medicine and Biomedical Sciences, University at Buffalo, State University of New York, and [4] Physicians Imaging Centers, Buffalo, NY

TABLE OF CONTENTS

1. ABSTRACT

A growing body of evidence indicates that irreversible tissue destruction including axonal and neuronal degeneration is a key component of the multiple sclerosis (MS) disease process. Magnetic resonance imaging (MRI) is a powerful technique that can be combined with semiautomated or automated computer assisted analysis approaches to detect progressive atrophy of the brain and spinal cord with high sensitivity and reproducibility. The pathophysiology of central nervous system (CNS) atrophy in MS is unknown but likely represents an epiphenomenon related to the effects of inflammation including chronic demyelination, axonal injury, neuronal loss and Wallerian degeneration. Other factors that may contribute to tissue atrophy include injury to the normal appearing gray and white matter by mechanisms such as loss of growth factors, altered electrical conduction and pathologic iron deposition. Prospective studies have suggested that atrophy in MS is predicted by previous inflammatory activity as measured by overt MRI lesions. Gadolinium (Gd)-enhancing lesions have shown a particularly strong predictive value in some but not all longitudinal studies of brain atrophy. Brain atrophy has also been related in cross-sectional and longitudinal studies to T2-hypointense lesions in deep grey matter, suggesting a link between tissue iron deposition and atrophy. The measurement of brain atrophy seems to be of growing clinical relevance as a biomarker of the MS disease process. Atrophy should now be included as a secondary endpoint in trials of therapies aimed at limiting disease progression. Currently available anti-inflammatory immunomodulatory agents and immunosuppressive treatments, while effective at preventing clinical deterioration, have shown at best partial effects in preventing CNS atrophy. Thus, there is a need to further validate atrophy as an outcome measure and ultimately develop treatment strategies that will protect against the destructive aspects of the disease process. This should in turn lead to better long term neurologic functioning and a better quality of life for patients with MS.

2. INTRODUCTION

Progressive brain atrophy in multiple sclerosis (MS) has been recognized for many years. It was shown in early computed tomography (CT) studies that atrophy can develop within one year of the diagnosis in MS patients with an aggressive clinical course (1). Other CT studies correlated atrophy with dementia (2). A study in 1995 suggested a link between atrophy and axonal dysfunction; reduced N-acetyl aspartate (NAA) levels detected by magnetic resonance spectroscopy (MRS) were associated with both cerebellar dysfunction and cerebellar atrophy in patients with MS (3). Three decades ago, histologic evidence emerged that demyelination alone results in a reduction of axonal diameter (4) suggesting that atrophy can occur by at least two mechanisms: loss of myelin per se and associated axonal shrinkage. However, only a few years ago magnetic resonance imaging (MRI) and pathological studies confirmed that atrophy may occur by at least two mechanisms, one potentially reversible (demyelination) and the other not likely to reverse (axonal loss) (5,6). The extent to which atrophy leads to neurologic impairment is probably influenced by both the underlying substrate of the damage that has occurred and the amount of reserve capacity present in relatively non-affected areas. When severe tissue destruction occurs, the central nervous system (CNS) responds by shrinkage and reorganization, leading to reduced volume of brain and spinal parenchyma

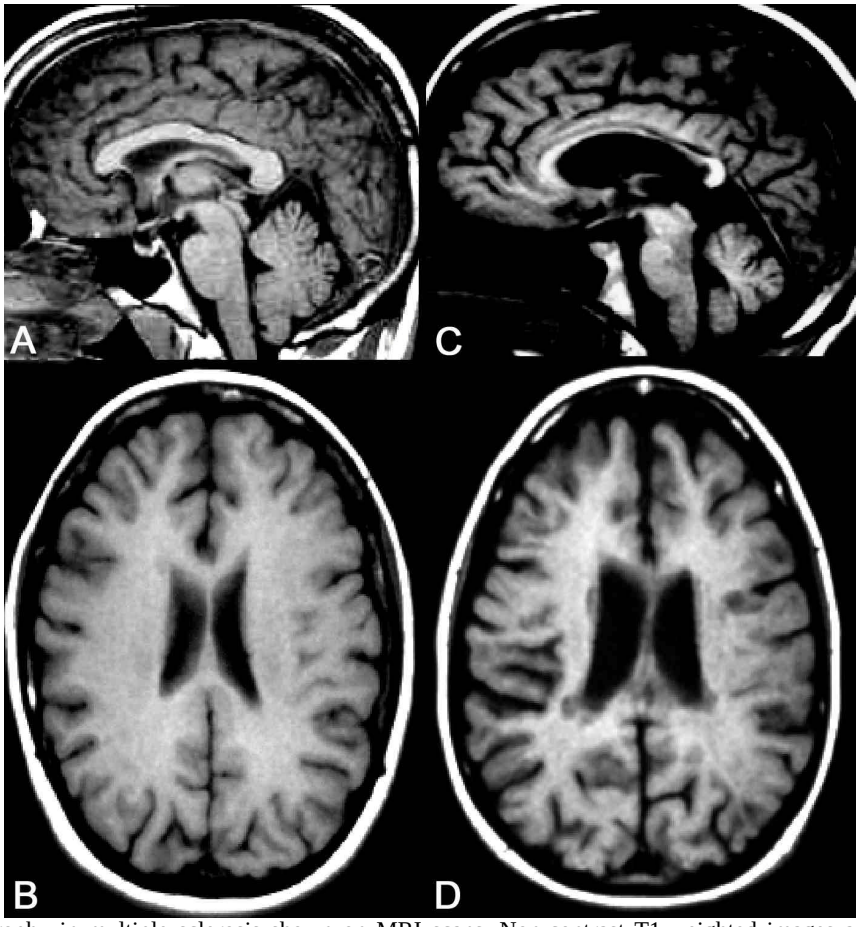

Figure 1. Brain atrophy in multiple sclerosis shown on MRI scans. Non-contrast T1-weighted images are shown of normal controls in the fifth decade (a-b) compared with age-matched patients with MS (c-d). Note thinning and decreased volume of corpus callosum and posterior fossa structures in MS (c). Note prominence of cortical sulci (suggesting cortical atrophy) and ventriculomegaly (indicating central atrophy) in MS (d). Using this type of visual analysis, ordinal rating systems can semiquantitatively measure atrophy (113).

and enlargement of adjacent cerebrospinal fluid (CSF) spaces. Several pathologic and imaging studies have revealed axonal/neuronal loss, Wallerian degeneration, and depletion of axonal/neuronal metabolites in the normal appearing white and gray matter (5-7). Another factor that has been linked to atrophy includes T2 hypointensity in the gray matter, a marker of pathologic iron deposition (8).

3. QUANTIFICATION OF BRAIN ATROPHY

There are several methods available to estimate the degree of brain atrophy by MRI analysis techniques. These involve absolute or normalized measurements of parenchyma or CSF volumes. If brain tissue is being replaced by CSF due to a disease process, then the tissue loss or atrophy will be associated with increases in CSF and a decrease in brain volume (Figure 1). A variety of brain atrophy measurement techniques are available. These include increased absolute or normalized volume of CSF spaces or decreased absolute or normalized volume of brain parenchymal structures (Figures 1-3). Normalization refers to the correction of raw volumes according to the head size (intracranial volume). The methods for quantification of atrophy can be classified as regional or global; manual; semiautomated and automated; 2D or 3D; absolute or normalized.

3.1. Regional methods

Recently, several regional brain atrophy measures have been applied in natural history studies and clinical trials of MS (9-23) (Table 1) (Figure 1, 4 and 5).

The bicaudate ratio is a normalized linear regional brain atrophy measure that reflects subcortical atrophy and is closely associated with cognitive dysfunction even after accounting for other MRI surrogate markers including whole brain atrophy (17) (Figure 5). In MS, atrophy of the corpus callosum is commonly detected by MRI (9) (Figure 1). In a two year natural history study of mildly disabled patients with relapsing-remitting (RR) MS loss of corpus callosum area occurred at a rate of 5.5% per year (9).

Many other regional brain atrophy measures have been tested in MS (see Table 1 and Figure 1 and 5). These include third (9,12) and lateral ventricle width (9),

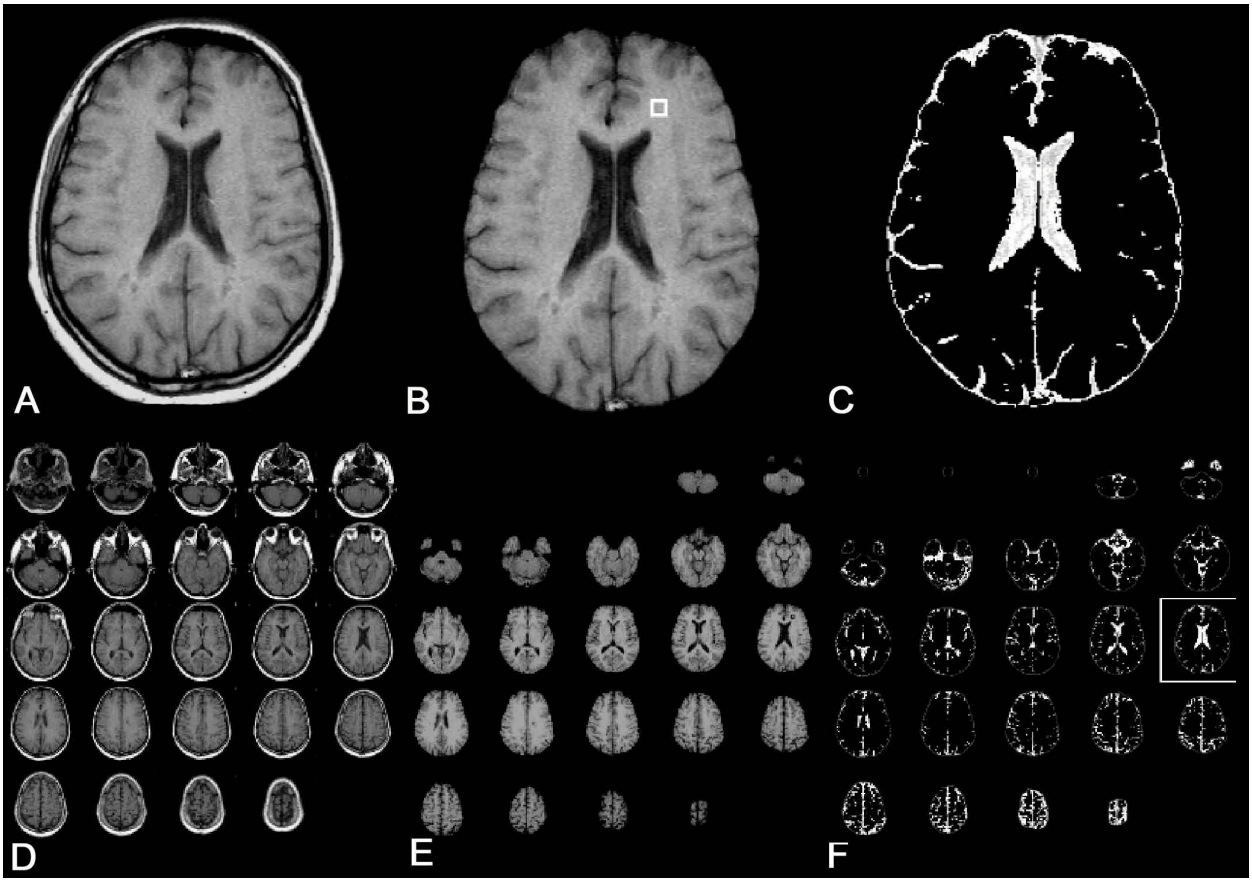

Figure 2. Semiautomated method of determining brain parenchymal fraction, a normalized measure of whole brain atrophy in MS, from the Buffalo Neuroimaging Analysis Center (adapted in part from ref. 37). (a-c): A single axial slice, spin-echo T1-weighted noncontrast, from an MS patient showing the raw image (a), after masking (removal) of extracranial tissue has been performed to isolating the outer brain contour (b), and after thresholding to separate the intracranial volume into brain parenchyma (black pixels) and CSF (white pixels) (c). (d-f): Shows that the same algorithm is applied to all axial images from the mid-cerebellum to the vertex. The image surrounded by the large white square (f) is used to identify normal appearing white matter on which a region-of-interest is placed in the normal appearing white matter (b, small white box) to determine the threshold for parenchyma vs. CSF segmentation (37). The BPF is the ratio of the brain parenchymal volume to the intracranial volume (volume within the surface contour including the subarachnoid space) and is thus a normalized measure. With recent software improvements, the total analysis time per patient is 10 minutes.

