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Abbreviations:

CSF = cerebrospinal fluid
EDSS = Expanded Disability Status Scale
MS = multiple sclerosis

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Brain Atrophy in Relapsing-Remitting Multiple Sclerosis: Fractional Volumetric Analysis of Gray Matter and White Matter¹

PURPOSE: To determine the fractional brain tissue volume changes in the gray matter and white matter of patients with relapsing-remitting multiple sclerosis (MS) and to correlate these measurements with clinical disability and total lesion load.

MATERIALS AND METHODS: Thirty patients with relapsing-remitting MS and 25 healthy control subjects underwent magnetic resonance imaging. Fractional brain tissue volumes (tissue volume relative to total intracranial volume) were obtained from the total segmented gray matter and white matter in each group and were analyzed.

RESULTS: The fractional volume of white matter versus that of gray matter was significantly lower (−6.4%) in patients with MS ($P < .0001$) than in control subjects. Neither gray matter nor white matter fractional volume measurements correlated with clinical disability in the patients with MS.

CONCLUSION: Loss of brain parenchymal volume in patients with relapsing-remitting MS is predominantly confined to white matter. Analysis of fractional brain tissue volumes provides additional information useful in characterizing MS and may have potential in evaluating treatment strategies.

Although brain atrophy in multiple sclerosis (MS) has been demonstrated with initial imaging and histopathologic reports of enlarged ventricles (1,2) and reduced size of corpus callosum (3), there is much current interest in the accurate and precise quantification of brain atrophy in MS by developing and improving quantitative magnetic resonance (MR) imaging techniques (4–6). The combination of advanced computer-assisted techniques and high-spatial-resolution MR imaging has allowed measurement of the amount of the brain tissue loss during the course of the disease. The quantitative assessment of brain atrophy in patients with MS is becoming an important consideration in monitoring the treatment effects in clinical trials, because brain volume loss may represent the net cumulative effect of all types of lesions or multiple abnormal processes of the entire brain (5,7).

As far as we are aware, the relative contribution of gray matter versus white matter volume loss to total brain parenchymal volume loss is not known. The results of several studies (8–10) suggest that MS is a disease not restricted to white matter but present also in gray matter. Because gray matter and white matter damage may differ in degree and pathophysiologic features, identifying loss of brain tissue and damage underlying separate tissues has implications for understanding the effect of the disease, as well as for monitoring its progression. Furthermore, because abnormal signal intensities from conventional MR imaging (eg, T2 lesion number, volume) alone may not enable prediction of future clinical benefit (11,12), increased attention has been focused on brain atrophy, which may indirectly indicate the total disease burden. The relationship between brain atrophy and clinical disability in patients with MS still is not well understood.

The purpose of our study was to determine the fractional brain tissue volume changes