Table 1. Recent methods for the determination of regional brain atrophy in multiple sclerosis

Method	**Reference**
• Third ventricle width	9
• Lateral ventricle width	9
• Corpus callosum area	9
• Cavalieri method for measurement of infratentorial structures	10
• Enlargement of subarachnoid spaces in various brain regions	11
• Ventricular enlargement and/or volume mesures	12-16
• Bicaudate ratio (intercaudate nucleus ratio)	17, 19
• Caudate atrophy	18
• Four central slices brain volume	20
• Seven contiguous slice method	21
• Normalized regional brain atrophy measures	22
• Thalamic atrophy	23

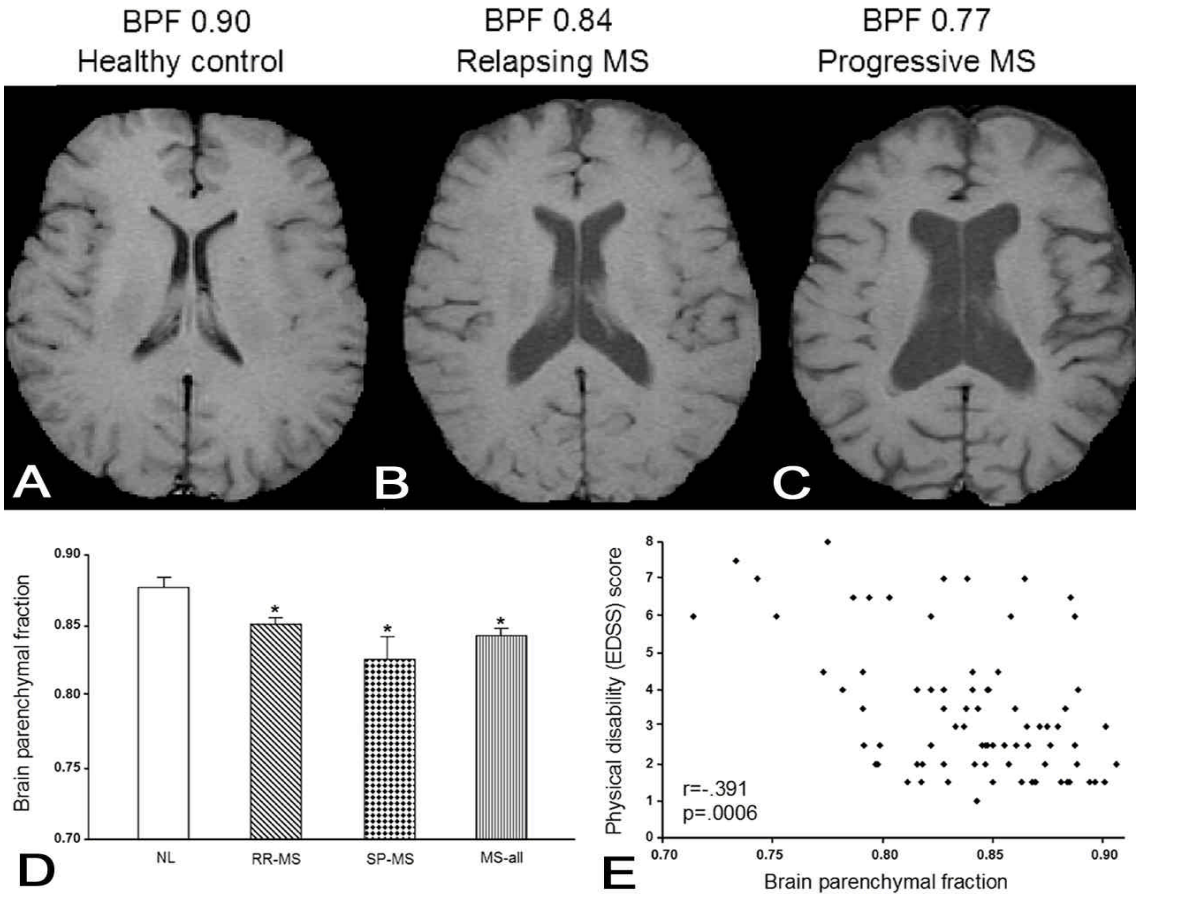

Figure 3. Whole brain atrophy in MS as measured by brain parenchymal fraction (BPF) from the Buffalo Neuroimaging Analysis Center (adapted in part from ref. 37). BPF is the ratio of the brain parenchymal to intracranial volume. Representative mid-ventricular axial MRIs are shown from non-contrast T1-weighted images of age-matched individuals in the fourth decade: (a) – normal volunteer; (b) –relapsing-remitting MS, mild-moderate physical disability, disease duration of 6 years; (c) – secondary progressive MS, moderate-severe disability, disease duration 12 years. Figures (d) and (e) depict whole brain atrophy in patients with MS vs. normal individuals (37). Bargraphs (d) show mean BPF and std. error of mean in MS (n=50, MS-all) and age-/sex-matched controls (n=17, NL). BPF was lower in MS ($p < .001$), indicating whole brain atrophy in MS. Both MS subgroups had lower BPF as compared to NL; BPF was lower in relapsing-remitting (RR) MS (n=40, p= .005. vs. NL) and in secondary progressive (SP) MS (n=10, p=.0005). Asterisks indicate a significant difference compared to NL. A scatter plot (e) shows the relationship between whole brain atrophy and physical disability in 78 patients with MS; brain parenchymal fraction was inversely related to overall physical disability (Expanded Disability Status Scale) score (r=-.391, p=.0006).

ventricular area or volume assessments (12-16), measurement of infratentorial structures (10), the volume of four to seven brain central slices (20,21) and parcellation of specific parenchymal structures in deep gray matter (18,23) (Figure 5).

Cerebellar and infratentorial atrophy is common in RR and secondary progressive (SP) MS (10,24). Significant reductions of 21% and 19% have been reported for the brainstem and cerebellum in comparison with controls in a series of 20 patients with RR and 20 patients with SP MS (24). Regional cortical atrophy has been also measured semiquantitatively based on sulcal enlargement (11) (Figure 1).

Differences in quantitative segmentation morphometry techniques of brain parenchymal regions, due to variations in software algorithms and anatomic landmarks, are challenges yet to be optimally addressed in the precise three dimensional determination of regional lobar and cortical atrophy (25). Recently, we developed a reproducible and sensitive regional brain atrophy method able to measure specific parenchymal regions of interest (ROIs) (Figure 4). Our method incorporates semiautomated and automated segmentation processes (22). For the outlining of the boundaries of each parenchymal ROI (Figure 4a, b and c) a semiautomated iterative morphologic method based on a digital 3D version of Harvard Medical School Atlas has been developed. This method creates an ROI containing parenchyma (Figure 4d, e and f) and CSF (Figure 4g, h and i) (intraregional ROI). Based on an automated segmentation algorithm for histogram thresholding analysis, the segmentation creates normalized and absolute regional brain atrophy measures. The proposed method achieved a good level of intra- and inter-rater reproducibility for various ROIs and required an acceptable amount of operator time.

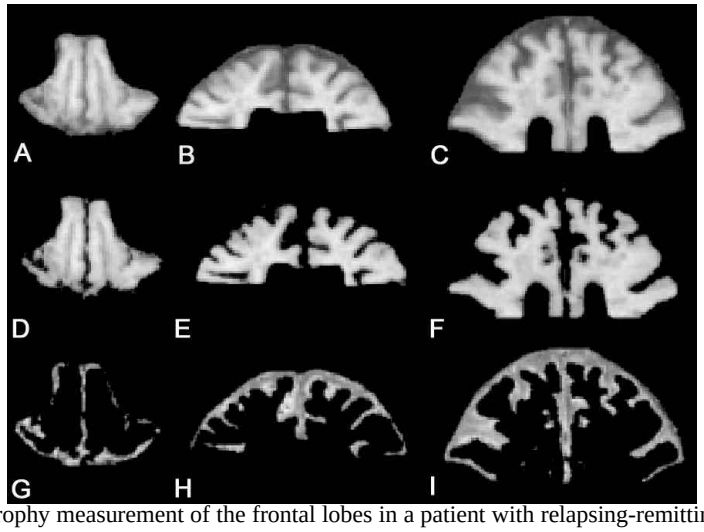

Figure 4. Regional brain atrophy measurement of the frontal lobes in a patient with relapsing-remitting multiple sclerosis. (a), (b) and (c) shows masked T1-weighted images of the frontal lobes segmented at various levels and obtained by semiautomated iterative morphologic outlining of the external cerebrospinal fluid (CSF) spaces; the next six images show the result of segmentation: (d), (e) and (f) are brain parenchyma-only images and (g), (h) and (i) are CSF-only images.

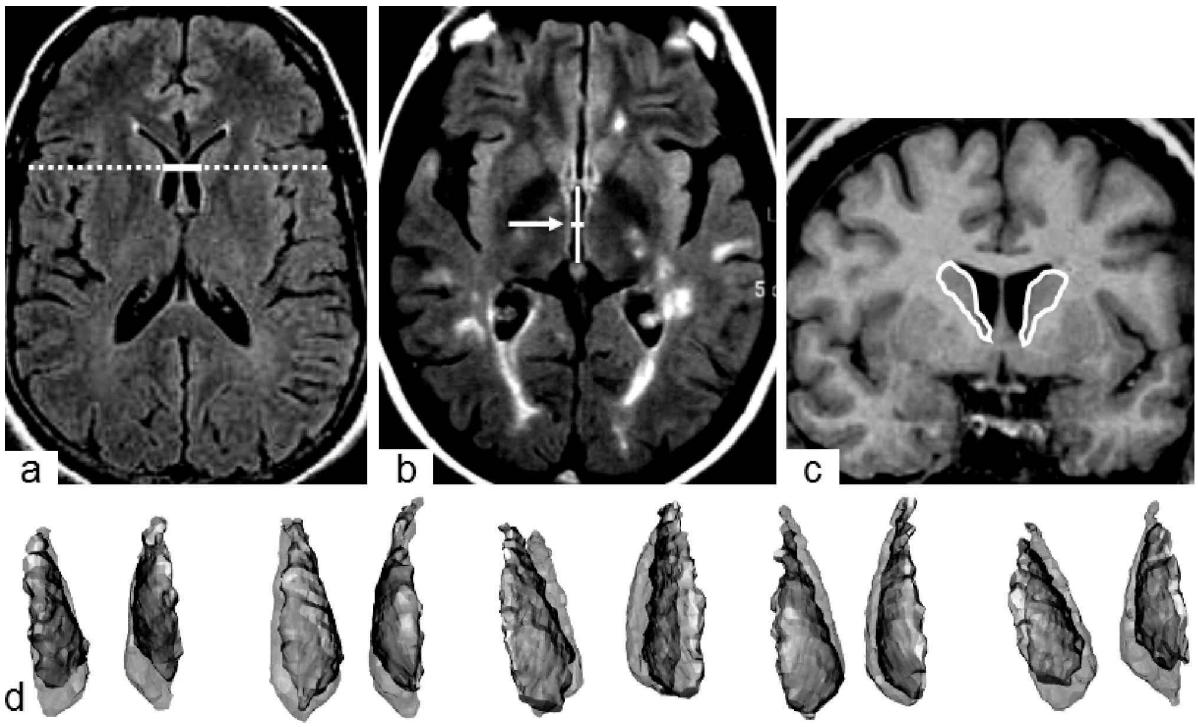

Figure 5. Quantitative regional atrophy analysis techniques in MS from the Buffalo Neuroimaging Analysis Center (adapted in part from refs. 17 and 18). (a): The bicaudate ratio (BCR) (17) or intercaudate nucleus ratio (19) is the minimum intercaudate distance (solid line) divided by brain width along the same line (dashed line). BCR is measured in an axial slice where the heads of the caudate nuclei are most visible and closest together. BCR is elevated in patients with MS, consistent with subcortical atrophy, and is independently associated with cognitive dysfunction (17). (b): A method (8,9) of measuring third ventricle width (3VW), a marker of central atrophy, is shown in a patient with MS. This involves drawing a line through the long axis of the third ventricle, parallel to the inter-hemispheric fissure. The width is measured by drawing a second line perpendicular to the first at its midpoint (arrow). Greater physical disability increments over one and two years in relapsing-remitting patients with MS have been associated with increasing 3VW (9). (c-d): Parcellation of the caudate nuclei in MS (18) involving manually tracing regions of interest delineating the caudate nuclei bilaterally in each slice in which they are visible on high resolution gradient echo coronal T1-weighted images (c). The region of interest files are then reconstructed, normalized, and compared with age-matched normal controls (NL). (d): Superior views of overlaid caudate nuclei from five representative MS (dark gray) and NL subjects (light gray) reveal caudate atrophy in MS; volumes were nearly 20% less than NL (18).

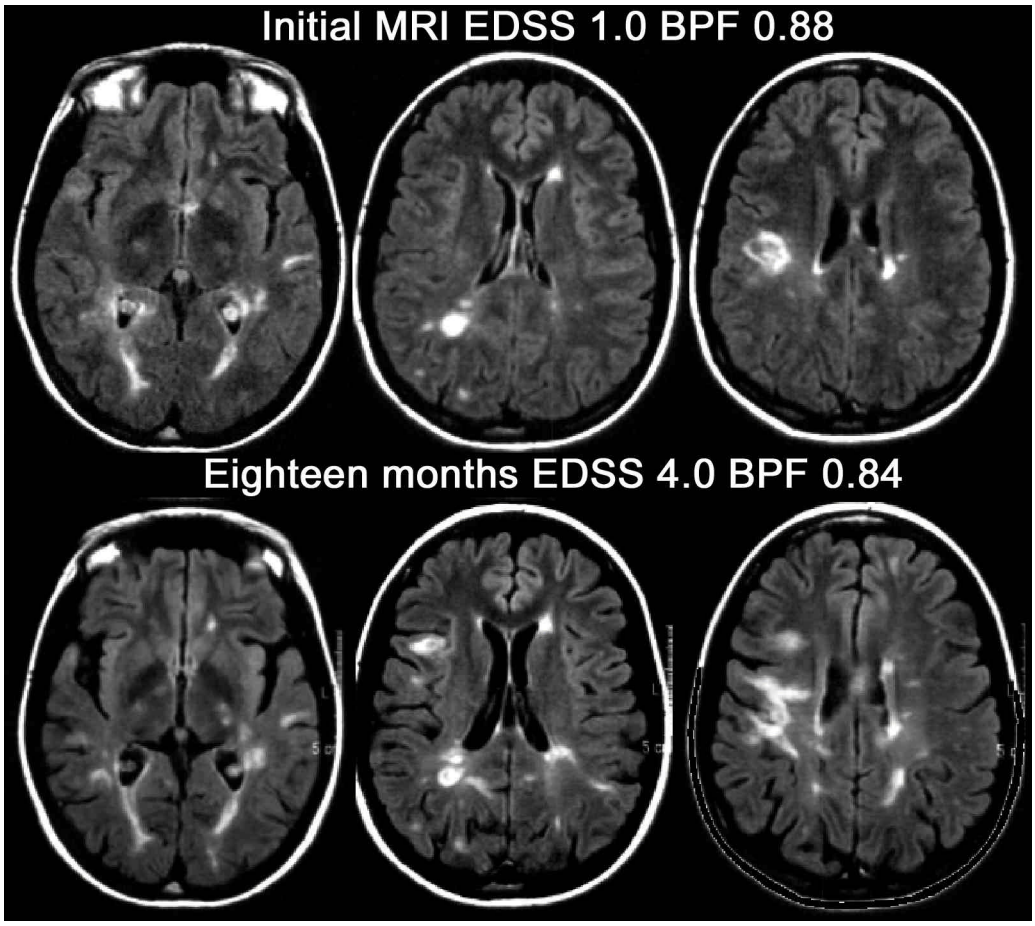

Figure 6. Progressive brain atrophy in MS over 18 months shown on representative MRI scans. A 22 year-old woman with relapsing-remitting MS and a disease duration of two years received MRI of the brain (top row). She had mild physical disability on the Expanded Disability Status Scale (EDSS) of 1.0. Brain parenchymal fraction (BPF), performed at the Buffalo Neuroimaging Analysis Center, was 0.88, which is normal for age. The patient experienced frequent relapses and disease progression during the next several months. Physical disability increased to a moderate level with an EDSS score of 4.0. A follow-up MRI was performed (remote from corticosteroid use) 18 months after the initial MRI. Note the marked enlargement of the cortical sulci, sylvian fissures, and third and lateral ventricles. BPF decreased by 4.5% to 0.84, which is ~5 standard deviations below normal.

3.2. Normalized vs. absolute methods

Absolute measurements of tissue or CSF volume have two main disadvantages. First, they have limited value for cross-sectional studies due to the pre-morbid (genetically-determined and body habitus related) differences in tissue size and morphology among individuals. Second, they are affected by scan-rescan variability due to changes in head position (26-28). To overcome these pitfalls, normalized measures of brain atrophy have been developed that correct for intracranial volume (head size) and may also minimize the effect of patient positioning in the scanner. In a recent cross-sectional study of 10 Alzheimer patients and 55 controls it was shown that normalization using intracranial volume increased the sensitivity in distinguishing disease from controls groups (26). There are several normalized methods available for measurement of whole brain atrophy (22,29-37) (Figure 2). These methods satisfy the essential requirements of being reproducible and sensitive in revealing atrophy compared to normal individuals (Figure 3) and in detecting disease related longitudinal changes (Figure 6). The magnitude of correlation between normalized measures of brain atrophy and physical disability or MRI lesion parameters in MS varies across different studies in which semiautomated (22,24,29-31,33,37) and automated (32,34-36) techniques have been applied. Recently, in a cross-sectional study, we evaluated whether normalized measures of regional brain atrophy correlate with MRI defined regional brain lesions better than the absolute measures of regional brain atrophy (22). Our data suggested that the regional brain parenchymal fraction is a reproducible and sensitive method for measuring regional brain parenchymal atrophy and that normalized regional brain atrophy measures are preferable to absolute regional measures in cross-sectional studies. There are no longitudinal studies that have compared absolute vs. normalized measurements of whole brain atrophy in MS.

3.3. Role of automation

To increase objectivity, reproducibility and efficiency, the segmentation algorithm should be computer-assisted and involve as little operator input as possible. There are a variety of currently available semiautomated (10,37-41) (Figure 2) and automated segmentation algorithms (42-52). The pros and cons of these segmentation algorithms have been discussed in a recently published review (25).

Manual outlining methods provide simple approaches to measuring change in volume over time (53,54). Manual segmentation of the whole brain is rarely used; it is more practical to outline small structures or specific regions (Figure 5). The common disadvantages of these methods include low precision, low reproducibility and heavy time demands.

Semiautomated methods of detecting brain atrophy have the potential advantage of increased accuracy of measurements due to the interaction between the operator and the images, ensuring precision throughout the algorithm. Such semiautomated methods have shown sensitivity in detecting whole brain atrophy in patients with MS versus normal controls (37,55) (Figure 3) with a magnitude of decreased brain parenchymal fraction (BPF) in MS patients vs. controls similar to that found by automated approaches (32,52). Decreases in BPF are seen even in the youngest of patients with MS (56). The use of regional segmentation algorithms, such as seed growing (40), knowledge-based thresholding (41) or local edge detection and contouring (38) can supplement and replace manual outlining methods, resulting in improved speed and reproducibility.

There are a variety of automated segmentation algorithms (42-52) which can be classified into two groups (25): (a) segmentation based methods (CSF volume measurement, BPF, whole brain ratio, brain intracranial capacity ratio, multi-spectral relaxometric imaging, image subtraction technique, fuzzy connectedness segmentation, statistical parametrical mapping-based segmentation, template driven segmentation, SIENAX and SIENA) and (b) registration-based methods (medical image display and analysis software method, voxel-based morphometry, and brain surface modeling).

3.4. Gray/white matter segmentation

It has been presumed that MS is a chronic inflammatory demyelinating disorder of the CNS that involves most prominently the white matter (WM) of the brain and spinal cord. However, recently, several neuroimaging studies (8,18,23,34,51,57-64) demonstrated lesional, atrophic, or diffuse gray matter (GM) changes in the brain. Thus, the disease process and, in particular, atrophy is not limited to the white matter. Using MRI analysis methods, the brain can be divided into GM, WM, and CSF compartments, providing more detailed information on the topography of atrophy. The segmentation of GM and WM volumes is usually performed by fully automated algorithms that use single-contrast or multi-spectral data (34,51,57,58). Methods include statistical parametric mapping (SPM)-based segmentation (32) (Figure 7), multiparametric segmentation (51), fuzzy connectedness (57) and partial volume estimation for tissue segmentation-SIENAX (52,58). The results of the first GM vs WM tissue segmentation study in patients with RR MS (57) suggested that the loss of brain parenchymal volume was predominantly confined to WM, suggesting that brain atrophy in MS does not represent classic cortical atrophy. However, recent reports demonstrate that GM damage and atrophy may occur early in the course of the RR disease (34,51,58). It is not completely clear whether GM atrophy is secondary to WM inflammation or is a consequence of direct pathologic mechanisms. A recent study reported that significant GM atrophy is present in primary progressive (PP) MS (58). It is not clear to what extent GM atrophy contributes to neurological impairment. Several reports failed to find any association between the loss of GM volume and accumulation of neurological impairment (EDSS) (18,34,51,57). Only in one study there was a significant relationship between the neocortical GM volume loss and disability in patients with RR ($r= -0.27$, $p= 0.04$) and PP ($r= -0.64$, $p < 0.0001$) MS (58). However, given the heavy weighting of disability measurements to spinal cord pathology, it may be more fruitful to examine correlations between gray matter atrophy and neuropsychological impairment as was successfully shown in one study (65). Further cross-sectional and longitudinal studies are warranted to examine the clinical relevance and predictive value of GM atrophy at different stages and across various subtypes of MS.

4. TECHNICAL ASPECTS OF ATROPHY MEASUREMENT

There are several approaches to the measurement of brain atrophy in MS. Although various whole brain atrophy studies utilized different measures, they consistently found significant brain atrophy. However, results differ among various studies, most likely due to differences in the techniques and patient populations. There is no accepted gold-standard for measuring brain tissue volumes and brain atrophy in patients with MS. The comparison of brain atrophy techniques is difficult from a methodological point of view because there are no standardized measurement guidelines. A variety of automated and semiautomated methods have been developed using commercially available and proprietary software packages but there has been no validation across different platforms.

There is a need for comparative studies of atrophy measured by different MRI pulse sequences and analysis algorithms to determine their relative precision, sensitivity, validity and reproducibility. A collaborative exchange of image databases and analysis tools may be a first step towards validation and calibration of any segmentation method.

Accuracy of the segmentation method is an important consideration in natural history studies and in clinical trials when estimation of brain atrophy is one of the

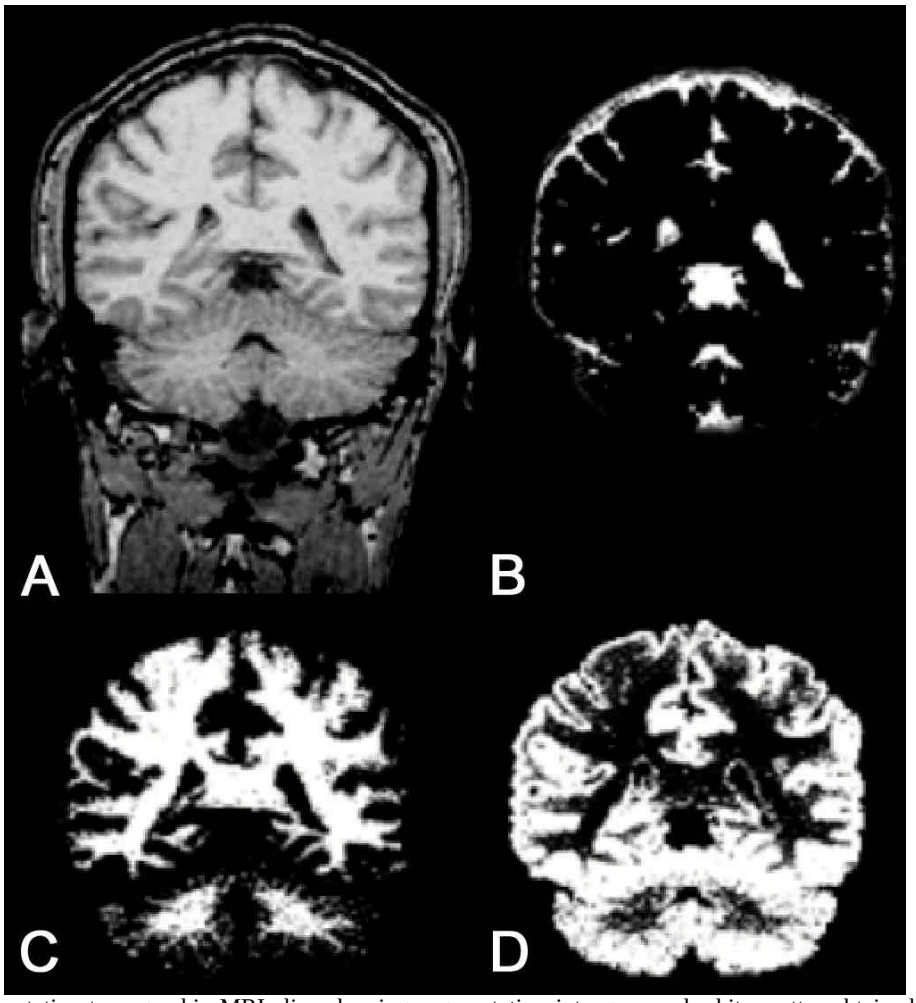

Figure 7. Representative tomographic MRI slice showing segmentation into grey and white matter obtained using Statistical parametrical mapping (SPM99) at the Buffalo Neuroimaging Analysis Center. Shown are (a) the source image (high-resolution T1-weighted gradient echo), (b) after masking and segmentation into brain parenchyma (black) and CSF (white), (c) segmentation into white matter (white areas), and (d) segmentation into grey matter (white areas).

endpoints. Segmentation methods may not be accurate and this makes it difficult to combine or compare the results of brain atrophy measurements that are obtained with different segmentation algorithms (27-52). There is no *in vivo* gold standard to determine the accuracy of the segmentation method; therefore, phantoms of known volumes similar in size and shape to a human brain are analysed to assess accuracy (37).