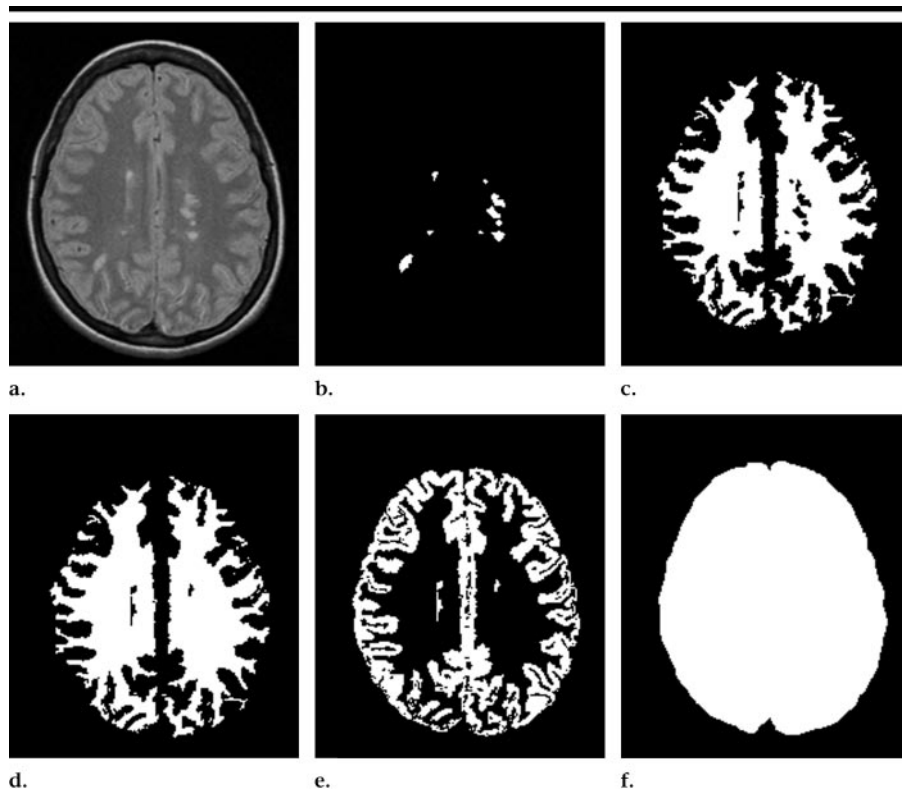


Figure 1. Double-echo fast spin-echo MR images from segmentation in one section (2,500/18, 90; one signal acquired; 3-mm thickness; 0.86-mm pixel size; and an echo train length of eight) in a 35-year-old woman with MS. (a) Intermediate-weighted MR image (2,500/18) and segmented volume images of (b) lesions, (c) normal-appearing white matter, (d) white matter, (e) gray matter, and (f) intracranial contents are shown. MR imaging-visible lesion voxels (b) were added to normal-appearing white matter (c) to get the actual total white matter volume (d). The fractional tissue volume is tissue volume (d or e) relative to total intracranial volume (f).

in the gray matter and white matter of patients with relapsing-remitting MS and to correlate these measurements with clinical disability and total lesion load.

MATERIALS AND METHODS

Subjects

Thirty patients (24 women and six men) with clinically definite relapsing-remitting MS and 25 age-matched healthy control subjects (15 women and 10 men) were examined. The patients were selected from a consecutively enrolled group of individuals with an MR imaging-based natural history of MS that was supported by the National Institutes of Health. All patients from this cohort who met the criterion for relapsing-remitting MS were chosen for study enrollment between 1995 and 1999 without regard to age, sex, or ethnicity. The patients were treated with short courses of steroids when clinically indicated for acute exacerbations; no other immunomodulating therapy (eg, interferon- β 1b, interferon- β 1a, and glatiramer acetate) was given. The

diagnosis in the patients with relapsing-remitting MS was made according to the following criterion: at least two relapses during the preceding 2 years, with relapses defined as new neurologic deficit or exacerbation of a previous deficit confirmed with examination, developing over 1–5 days and lasting at least 48 hours in a patient with previous neurologic stability.

The mean age of the patients was 34.9 years (age range, 26–52 years), and the mean disease duration was 3.8 years (range, 1.0–14.9 years). The mean age of the control subjects was 32.9 years (age range, 21.0–53.0 years). The mean Kurtzke Expanded Disability Status Scale (EDSS) score (13) for the patients with MS was 2.0, which was determined at the time of MR imaging. Informed consent was obtained from all patients and control subjects, and the protocol was approved by the institutional review board of the University of Pennsylvania.

MR Imaging

All patients and control subjects underwent MR imaging with a 1.5-T unit

(Signa; GE Medical Systems, Milwaukee, Wis) with a quadrature transmit/receive head coil. Whole-brain transverse double-echo fast spin-echo images were acquired with 2,500/18, 90 (repetition time msec/echo times msec), a 3-mm section thickness, a 22-cm field of view, one signal acquired, and a 192×256 matrix. The echo train length was eight, and the pixel size was 0.86 mm. More than 50 sections were obtained for each patient to cover the whole brain.