There are also no standardized established methods available to evaluate in-vivo the precision of various atrophy and volumetric measurement techniques. Although the judgement of precision is completely subjective and therefore observer-dependent, in a recently completed study (66) we compared the precision of various semiautomated and automated techniques for detecting whole brain atrophy (BPF) in MS. The semiautomated methods (Trieste and Buffalo) showed a higher precision than the automated SIENAX method (Figure 8), especially in the segmentation of infratentorial and cortical regions where operator interaction during the segmentation processes was helpful. This manual input allows the user to ensure accuracy and precision of various steps in the algorithm, such as masking, surface contour tracing, and CSF-tissue segmentation (elimination of under-sampling or over-sampling). We hypothesized that differences in precision may explain significantly stronger correlations with physical disability and lesion volumes obtained with the semiautomated than with the automated methods (66).

Acquisition (pulse) sequence (e.g. T2, T1, FLAIR) and MRI parameters (image resolution, image contrast, spin-echo vs. gradient echo, inter-slice gaps, slice thickness) may introduce errors in atrophy measurement. Measurement of brain atrophy is more difficult than the measurement of cord atrophy, because it involves the extraction of the brain from overlying tissues (scalp, skull, and meninges), the delineation of the lower border of brain, and segmentation of brain vs. CSF. Four recent studies (35,36,66,67) pointed out that different acquisition and

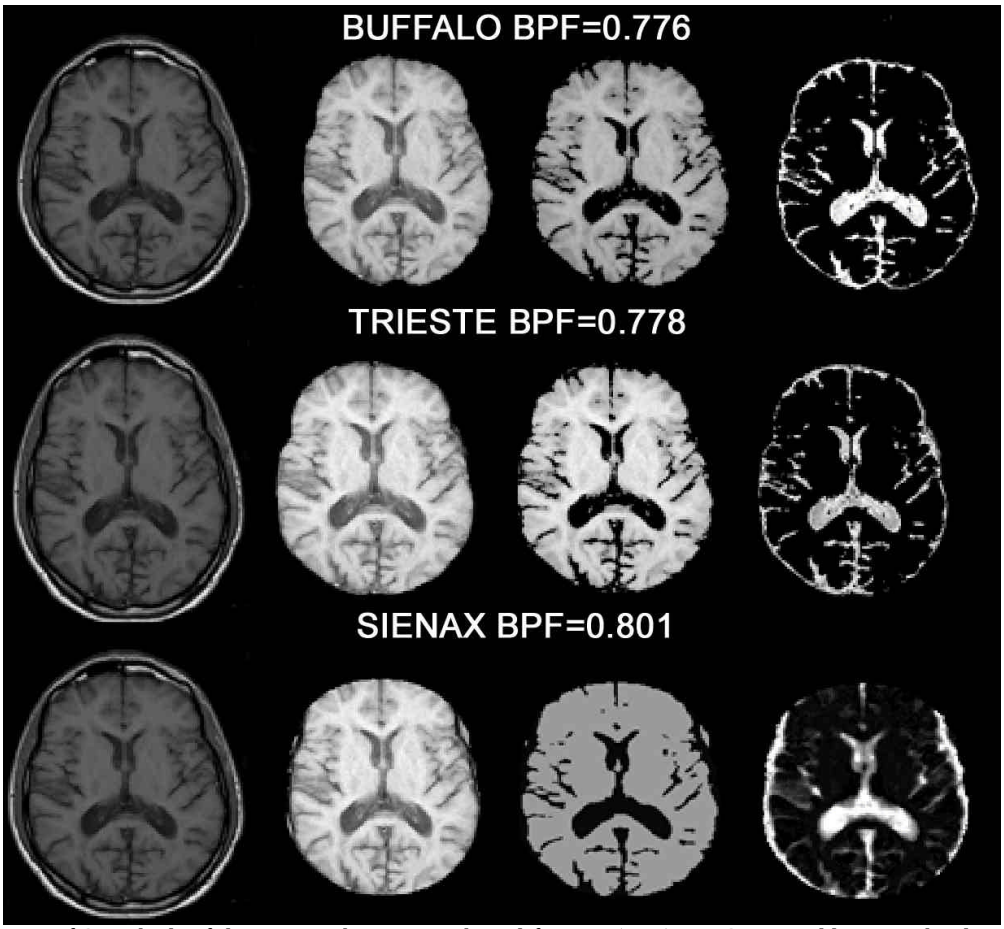

Figure 8. Output of 3 methods of determining brain parenchymal fraction (BPF) in a 32-year old man with relapsing-remitting MS. BPF was quantified on conventional spin-echo T1-weighted axial sequences by 3 software approaches. The Buffalo (37) and Trieste (41) methods were semiautomated, while SIENAX (52) was fully automated. Axial T1-weighted images (far left), scalped- (left middle), brain parenchyma- (right middle) and CSF- only images (far right), are presented. There are clear differences in how semiautomated (Buffalo and Trieste) and automated (SIENAX) algorithms segmented the images into brain parenchyma- and CSF-only images, translating to differences in clinical correlation and precision (66).

segmentation methods can influence the measurement of whole brain atrophy.

Measurement may be affected by confounding factors that are known to influence cerebral volume such as: inflammation and edema (68), steroid therapy (69-72), nutritional status (73), intracranial hypertension (74), alcohol (75), anorexia (76), and dehydration (77).

In a recently presented study in which more than 20 serial scans were performed over a 1-year period, short-term changes of BPF in individuals were larger than the average change seen over the whole 12-month period (78). In RR MS, greater short-term fluctuations of brain volume were observed (15,21,79-81) than in progressive patients (18,82,83). One of the explanations for this is the decreased rate of WM inflammatory edematous lesions in progressive vs. RR stages of disease (84).

The net effect of intravenous methylprednisolone (IVMP) on brain volume fluctuations in MS patients is not completely clear. To understand the impact of corticosteroids on brain volume in other non-CNS diseases, it has been suggested that the action of steroids is twofold: steroid-induced protein catabolism and the reduction of vasogenic edema due to decreased vascular permeability. In the first case, the effects could be systemic and induced by long term treatment, whereas in the latter there could be cerebral dehydration with short-term treatment or rapid dose changes. The second hypothesis has been confirmed by experimental studies that examined electrolyte balance and changes of osmolarity in humans (74,77). However, steroids may have a protective effect on brain volume (limiting atrophy) over the long term due to immunomodulatory and anti-inflammatory effects that impact on disease progression (85). In a recently published study (70), we evaluated the long-term effect of treatment with pulses of IVMP in RR MS patients and demonstrated that IVMP limits the development of global brain atrophy over a five year period. The short-term effect of IVMP on brain volume has been studied in two recently published studies (71,72). In 26 RR MS patients who were followed

by monthly MRI, a total of 56 courses of IVMP were given during the follow-up, 40 while taking interferon-beta and 16 while on no other disease modifying treatment (71). In the 3 months prior to IVMP treatment, brain fractional volume was stable, but when all 56 events were combined, there was a significant reduction at month 1 and 2 following IVMP. A significant reduction in brain fractional volume was also observed when patients were classified according to prior treatment status. The study suggests that MRI should be delayed by 2 months following IVMP to avoid a reversible steroid effect on brain volume. Serial short- and long-term MRI studies that account for the effects of fluid status, nutrition, and body habitus on brain volume fluctuations in MS are lacking.

5. PATHOGENESIS AND PROGNOSIS

The pathological processes responsible for the development of brain atrophy in MS are uncertain, but a combination of demyelination and axonal loss most likely play a major role. There may also be a contribution from reduced axonal diameter and tissue contraction caused by astrogliosis. It is not entirely clear, however, whether processes other than demyelination and axonal loss contribute to the progressive development of atrophy. Some authors hypothesized that mechanisms of neurotoxicity separate from inflammation, such as: chronic demyelination (15,86), loss of tropic support of the axons (87), altered electrical conduction (88), and iron deposition in grey matter (8), might contribute to axonal loss in MS patients. Although suspected as a significant factor in the clinical manifestation and progression of disability in MS patients, axonal injury, including axonal transection, only recently has been reported within acute inflammatory lesions (5,6). Furthermore, this finding has been supported by studies showing reduced NAA in acute MS lesions (89). These observations are consistent with reports indicating that relapse rate (10) and lesion activity visible on MRI (14,16,22,90) are related to the rate of brain atrophy. Neuronal degeneration in gray matter also appears to play a role in brain atrophy in MS. Substantial neuronal degeneration, metabolite depletion, increased water diffusion, and macroscopic volume loss has been described in the deep gray matter of patients with MS (18,23,63). The degree of deep gray matter atrophy is unrelated to overt lesion load in white matter (18), suggesting that gray matter is not simply due to Wallerian degeneration from white matter injury and that direct mechanisms of gray matter injury may play a role.

T2 hypointensity, an MRI marker of pathologic iron deposition, has been described in the brain of patients with MS (Figure 9) and is related to clinical course, physical disability, and MRI lesion load (8,91,92). A relationship between T2 hypointensity and brain atrophy has been shown in both cross-sectional (8,91,92) and longitudinal studies (93). These studies indicate that T2 hypointensity is the best predictor of brain atrophy even after accounting for the influence of overt MRI lesions. Thus, iron deposition in the brain may play a role in the pathophysiology of neurodegeneration in MS, perhaps through the development of free radicals and lipid peroxidation. Alternatively, iron deposition may represent an epiphemenon related to tissue degeneration.

A number of studies examined clinical factors underlying the relationship between disease course in MS and the rate of brain atrophy accumulation, but their results were contradictory. Some reports suggested that the rate of atrophy progression may be greater in earlier RR phases, as compared with later SP stages of disease (6), but other authors reported the opposite (30). Recent reports (14-16,41,84) showed that MS patients exhibit significant brain volume decline in the earliest stages of disease and provided evidence that brain atrophy and axonal loss may develop at a faster rate in the first few years of the disease (24,94). Later, the clinical and MRI activity decreases and patients experience the progression of disability. However, this progression seems unaffected by relapses when a clinical threshold of irreversible disability has been reached (95). Therefore, there are reasons to believe that the atrophy rate varies within each stage of the disease in the same individual, and that there is almost certainly heterogeneity across subsets of patients (9,96).

Several studies looked for a correlation between brain atrophy and clinical, demographic and MRI measures. The clinical heterogeneity of patients and limited sample size are the most important flaws of these studies. However, although no clear factor highly predictive of brain atrophy has been identified, there are some suggestions that gadolinium (Gd)-enhancing lesions, (14,21,32,79,97,98), lesions on T1- (71,82,84,99) and T2-weighted images (31,32,41) and T2-hypointensities in deep grey matter (see above) (8,91-93) have predictive value.

Gd-enhancing lesions on T1-weighted images represent disruption of the blood-brain-barrier and acute inflammation. It is not completely clear if inflammation is the triggering event that causes demyelination and axonal degeneration; although, previous longitudinal studies demonstrated that the presence of active lesions on serial scans carries a high risk of subsequent disease activity (96,100). A meta-analysis study performed by Kappos *et al.* (101) demonstrated that Gd-enhancement is a good predictor of the occurrence of relapses, but not a strong predictor of the development of long term impairment or disability, indicating that different pathogenetic mechanisms are operative in the occurrence of relapses and in the development of long-term disability in MS.

The concept that early short-term inflammatory events lead immediately to irreversible damage associated with loss of brain parenchyma has not been established. Moreover, it is not completely clear whether early acute inflammatory disease activity triggers a cascade of events, which contributes to progressive brain volume decline in long term follow-up. The clarification of this issue is of great importance for the selection of the most appropriate treatment strategies.

In a recently published review (102), we investigated hypothetical pathological mechanisms proposed as determinants of brain atrophy. We considered

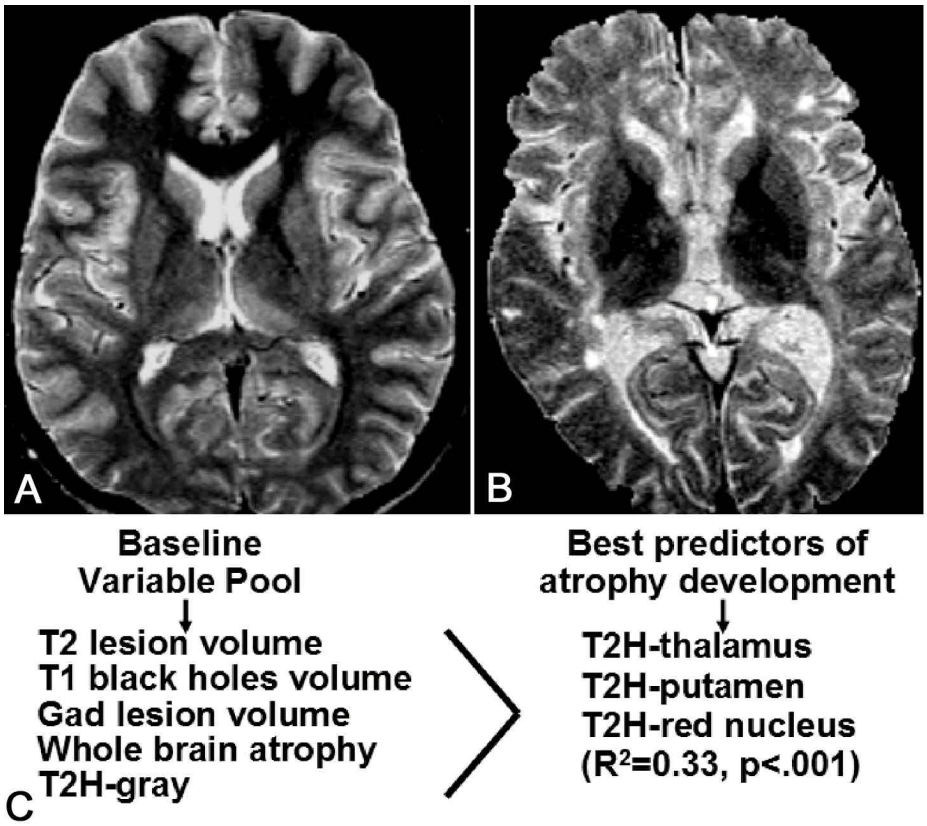

Figure 9. MRI T2 hypointensity (T2H) in the gray matter and brain atrophy in MS. Hypointensity on T2-weighted images has been described in the gray matter of patients with MS and is related to physical disability, clinical course, MRI lesion load, and brain atrophy (8,91,92). The finding most likely represents pathologic iron deposition. Conventional spin-echo T2-weighted images are shown of a normal volunteer (a) and an age-matched patient with secondary progressive MS early in the fourth decade of life (b). In the latter, note the marked hypointensity of the deep gray matter nuclei, including the thalamus and putamen. A natural history longitudinal study (93) of 68 relapsing-remitting patients revealed that T2 hypointensity (T2H) in the caudate, putamen, globus pallidus, red nucleus, and thalamus correlated with baseline brain parenchymal fraction (BPF) (r=.19-.39, p=.001-.029). Baseline T2H in the thalamus, putamen, and globus pallidus predicted 2-year BPF change (r=.26-.33, p=.006-.032) and T2H was chosen in regression modeling as the best predictor of BPF change after accounting for all MRI lesion variables (c). Thus, gray matter T2H predicts progression of brain atrophy suggesting a relationship between iron deposition and tissue destruction in MS.

only studies with the following characteristics: prospective design with natural history of MS patients and/or placebo treated patients of therapeutic trials, in which patients underwent baseline and follow-up scans, T1-weighted single dose (0.1 mmol/kg bodyweight) Gd-enhanced MRI and the relationship between enhancement and progression of brain atrophy was assessed. Five hundred thirty two patients of five natural history studies and five therapeutic trial studies participated in the review. The main observation was that in RR patients there was a predictive relationship between Gd-enhancement and brain atrophy progression. This relationship remained after adjusting for demographic and clinical data. The examination of pathological mechanisms proposed in the reviewed studies led us to believe that inflammation is significantly but only partly responsible for the development of brain atrophy. This conclusion may have an implication for the strategies of tissue protection advocated in the early stages of RR course.

Distinct Gd-enhancement patterns may suggest differences in the underlying pathology of lesions. Concentric ring-enhancing lesions with central contrast pallor arise in previously damaged areas or in areas of accelerated local inflammation (79,103-108). They are larger and of longer duration than homogeneously enhancing lesions (79,103,104,106). The magnetization transfer ratios (105-107) and trace apparent diffusion coefficients (108) are lower in ring-enhancing lesions than in homogeneously enhancing lesions. Moreover, ring-enhancing lesions weakly predict the persistence of T1 hypointense lesions (109). Therefore, ring-enhancing lesions are thought to be related to high disease activity and extensive tissue damage (79,103) and may mark a type of inflammation in more aggressive forms of disease. In a recent short-term observation study we assessed the value of different Gd-enhancement patterns shown on monthly MRI as predictors of brain atrophy (110). In ring-enhancement positive patients there was a significant reduction of BPF over

Table 2. Long-term longitudinal correlations (= 5 years) between the development of brain atrophy and accumulation of subsequent neurological impairment in patients with relapsing-remitting multiple sclerosis

Atrophy measure vs. neurological impairment	r	p	Years	Reference
BPAV vs. EDSS	-0.38	0.025	5	65
SBV vs. EDSS	-0.46	0.001	6	109
BPF vs. EDSS	-0.31	0.0005	8	110
BPF vs. MSFC	-0.30	0.001	8	110

BPAV- brain parenchymal absolute volume; SBV-supratentorial brain volume; BPF- brain parenchymal fraction; EDSS-Expanded Disability Status Scale; MSFC- Multiple Sclerosis Functional Composite

the next 3 months. Moreover, this decrease was significantly greater than that of patients with homogeneously enhancing lesions. At baseline, BPF correlated inversely with ring-enhancing lesions and ring-enhancing lesion volume and longitudinally there was a trend between change of ring-enhancing lesion volume and subsequent reduction of BPF. Moreover, multiple regression analysis demonstrated that the only baseline and on-study variables that predicted changes of BPF were ring-enhancing lesions and the change of ring-enhancing lesion volume. The predictive value of ring-enhancement on brain atrophy progression observed in this short-term study may suggest that ring-enhancement indicates a more aggressive subtype of disease even at the earliest stages of RR MS. We believe that this is an important observation because if ring-enhancing Gd-enhancement is associated with accelerated rates of brain atrophy early in the disease course, these patients are candidates for more aggressive early therapy.

6. CLINICAL RELEVANCE OF BRAIN ATROPHY

The measurement of brain atrophy seems to be of growing clinical relevance as a biomarker of the MS disease process (25). There is a consensus developing that the assessment of brain atrophy by serial MRI represents a potentially powerful tool for monitoring disease progression in MS (32,34,55). When compared to conventional MRI lesion measurements, brain atrophy is a better predictor of clinical impairments such as cognitive dysfunction (17,33,41,65,111), physical disability (33,70,112-116), mood disturbances (11,117,118) and impaired quality of life in cross-sectional studies (119-121). Longitudinal studies (= 5 years) demonstrated that the development of brain atrophy is a good predictor of subsequent neurological impairment accumulation in the long-term (Table 2) (70,114,115). These findings indicate that once brain atrophy has occurred, irreversible damage has occurred. The ability of the CNS to compensate for the loss of axons and neurons is likely dependent on the location of atrophy and the available brain reserve capacity. We hypothesize that once the level of atrophy reaches a critical threshold, patients begin to suffer clinical impairments and disease progression.

7. ATROPHY MEASUREMENT AND TREATMENT EVALUATION

Atrophy should now be included as a secondary outcome measure in trials of disease modifying agents aimed at limiting disease progression. Currently available anti-inflammatory immunomodulatory agents and immunosuppressive treatments, while effective at preventing relapses, have shown at best partial effects on preventing CNS atrophy. Thus, there is a need to further validate atrophy as an outcome measure and ultimately develop treatment strategies that will protect against the destructive aspects of this and in turn will lead to better long term neurologic function and quality of life for patients with MS. Several studies have evaluated the effect of disease modifying agents on longitudinal brain atrophy in MS patients (21,32,68,70,81,114,122-124), but only in five studies was a treatment effect demonstrated 32,70,125-127).

The modest correlation between MRI enhancing lesions and brain atrophy observed in the placebo groups of several trials fits with the concept that inflammatory activity is only partially associated with the global neurodegenerative disease (129). Consistent with this hypothesis, several immunomodulating and immunosuppressive treatments that are known to reduce MRI-measurable inflammatory activity have exerted relatively little effect on progressive tissue loss. CNS atrophy measurements may be useful to test the efficacy of experimental MS treatments targeted to counteract neurodegenerative components of the disease.

8. SPINAL CORD ATROPHY

MRI of the spinal cord often shows characteristic findings and thus contributes to the diagnosis of MS (129). The number and extent of T2-weighted lesions shows a partial relationship with disability (130-131). However, atrophy of the spinal cord in MS has particular importance in the development of physical disability. This atrophy may be focal or generalized (132). The first quantitative study of spinal cord atrophy in MS employed a T2-weighted gradient echo sequence and evaluated four vertebral levels (fifth cervical, second, seventh and eleventh thoracic) (130). Atrophy was considered to be present when the measured area was two standard deviations below the mean of healthy controls. Patients with atrophy had higher levels of disability measured by EDSS than patients without atrophy ($p = 0.006$). No difference in cord cross-sectional area was found among SP, PP and benign MS groups. A longitudinal study of PP and SP MS showed a decrease in mean cord area over 1 year, but there was no correlation with clinical progression (131). A separate study (133) carried out by the same investigators, assessed RR MS patients over 1 year but failed to show any change in cord cross-sectional area.

Loseff *et al.* (134) developed a technique which measured the cord cross-sectional area at the level of the

intervertebral disc between the second and third vertebrae (scan-rescan coefficient of variation-COV was 0.79%). It used an inversion prepared fast spoiled gradient-echo T1-weighted sequence, which nulls the CSF. The cord cross-sectional area of PP and SP MS patient groups were significantly lower than RR MS patients. Cord atrophy correlated strongly with disability ($r = -0.7$, $p < 0.0001$) and with disease duration ($r = -0.52$, $p < 0.0001$). Stevenson *et al.* (135) found increasing atrophy over a 12-month period in RR, PP and SP MS groups. Spinal cord atrophy showed a close association with EDSS score, whereas T2-lesion volume and T1 hypointensity lesion volume did not (135,136). Ingle *et al*. (136) studied clinical and MRI changes in 190 patients with PP or transitional progressive (TP) MS over 2 years. Patients underwent clinical and MRI examination at baseline, year one and year two. Significant increases were seen in lesion load, while brain and cord volume decreased. On the contrary, significantly higher T2 lesion volumes were found in PP MS patients who presented with non-cord clinical syndromes when compared to patients with syndromes and there was a trend to greater brain atrophy in those who deteriorated clinically over the course of the study compared to those who remained stable. Significant cord atrophy was only seen in those with a myelopathic clinical presentation. These data suggest a mechanism of diffuse progressive axonal loss in PP MS patients. Decreased magnetization transfer ratio and a smaller cervical cord area also contribute to neurological impairment in PP MS (137).

The spinal cord is mobile and at C5 is influenced by neck extension. CSF flow is often turbulent at C5, which causes flow artifacts. Thus, three dimensional global measurements of spinal cord size (the volume of the whole cervical cord) may have more utility than 2D methods (138).

9. SUMMARY

In conclusion, it is now possible to detect progressive atrophy of both the brain and spinal cord using semiautomated and automated, accurate and reproducible MRI analysis techniques. Atrophy is most likely a complex phenomenon reflecting the effects of inflammatory tissue injury, demyelination, and axonal loss. Atrophy of the central nervous system appears to closely relate to clinical impairment, disease progression and accumulation of physical and neuropsychological disability. The measures of atrophy are sensitive to longitudinal change and show fairly strong predictive value and correlations with clinical status. Therefore, quantitative assessment of atrophy plays a role in the monitoring of clinically relevant disease progression and should be used in MS clinical trials.

10. ACKNOWLEDGEMENTS

This work was supported in part by research grants to R. Bakshi from the National Institutes of Health (NIH-NINDS 1 K23 NS42379-01), National Multiple Sclerosis Society (RG 3258A2/1), and National Science Foundation (DBI-0234895). We thank Christopher Tjoa, Jitendra Sharma, Kelly Watts, Sarah Ludwig, and Mike Dwyer for technical support.

11. REFERENCES

1. Gross, K. R. Tomberg, T. A. Kokk, A. A. & A. E. Kaasik: The prognosis of multiple sclerosis: computed tomographic comparisons. *Zh Nevropatol Psikhiatr* 93, 32-35 (1993)

2. Huber, S. J. Paulson, G. W. Shuttleworth, E. C. Chakeres, D. Clapp, L. E. Pakalnis, A. Weiss, K. & K. Rammohan: Magnetic resonance imaging correlates of dementia in multiple sclerosis. *Arch Neurol* 44, 732-736 (1987)

3. Davie, C. A. Barker, G. J. Webb, S. Tofts, P. S. Thompson, A. J. Harding, A. E. McDonald, W. I. & D. H. Miller: Persistent functional deficit in multiple sclerosis and autosomal dominant cerebellar ataxia is associated with axon loss. *Brain* 118, 1583-1592 (1995)

4. Prineas, J. W. & F. Conell: The fine structure of chronically active multiple sclerosis plaques. *Neurology* 28, 68-75 (1978)

5. Ferguson, B. Matyszak, M. K. Esiri, M. M. & V. H. Perry: Axonal damage in acute multiple sclerosis lesions. *Brain* 120, 393-399 (1997)

6. Trapp, B. D. Peterson, J. Ransohoff, R. M. Rudick, R. Mork S. & L. Bo: Axonal transection in the lesions of multiple sclerosis. *N Eng J Med* 338, 278-285 (1998)

7. Fu, L. Matthews, P. M. De Stefano, N. Worsley, K. J. Narayanan, S. Francis, G. S. Antel, J. P. Wolfson, C. & D. L. Arnold: Imaging axonal damage of normal appearing white matter in multiple sclerosis. *Brain* 121, 103-113 (1998)

8. Bakshi, R. Benedict, R. B. H. Bermel, R. A. Caruthers, S. D. Puli, S. R. Toja,C. W. Fabiano, A. J & L. Jacobs: T2 hypointensity in deep grey matter of patients with multiple sclerosis. *Arch Neurol* 59, 62-68 (2002)

9. Simon, J. H. Jacobs, L. D. Campion, A. Rudick, R. A. Cookfair, D. L. Herndon, R. M. Richert, J. R. Salazar, A. M. Fischer, J. S. Goodkin, D. E. Simonian, N. Lajaunie, M. Miller, D. E. Wende, K. Martens-Davidson, A. Kinkel, R. P. Munschauser F. E. 3rd & C. M. Brownscheidle: A longitudinal study of brain atrophy in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). *Neurology* 53, 139-148 (1999)

10. Edwards, S. G. M. Gong, Q. Y. Liu, C. Zvartau, M. E. Jaspen, T. Roberts, N. & L. D. Blumhardt: Infratentorial atrophy on magnetic resonance imaging and disability in multiple sclerosis. *Brain* 122, 291-301 (1999)

11. Bakshi, R. Czarnecki, D. Shaikh, A. Priore, R. L. Janardhan, V. Kaliszky, Z. & P. R. Kinkel: Brain MRI lesions and atrophy are related to depression in multiple sclerosis. *Neuroreport* 11, 1153-1158 (2000)

12. Turner, B. Ramli, N. Blumhardt, L. D. & T. Jaspen: Ventricular enlargement in multiple sclerosis: a comparison of three-dimensional and linear MRI estimates. *Neuroradiology* 43, 608-614 (2001)

13. Berg, D. Mäurer, M. Warmuth-Metz, M. Rieckmann, P. & G. Becker: The correlation between ventricular diameter measured by transcranial sonography and clinical disability

and cognitive dysfunction in patients with multiple sclerosis. *Arch Neurol* 57, 1289-1292 (2000)
14. Luks, T. L. Goodkin, D. E. Nelson, S. J. Majumdar, S. Bacchetti, P. Portnoy, D. & R. Sloan: A longitudinal study of ventricular volume in early relapsing-remitting multiple sclerosis. *Mult Scler* 6, 322-327 (2000)
15. Brex, P. A. Jenkins, R. Fox, N. C. Crum, W. R. O'Riordan, J. Plant, G. T. & D. H. Miller: Detection of ventricular enlargement in patients at the earliest clinical stage of MS. *Neurology* 54, 1689-1691(2000)
16. Redmond, I. T. Barbosa, S. Blumhardt, L. D. & N. Roberts: Short-term ventricular volume changes on serial MRI in multiple sclerosis. *Acta Neurol Scand* 102, 99-105 (2000)
17. Bermel, R. A. Bakshi, R. Tjoa, C. Puli, S. R. & L. Jacobs: Bicaudate ratio as a magnetic resonance imaging marker of brain atrophy in multiple sclerosis. *Arch Neurol* 59, 275-280 (2002)
18. Bermel, R. A. Innus, M. D. Tjoa, C. W. & R. Bakshi: Selective caudate atrophy in multiple sclerosis: a 3D MRI parcellation study. *Neuroreport* 14, 335-339 (2003)
19. Caon, C. Zvartau-Hind, M. Ching, W. Lisak, R. P. Tselis, A. C. & O. A. Khan: Intercaudate nucleus ratio as a linear measure of brain atrophy in multiple sclerosis. *Neurology* 60, 323-325 (2003)
20. Losseff, N. A. Wang, L. Lai, H. M. Yoo, D. S. Gawne-Cain, M. L. McDonald, W. I. Miller, D. H. & A. J. Thompson: Progressive cerebral atrophy in multiple sclerosis. A serial MRI study. *Brain* 119, 2009-2019 (1996)
21. Rovaris, M. Comi, G. Rocca, M. A. Wolinsky, J. S. Filippi, M. & European/Canadian Glatiramer Acetate Study Group: Short-term volume change in relapsing-remitting multiple sclerosis. Effect of glatiramer acetate and implications. *Brain* 124, 1803-1812 (2001)
22. Zivadinov, R. Locatelli, L. Stival, B. Bratina, A. Grop, A. Nasuelli, D. Brnabic-Razmilic, O. & M. Zorzon: Normalized regional brain atrophy measures in multiple sclerosis. *Neuroradiology* 2003 (in press)
23. Cifelli, A. Arridge, M. Jezzard, P. Esiri, M. M. Palace, J. & P. M. Matthews: Thalamic neurodegeneration in multiple sclerosis. *Ann Neurol* 52, 650-653 (2002)
24. Liu, C. Edwards, S. Gong, Q. Roberts, N. & L. D. Blumhardt: Three dimensional MRI estimates of brain and spinal cord atrophy in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 66, 323-330 (1999)
25. Miller, D. H. Barkhof, F. Frank, J. A. Parker, G. J. M. & A. J. Thompson: Measurement of atrophy in multiple sclerosis: pathological basis, methodological aspects and clinical relevance. *Brain* 125, 1676-1695 (2002)
26. Whitwell, J. L. Crum, W. R. Watt, H. C. & N. C. Fox: Normalization of cerebral volumes by use of intracranial volume: implications for longitudinal quantitative MR imaging. *AJNR Am J Neuroradiol* 22, 1483-1489 (2001)
27. Hartmann, P. Ramseier, A. Gudat, F. Mihatsch, M. J. & W. Polasek: Normal weight of the brain in adults in relation to age, sex, and weight. *Pathologe* 15, 165-170 (1994)
28. Schwartz, M. Creasey, H. Grady, C. L. DeLeo, J. M. Frederickson, H. A. Cutler, N. R. & S. I. Rapoport: Computed tomographic analysis of brain morphometrics in 30 healthy men, aged 21 to 81 years. *Ann Neurol* 17, 146-157 (1985)
29. Ge, Y. Grossman, R. I. Udupa, J. K. Wei, L. Mannon, L. J. Polansky, M. & D. L. Kolson: Brain atrophy in relapsing remitting multiple sclerosis and secondary progressive multiple sclerosis: longitudinal quantitative analysis. *Radiology* 214, 665-670 (2000)
30. Saindane, A. M. Ge, Y. Udupa, J. K. Babb, J. S. Mannon, L. J. & R. I. Grossman: The effect of gadolinium-enhancing lesions on whole brain atrophy in relapsing-remitting MS. *Neurology* 55, 61-65 (2000)
31. Phillips, M. D. Grossman, R. I. Miki, Y. Wei, L. Kolson, D. L. van Buchem, M. A. Polansky, M. McGowan, J. C. & J. K Udupa: Comparison of T2 lesion volume and magnetization transfer ratio histogram analysis and of atrophy and measures of lesion burden in patients with multiple sclerosis. *AJNR Am J Neuroradiol* 19, 1055-1060 (1998)
32. Rudick, R. A. Fisher, E. Lee, J. C. Simon, J. & L. Jacobs: Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. the Multiple Sclerosis Collaborative Research Group. *Neurology* 53, 1698-1704 (1999)
33. Zivadinov, R. De Masi, R. Nasuelli, D. Bragadin, L. M. Ukman, M. Pozzi-Mucelli, R. S. Grop, A. Cazzato, G. & M. Zorzon: Magnetic resonance imaging techniques and cognitive impairment in early phase of relapsing-remitting multiple sclerosis. *Neuroradiology* 43, 272-78 (2001)
34. Chard, D. T. Griffin, C. M. Parker, G. J. M. Kapoor, R. Thompson, A. J. & D. H. Miller: Brain atrophy in clinically early relapsing-remitting multiple sclerosis. *Brain* 125, 327-337 (2002)
35. Stevenson, V. L. Smith, S. M. Matthews, P. M. Miller, D. H. & A. J. Thompson: Monitoring disease activity and progression in primary progressive multiple sclerosis using MRI: sub-voxel registration to identify lesion changes and to detect cerebral atrophy. *J Neurol* 249, 171-177 (2002)
36. Leigh, R. Ostuni, J. L. Pham, D. Goldszal, A. Lewis, B. K. Howard, T. Richert, N. McFarland, H. & J. A. Frank: Estimating cerebral atrophy in MS patients from various MR pulse sequences. *Mult Scler* 8, 420-429 (2002)
37. Bermel, R. A. Sharma, J. Tjoa, C. W. Puli, S. R. & R. Bakshi: A semiautomated measure of whole-brain atrophy in multiple sclerosis. *J Neurol Sci* 208, 57-65 (2003)
38. Plummer, D. L: DispImage: a display and analysis tool for medical images. *Rivist Neuroradiol* 5, 89-95 (1992)
39. Nijeholt, G. J. van Walderveen, M. A. Castelijns, J. A. van Waesberghe, J. H. Polman, C. Scheltens, P. Rosier, P. F. Jongen, P. J. & F. Barkhof: Brain and spinal cord abnormalities in multiple sclerosis. Correlation between MRI parameters, clinical subtypes and symptoms. *Brain* 121, 687-697 (1998)
40. Rovaris, M. Inglese, M. van Schijndel, R. A. Sormani, M. P. Rodegher, M. Comi, G. & M. Filippi: Sensitivity and reproducibility of volume change measurement of different brain portions on magnetic resonance imaging in patients with multiple sclerosis. *J Neurol* 247, 960-965 (2000)
41. Zivadinov, R. Sepcic, J. Nasuelli, D. De Masi, R. Bragadin, L. M. Tommasi, M. A. Zambito-Marsala, S. Moretti, R. Bratina, A. Ukmar, M. Pozzi-Mucelli, R. S. Grop, A. Cazzato, G. & M. Zorzon: A longitudinal study of brain atrophy and cognitive disturbances in the early phase of relapsing-remitting multiple sclerosis. *J Neurol Neurosurg Psychiatry* 70, 773-780 (2001)
42. Asburner, J. & K. J. Friston: Voxel-based morphometry – the methods. Review. *Neuroimage* 11, 805-821(2000)

43. Collins, D. L. Montagnat, J. Zijdenbos, A. P. Evans, A. C. & D. L. Arnold: Automated estimation of brain volume in multiple sclerosis with BICCR. *Lect Notes Comput Sci* 2082, 141-147 (2001)
44. Fisher, E. Cothren, R. M. Tkach, J. A. Masaryk, T. J. & J. F. Cornhill: Knowledge-based 3D segmentation of the brain in MR images for quantitative multiple sclerosis lesion tracking. *SPIE Med Imag* 3034, 19-25 (1997)
45. Smith, S. M. DeStefano, N. Jankinson, M. & P. M. Matthews: Normalized accurate measurement of longitudinal brain change. *J Comput Assist Tomogr* 25, 466-475 (2001)
46. Zijdenbos, A. Evans, A. Riahi, F. Sled, J. Chui, J. & V. Kollokian: Automatic quantification of multiple sclerosis lesion volume using stereotaxic space. *Lect Notes Comput Sci* 1131, 439-448 (1996)
47. Udupa, J. K. Wei, L. Samarasekera, S. Miki, Y. van Buchem, M. A. & R. I. Grossman: Multiple sclerosis lesion quantification using fuzzy-connectedness principles. *IEEE Trans Med Imag* 16, 598-609 (1997)
48. Pham, D. L. & J. L. Prince: An adaptive fuzzy C-means algorithm for image segmentation in the presence of intensity inhomogeneities. *Patt Recog Lett* 20, 57-68 (1999)
49. Kikinis, R. Guttmann, C. R. Metcalf, D. Wells, W. M. 3rd Ettinger, G. J. Weiner, H. L. & F. A. Jolesz: Quantitative follow-up of patients with multiple sclerosis using MRI:technical aspects. *J Magn Reson Imag* 9, 519-530 (1999)
50. Zhang, J. Brady, M. & S. Smith: Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Trans Med Imag* 20, 45-57 (2001)
51. Quarantelli, M. Ciarmiello, A. Morra, V. B. Orefice, G. Larobina, M. Lanzillo, R. Schiavone, V. Salvatore E. Alfano, B. & A. Brunetti: Brain tissue volume changes in relapsing-remitting multiple sclerosis: correlation with lesion load. *Neuroimage* 18, 360-366 (2003)
52. Smith, S. M. Zhang, Y. Jenkinson, M. Chen, J. Matthews, P. M. Federico, A. & N. De Stefano: Accurate, robust and automated longitudinal and cross-sectional brain change analysis. *Neuroimage* 17, 479-489 (2002)
53. Filippi, M. Mastronardo, G. Rocca, M. A. Pereira, C. & G. Comi: Quantitative analysis of brain magnetic resonance imaging from patients with multiple sclerosis. *J Neurol Sci* 158, 148-153 (1998)
54. Inglese, M. Rovaris, M. Giacomotti, L. Mastronardo, G. Comi, G. & M. Filippi: Quantitative brain volumetric analysis from patients with multiple sclerosis: a follow-up study. *J Neurol Sci 171*, 8-10 (1999)
55. Fox, N. C. Jenkins, R. Leary, S. M. Stevenson, V. L. Losseff, N. A. Crum, W.R. Harvey, R. J. Rossor, M. N. Miller, D. H.& A. J. Thompson: Progressive cerebral atrophy in MS: a serial study using registered, volumetric MRI. *Neurology* 54, 807-812 (2000)
56. Kassubek, J. Tumani, H. Ecker, D. Kurt, A. Ludolph, A. C. & F. D. Juengling: Age-related brain parenchimal fraction is significantly decreased in young multiple sclerosis patients: a quantitative MRI study. *Neuroreport* 14, 427-430 (2003)
57. Ge, Y. Grossman, R. I. Udupa, J. K. Babb, J. S. Nyul, L. G. & D. L. Kolson: Brain atrophy in relapsing-remitting multiple sclerosis: fractional volumetric analysis of gray matter and white matter. *Radiology* 220, 606-610 (2001)
58. De Stefano, N. Matthews, P. M. Filippi, M. Agosta, F. De Luca, M. Bartolozzi, M. L. Guidi, L. Ghezzi, A. Montanari, E. Cifelli, A. Federico, A & S.M. Smith: Evidence of early cortical atrophy in MS. Relevance to white matter changes and disability. *Neurology* 60, 1157-1162 (2003)
59. Cercignani, M. Bozzali, M. Iannucci, G. Comi, G. & M. Filippi: Magnetisation transfer ratio and mean diffusivity of normal appearing white and grey matter from patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry* 70, 311-317 (2001)
60. Sharma, R. Narayana, P. A. & J. S. Wolinsky: Grey matter abnormalities in multiple sclerosis: proton magnetic resonance spectroscopic imaging. *Mult Scler* 7, 221-226 (2001)
61. Rovaris, M. Bozzali, M. Iannucci, G. Ghezzi, A. Caputo, D. Montanari, E. Bartolotto, A. Bergamaschi, R. Capra, R. Mancardi, G. L Martinelli, V. Comi, G. & M. Filippi: Assessment of normal appearing white and gray matter in patients with primary progressive multiple sclerosis: a diffusion tensor magnetic resonance imaging study. *Arch Neurol* 59, 1406-1412 (2002)
62. Bakshi, R. Ariyaratana, S. Benedict, R. H. B. & L. Jacobs: Fluid-attenuated inversion recovery magnetic resonance imaging detects cortical and juxtacortical multiple sclerosis lesions. *Arch Neurol* 58, 742-748 (2001)
63. Fabiano, A. J. Sharma, J. Weinstock-Guttman, B. Munschauer, F. E. Benedict, R. H. B. Zivadinov & R. Bakshi: Thalamic involvement in multiple sclerosis: a diffusion-weighted magnetic resonance imaging study. *J Neuroimaging* (2003) (in press).
64. Bakshi, R. Miletich, R. S. Kinkel, P. R. Emmet, M. L. & W. R. Kinkel: High-resolution fluorodeoxyglucose positron emission tomography shows both global and regional cerebral hypometabolism in multiple sclerosis. *J Neuroimaging* 1998 8, 228-234 (1998)
65. Benedict, R. H. B. Bakshi, R. Simon, J. H. Priore, R. Miller, C. & F. E. 3rd Munschauer Frontal cortex atrophy predicts cognitive impairment in multiple sclerosis. *J Neuropsy and Clin Neurosci* 14, 44-51 (2002)
66. Zivadinov, R. Bakshi, R. Grop, A. Sharma, J. Bratian, A. Kuwata, J. M. Nasuelli, D. Tjoa, C. V. & M. Zorzon: A comparison of different quantitative MRI techniques to measure whole brain atrophy in relapsing-remitting multiple sclerosis (abstract). *Mult Scler* 28 (Suppl 1), 99 (2002)
67. Horsfield, M. A. Rovaris, M. Rocca, M. A. Rossi, P. Filippi, M. & R. Bakshi: Whole brain atrophy in multiple sclerosis measured from various MRI sequences. *J Neurol Sci* (in press).
68. Molyneux, P. D. Kappos, L. Polman, C. Pozzilli, C. Barkhof, F. Filippi, M. Yousry, T. Hahn, D. Wagner, K. Ghazi, M. Beckmann, K. Dahlke, F. Losseff, N. Barker, G. J. Thompson, A. J. & D. H. Miller: The effect of interferon beta-1b treatment on MRI measures of cerebral atrophy in secondary progressive multiple sclerosis. *Brain* 123, 2256-2263 (2000)
69. Benston, J. Reza, M. Winter, J. & G. Wilson: Steroids and apparent cerebral atrophy on computed tomography scans. *J Comput Assist Tomogr* 2, 16-23 (1978)

70. Zivadinov, R. Rudick, R. A. De Masi, R. Nasuelli, P. Ukmar, M. Pozzi-Mucelli, R. S. Grop, A Cazzato, G. & M. Zorzon: Effects of intravenous methylprednisolone on brain atrophy in relapsing-remitting multiple sclerosis. *Neurology* 57, 1239-1247 (2001)
71. Rao, A. B. Richert, N. D. Howard, T. Lewis, B. K. Bash, C. N. McFarland, H. F. & J. A. Frank: Methylprednisolone effects on brain volume and enhancing lesions before and during IFNbeta-1b. *Neurology* 59, 688-694 (2002)
72. Hoogervorst, E. L. J. Polman, C. H. & F. Barkhof: Cerebral volume changes in multiple sclerosis patients treated with high-dose intravenous methylprednisolone. *Mult Scler* 8, 415-419 (2002)
73. Patel, N. V. & C. E. Finch: The glucocorticoid paradox of caloric restriction in slowing brain aging. *Neurobiol Aging* 23, 707-717 (2002)
74. Neville, B. G. R. & J. Wilson: Benign intracranial hypertension following corticosteroid withdrawal in childhood. *Br Med J* 3, 554-555 (1970)
75. Ron, M. A. Acker, W. Shaw, G. K. & W. A. Lishman: Computerized tomography of the brain in chronic alcoholism. A survey and follow-up study. *Brain* 105, 497-514 (1982)
76. Kohlmeyer, K. Lehmkuhl, G. & F. Poutska: Computed Tomography of Anorexia Nervosa. *AJNR Am J Neuroradiol* 4, 437-438 (1983)
77. Mellanby, A. R. & M. A. Reveley: Effects of acute dehydration on computerised tomographic assessment of cerebral density and ventricular volume. *Lancet* 16, 874 (1982)
78. Guttmann, C. R. Warfield, S. K. Guimond, A. Kikinis, R. Albert, M. S. & F. A. Jolesz: Variability of brain atrophy estimates in multiple sclerosis (abstract). *Proc Int Soc Magn Reson Med* 8, 1188 (2000)
79. Leist, T. P. Gobbibi, M. I. Frank, J. A. & H. F. McFarland: Enhancing magnetic resonance imaging lesions and cerebral atrophy in patients with relapsing multiple sclerosis. *Arch Neurol* 58, 57-60 (2001)
80. Thorpe, J. W. Kidd, D. Moseley, I. F. Kenndall, B. E. Thompson, A. J. MacManus, D. G. McDonald W. I. & D. H. Miller: Serial gadolinium-enhanced MRI of the brain and spinal cord in early relapsing-remitting multiple sclerosis. *Neurology* 46, 373-378 (1996)
81. Turner, B. Lin, X. Calmon, G. Roberts, N. & L. D. Blumhardt: Cerebral atrophy and disability in relapsing-remitting and secondary progresive multiple sclerosis over four years. *Mult Scler* 9, 21-27 (2003)
82. Sailer, M. Losseff, N. A. Wang, L. Gawne-Cain, M. L. Thompson A. J. & D. H. Miller: T1 lesion load and cerebral atrophy as a marker for clinical progression in patients with multiple sclerosis. A prospective 18 months follow-up study. *Eur J Neurology* 8, 37-42 (2001)
83. Paolillo, A. Coles, A. J. Molyneux, P. D. Gawne-Cain, M. MacManus, D. Barker, G. J. Compston, D. A. & D. H. Miller: Quantitative MRI in patients with secondary progressive MS treated with monoclonal antibody Campath 1H. *Neurology* 53, 751-757 (1999)
84. Dalton, C. M. Brex, P A. Jenkins, R. Fox, N.C. Miszkiel, K. A. Crum, W. R. O'Riordan, J. I. Plant, G. T. Thompson, A. J. & D. H. Miller: Progressive ventricular enlargement in patients with clinically isolated syndromes is associated with the early development of multiple sclerosis. *Ann Neurol* l73, 141-147 (2002)
85. Miller, D. M. Weinstock-Guttman, B. Bethoux, F. Lee, J. C. Beck, G. Block, V. & L. Durelli: A meta-analysis of methylprednisolone in recovery from multiple sclerosis exacerbations. *Mult Scler* 6, 267-273 (2000)
86. Trapp, B. D. Ransohoff, R. M. Fisher, E. & R. A. Rudick: Neurodegeneration in multiple sclerosis: relationship to neurological disability. *Neuroscientist* 5, 48-57 (1999)
87. Kaplan, M. R. Meyer-Franke, A. Lambert, S. Bennett, V. Duncan, I. D. Levinson, S. R. & B. A. Barres: Induction of sodium channel clustering by oligodendrocytes. *Nature* 386, 724-728 (1997)
88. Pfrieger, F. W. & B. A. Barres: Synaptic efficacy enhanced by glial cells in vitro. *Science* 277, 1684-1687 (1997)
89. Arnold, D. L. Wolinsky, J. S. Matthews, P. M. & A. Falini: The use of magnetic resonance spectroscopy in the evaluation of the natural history of multiple sclerosis. *J Neurol Neurosurg Psychiatry* 64 S(1) 94-101 (1998)
90. Lin, X. & L. D. Blumhardt: Inflammation and atrophy in multiple sclerosis: MRI association with disease course. *J Neurol Sci* 189, 99-104 (2001)
91. Bakshi, R. Dmochowski, J. Shaikh, Z. A. & L. Jacobs: Gray matter T2 hypointensity is related to plaques and atrophy in the brains of multiple sclerosis patients. *J Neurol Sci* 185, 19-26 (2001)
92. Bakshi, R. Shaikh, Z. A. & V. Janardhan: MRI T2 shortening (black T2) in multiple sclerosis: frequency, location, and clinical correlation. *Neuroreport* 11, 15-21 (2000)
93. Bermel, R. A. Puli, S. R. Fabiano, A. J. Rudick, R. A. Weinstock-Guttman, B. Simon, J. H. F. E. 3rd Munschauer & R. Bakshi: Gray matter MRI T2 hypointensity predicts longitudinal brain atrophy in multiple sclerosis: effect of intramuscular interferon beta-1a treatment (abstract). *Neurology* 60 (suppl 1), A422. (2003)
94. De Stefano, N. Matthews, P. M. Fu, L. Narayanan, S. Stanley, J. Francis, G. S. Antel, J. P. & D. L. Arnold: Axonal damage correlates with disability in patients with relapsing-remitting multiple sclerosis. Results of longitudinal magnetic resonance spectroscopy study. *Brain* 121, 1469-1504 (1998)
95. Confavreux, C. Vukusic, S. Moreau, T. & P. Adeleine: Relapses and progression of disability in multiple sclerosis. *N Eng J Med* 343, 1430-1438 (2000)
96. J. H. Simon: Brain and spinal cord atrophy in multiple sclerosis. *Advances in Multiple Sclerosis* 10, 753-769. (2000)
97. Simon, J. H: From enhancing lesions to brain atrophy in relapsing MS. *J of Neuroimmunology* 98, 7-15 (1999)
98. Simon, J. H. Lull, J. Jacobs, L. D. Rudick, R. A. Cookfair, D. L. Herndon, R. M. Richert, J. R. Salazar, A. M. Sheeder, J. Miller, D. McCabe, K. Serra, A. Campion, M. K. Fischer, J. S. Goodkin, D. E. Simonian, N. Lajawnie, M. Wende, K. Martens-Davidson, A. Kinkel, R. P. & F. E. 3rd Munschauer: A longitudinal study of T1-hypointense lesions in relapsing MS: Results from the MSCRG trial of interferon beta-1a.Multiple Sclerosis Collaborative Research Group. *Neurology* 55, 185-192 (2000)
99. Paolillo, A. Pozzilli, C. Gasperini, C. Giugni, E. Maiero, C. Giuliani, S. Tomassini, V. Millefiorini, E. & S.

Bastianello: Brain atrophy in relapsing–remitting multiple sclerosis. Relationship with black holes, disease duration and clinical disability. *J Neurol Sci* 174, 85-91 (2000)
100. Molyneux, P. D. Filippi, M. Barkhof, F. Gasperini, C. Yousry, T. A. Truyen, L. Lai, H. M. Rocca, M. H. Moseley, I. F. & D. H. Miller: Correlations between monthly enhanced MRI lesion rate and changes in T2 lesion volume in multiple sclerosis. *Ann Neurol* 43, 332-339 (1998)
101. Kappos, L. Moeri, D. Radue, E. W. Schoetzau, A. Schweikert, K. Barkhof, F. Miller, D. Guttmann, C. R. Weiner, H. L. Gasperini, C. & M. Filippi: Predictive value of gadolinium-enhanced magnetic resonance imaging for relapse rate and changes in disability or impairment in multiple sclerosis: a meta-analysis. *Lancet* 353, 964-969 (1999)
102. Zivadinov, R. & M. Zorzon: Is gadolinium enhancement predictive of the development of brain atrophy in multiple sclerosis? A review of the literature. *J Neuroimaging* 12, 302-309 (2002)
103. Morgen, K. Jeffries, N. O. Stone, R. Martin, R. Richert, N. D. Frank, J. A. & H. F. McFarland: Ring-enhancement in multiple sclerosis: marker of disease severity. *Mult Scler* 7, 167-171 (2001)
104. He, J. Grossman, R. I. Ge, Y. & L. J. Mannon: Enhancing patterns in multiple sclerosis: evolution and persistence. *AJNR Am J Neuroradiol* 22, 664-669 (2001)
105. Rovira, A. Alonso, J. Cucurella, G. Nos, C. Tintore, M. Pedraza, S. Rio, J. & X. Montalban: Evolution of multiple sclerosis lesions on serial contrast-enhanced T1-weighted and magnetization-transfer MR images. *AJNR Am J Neuroradiol* 20, 1939-1945 (1999)
106. Guttman, C. R. Ahn, S. S. Hsu, L. Kikinis, R. & F. A. Jolesz: The evolution of multiple sclerosis lesions on serial MR. *AJNR Am J Neuroradiol* 16, 1481-1491 (1995)
107. Petrella, J. R. Grossman, R. I. McGowan, J. C. Campbell, G. & J. A. Cohen: Multiple sclerosis lesions: relationship between MR enhancement pattern and magnetization transfer effect. *AJNR Am J Neuroradiol* 17, 1041-1049 (1996)
108. Roychowdhury, S. Maldijian, J. A. & R. I. Grossman: Multiple sclerosis: comparison of trace apparent diffusion coefficients with MR enhancement pattern of lesions. *AJNR Am J Neuroradio*l 21, 869-874 (2000)
109. van Waesberghe, J. H. van Walderveen, M. A. Castelijns, J. A. Scheltens, P. Nijeholt, G. T. Polman, C. H. & F. Barkhof: Patterns of lesion development in multiple sclerosis: longitudinal observations with T1-weighted spin-echo and magnetization transfer MR. *AJNR Am J Neuroradiol* 19, 675-683 (1998)
110. Locatelli, L. Zivadinov, R. Bagnato, F. Nasuelli, D. Bastianello, S. Bratina, A, Finamore, L. Catalan, M. Clemenzi, A. Grop, A. Millefiorini, E. & M. Zorzon: Ring-Enhancement Pattern Predict Short-Term Brain Atrophy Changes in Relapsing-Remitting Multiple Sclerosis (abstract). *Neurology* 60 (Suppl 1):A422 (2003)
111. Hohol, M. J. Guttmann, C. R. Orav, J. Mackin, G. A. Kikinis, R. Khoury, S. J. Jolesz, F. A. & H. L. Weiner: Serial neuropsychological assessment and magnetic resonance imaging analysis in multiple sclerosis. *Arch Neurol* 54, 1018-1025 (1997)
112. Filippi, M. Rovaris, M. Iannucci, G. S. Mennea, M. P. Sormani & G. Comi: Whole brain volume changes in patients with progressive MS treated with cladibrine. *Neurology* 55, 1714-1718 (2000)
113. Bakshi, R. Benedict, R. H. B. Bermel, R. A. & L. Jacobs: Regional brain atrophy is associated with physical disability in multiple sclerosis: semiquantitative MRI and relationship to clinical findings. *J Neuroimaging* 11, 129-136 (2001)
114. Paolillo, A. Pozzilli, E. Giugni, E. Tomassini, V. Gasperinic, C. Fiorelli, M. Mainero, C. Horsfield, M. Galgani, S. Bastianello, S. & C. Buttinelli: A 6-year clinical and MRI follow-up study of patients with relapsing-remitting multiple sclerosis treated with Interferon-beta. *Eur J Neurol* 9, 1-11 (2002)
115. Fisher, E. Rudick, R. A. Simon, J. H. Cutter, G. Baier, M. Lee, J. C. Miller, D. Weinstock-Guttman, B. Mass, M. K. Dougherty, D. S. & N. A. Simonian: Eight-year follow-up study of brain atrophy in patients with MS. *Neurology* 59, 1412-1420 (2002)
116. Kalkers, N. F. Ameziane, N. Bot, J. C. Minneboo, A. Polman, C. H. & F. Barkhof: Longitudinal brain volume measurement in multiple sclerosis: rate of brain atrophy is independent of the disease subtype. *Arch Neurol* 10, 172-176 (2002)
117. Zorzon, M. de Masi, R. Nasuelli, D. Ukmar, M. Mucelli, R. P. Cazzato, G. Bratina A. & R. Zivadinov: Depression and anxiety in multiple sclerosis. A clinical and MRI study in 95 subjects. *J Neurol* 248, 416-421 (2001)
118. Zorzon, M. Zivadinov, R. Nasuelli, D. Ukmar, M. Bratina, A. Tommasi, M. A. Mucelli, R. P. Brnabic-Razmilic, O. Grop, A. Bonfigli, L. & G. Cazzato: Depressive symptoms and MRI changes in multiple sclerosis. *Eur J Neurol* 9, 491-496 (2002)
119. Janardhan, V. & R. Bakshi: Quality of life and its relationship to brain lesions and atrophy on magnetic resonance images in 60 patients with multiple sclerosis. *Arch Neurol* 57, 1485-1491 (2000)
120. Zorzon, M. Zivadinov, R. Locatelli, L. B. Stival, F. Monti, D. Nasuelli, M.A. Tommasi, A. Bratina & G. Cazzato: Sexual dysfunction in multiple sclerosis: brain magnetic resonance imaging and urodynamic findings. *Mult Scler* 9, 108-110 (2003)
121. Zivadinov, R. Zorzon, M. Locatelli, L. Stival, B. Monti, F. Nasuelli, D. Tommasi, M. A. Bratina, A. & G. Cazzato: Sexual dysfunction in multiple sclerosis. A MRI, neurophysiological and urodynamic study. *J Neurol Sci* 210, 73-76 (2003)
122. Kalkers, N. F. Barkhof, F. Bergers, E. van Schijndel, R. & C. H. Polman The effect of the neuroprotective agent riluzole on MRI parameters in primary progressive multiple sclerosis: a pilot study. *Mult Scler* 8, 532-533 (2002)
123. Jones, C. K. Riddehough, A. Li, D. K. B. Zhao, G. Paty, D. W. PRISMS Study Group & UBC MS/MRI Research Group: MRI cerebral atrophy in relapsing-remitting MS: results from the PRISMS trial (abstract). *Neurology* 56 (suppl 3) A379 (2001)
124. Gasperini, C. Paolillo, A. Giugni, E. Galgani, S. Bagnato, F. Mainero, C. Onesti, E. Bastianello, S. & C. Pozzillo: MRI brain volume changes in relapsing-remitting multiple sclerosis patients treated with interferon beta-1a. *Mult Scler* 8, 119-123 (2002)
125. Ge, Y. Grossman, R. I. Udupa, J. K. Fulton, J. Constantinescu, C. S. Gonzales-Scarano, F. Babb, J. S.

Mannon, L. J. Kolson, D. L. & J. A. Cohen: Glatiramer acetate (Copaxone) treatment in relapsing-remitting MS: quantitative MR assessment. *Neurology* 54, 813-817 (2000)
126. Comi, G. Inglese, M. De Stefano, N. Smith, S. Barkhof, F. Durelli, L. Edan, G. Fernandez, O. Hartung, H. P. Matthews, P. M. Seeldrayers, P. Sorensen, P. S. Martinelli, V. Hommes, O. R. & M. Filippi: Brain volume changes in patients at presentaion with suspected multiple sclerosis: results from the ETOMS study (abstract). *Mult Scler* 8 (suppl 1) S10 (2002)
127. Hardmeier, M. Wagenpfeil, S. Fisher, E. Rudick, R. A. Kooijmans, M. Clanet, M. Radue E. W. & L. Kappos: Predictors of atrophy during treatment with rIFN-beta 1a IM in relapsing multiple sclerosis: a three year follow-up (abstract). *Neurology* 60 (suppl 1) A300 (2003)
128. Rovaris, M. & M. Filippi: Interventions for the prevention of brain atrophy in multiple sclerosis: current status. *CNS Drugs* 17, 563-575 (2003)
129. Bakshi, R. Kinkel, P. R. Mechtler, L. L. Bates, V. E. Lindsay, B. D. Esposito, S. E. & W. R. Kinkel: Magnetic resonance imaging findings in 22 cases of myelitis: comparison between patients with and without multiple sclerosis. *Eur J Neurol* 5, 35-48 (1998)
130. Kidd, D. Thorpe, J. W. Thompson, A. J. Kendall, B. E. Moseley, I. F. MacManus, D. G. McDonald, W. I. & D. H. Miller: Spinal cord MRI using multi-array coils and fast spin echo. II. Findings in multiple sclerosis. *Neurology* 43, 2632-2637 (1993)
131. Kidd, D. Thorpe, J. W. Kendall, B. E. Barker, G. J. Miller, D. H. McDonald, W. I. & A. J. Thompson: MRI dynamics of brain and spinal cord in progressive multiple sclerosis. *J Neurol Neurosurg Psychiatry* 60, 15-19 (1996)
132. Tartaglino, L. M. Friedman, D. P. Flanders, A. E. Lublin, F. D. Knobler, R. L. & M. Liem: Multiple sclerosis in the spinal cord: MR appearance and correlation with clinical parameters. *Radiology* 195, 725-732 (1995)
133. Thorpe, J. W. Kidd, D. Moseley, I. F. Kenndall, B.E. Thompson, A. J. MacManus, D.G. McDonald, W. I. & D. H. Miller: Serial gadolinium-enhanced MRI of the brain and spinal cord in early relapsing-remitting mutiple sclerosis. *Neurology* 46, 373-378 (1996)
134. Losseff, N. A. Webb, S. L. O'Riordan, J. I. Page, R. Wang, L. Barker, G. J. Tofts, P. S. McDonald, W. I. Miller, D. H. & A. J. Thompson: Spinal cord atrophy and disability in multiple sclerosis. A new reproducible and sensitive MRI method with potential to monitor disease progression. *Brain* 119, 701-708 (1996)
135. Stevenson, V. L. Miller, D. H. Rovaris, M. Barkhof, F. Brochet, B. Dousset, V. Filippi, M. Montalban, X. Polman, C. H. Rovira, A. De Sa, J. & A. J. Thompson: Primary and transitional progressive multiple sclerosis; a clinical and MRI cross sectional study. *Neurology* 52, 839-845 (1999)
136. Ingle, G. T. Stevenson, V. L. Miller, D. H. Leary, S. M. Rovaris, M. Barkhof, F. Brochet, B. Dousset, V. Filippi, M. Montalban, X. Kalkers, N. F. Polman, C. H. Rovira, A. & A. J. Thompson: Two-year follow-up study of primary and transitional progressive multiple sclerosis. *Mult Scler* 2, 108-114 (2002)
137. Rovaris, M. Bozzali, M. Santuccio, G. Ghezzi, A. Caputo, D. Montanari, E. Bertolotto, A. Bergamaschi, R. Capra, R. Mancardi, G. Martinelli, V. Comi, G. & M. Filippi: In vivo assessment of the brain and cervical cord pathology of patients with primary progressive multiple sclerosis. *Brain* 124, 2540-2549 (2001)
138. Coulon, O. Hickman, S. J. Parker, G. J. Barker, G. J. Miller, D.H. & S. R. Arridge: Quantification of spinal cord atrophy from magnetic resonance images via a B-spline active surface model. *Magn Reson Med* 47, 1176-1185 (2002)

Key Words: Multiple sclerosis, Magnetic resonance imaging, Brain atrophy, Spinal cord atrophy, Neurodegeneration, Inflammation, Review

Send correspondence to: Rohit Bakshi, MD, Center for Neurological Imaging, Harvard Medical School, 221 Longwood Avenue, Boston, MA 02115, USA, Tel: 617-732-8600, Fax: 617-264-5154, E-mail: mri@drbakshi.com