Image Processing and Analysis

The first (intermediate-weighted) and second (T2-weighted) echoes of the fast spin-echo sequence in each study were transferred directly to a workstation (Sun Sparc; Sun Microsystems, Mountain View, Calif) by means of the picture archiving and communications system of our radiology department. Gray matter, normal-appearing white matter without T2-weighted lesions, and lesion and cerebrospinal fluid (CSF) were segmented by using the 3DVIEWS software system (Medical Image Processing Group, University of Pennsylvania, Philadelphia) (14) and by analyzing the intermediate- and T2-weighted images. The process began with segmentation of the brain by using the theory of “fuzzy connectedness” (15). All segmented brain volume images, more than 50 sections in each study, were individually reviewed, and any residual extracranial components were excluded, if necessary, by an experienced neuroradiologist (Y.G.). Lesions (if present), gray matter, normal-appearing white matter, and CSF were identified as three-dimensional fuzzy-connected objects (Fig 1) according to their “affinity,” “fuzzy adjacency,” and “hanging togetherness” (15,16). This semiautomated technique created a volume image, or binary image, by using thin-section double-echo intermediate- and T2-weighted images for the gray matter, normal-appearing white matter, CSF, and lesions (in patients), as well as intracranial contents (Fig 1) from all sections that covered the whole brain. It has been shown that the intra- and interobserver variability is less than 1% for the total lesion volume (16).

To determine the reproducibility of the various volume estimations, we performed repeated MR imaging within 1 week after the previous imaging examination in 12 randomly chosen patients. The previously described parameters were used to obtain the repeat images. Finally, the total gray matter and white matter volume, including the cited lesions, was calculated by adding T2-lesion volume to

compare with those in the control subjects (Fig 1). To minimize the variation of the whole-brain parenchymal volume calculation, only those sections from the MR image sets that were obtained beginning at the section just before the cerebellum appeared at the bottom of the brain and extended to the last section at the top of the brain were included.

Direct comparison of absolute brain parenchymal volume between the patients and healthy control subjects may be obscured by differences in head size, particularly across sex (5). We normalized for head size variability by using fractional gray matter volume and fractional white matter volume, which were computed as percentages of the intracranial volume; for example, fractional gray matter = gray matter/(gray matter + white matter + CSF).

Statistical Methods

Least squares regression was used to determine whether there were differences between the patients with relapsing-remitting MS and the unaffected control subjects in fractional gray matter and fractional white matter volume after adjusting for differences attributable to age and sex. For these analyses, the dependent variables, fractional gray matter volume and fractional white matter volume, were modeled as a linear function of patient age in years; the model included dummy variables identifying the sex and disease status (relapsing-remitting MS vs unaffected) of the subjects. Associations among measurements in patients with relapsing-remitting MS were assessed by using Spearman rank correlation coefficients.

RESULTS

The median coefficient of variation in repeat MR imaging test was 2.1% for gray matter volume, 1.9% for normal-appearing white matter volume, and 1.5% for CSF volume. The fractional and absolute gray matter volumes and white matter volumes in the patients and control subjects are summarized in Table 1.

With respect to white matter volume, there was no significant difference between sexes ($P = .22$) and no significant association with age ($P = .31$), but there was a highly significant difference between the patients and control subjects ($P < .0001$). Specifically, the patients had a significantly lower average white matter volume (mean \pm SD, 425.8 mL \pm 68.3) than did the control subjects (516.6 mL \pm 84.6). Similar results were obtained for fractional

TABLE 1
Comparison of Gray Matter and White Matter Volume Measurements in Patients and Control Subjects

Volumes	Mean \pm SD	Median
Control subjects		
GM (mL)	711.7 \pm 102.1	717.7
Fractional GM (%)	52.0 \pm 4.5	51.5
WM (mL)	516.6 \pm 84.6	503.7
Fractional WM (%)	37.9 \pm 5.3	38.1
Patients		
GM (mL)	686.7 \pm 102.3	708.1
Fractional GM (%)	50.7 \pm 5.7	5.7
WM (mL)	425.8 \pm 68.3	68.3
Fractional WM (%)	31.4 \pm 3.9	3.9

Note.— P values represent the significance level of a two-sided test of the difference between control subjects and patients with relapsing-remitting MS. For gray matter (GM), $P = .37$; for white matter (WM), $P < .0001$.

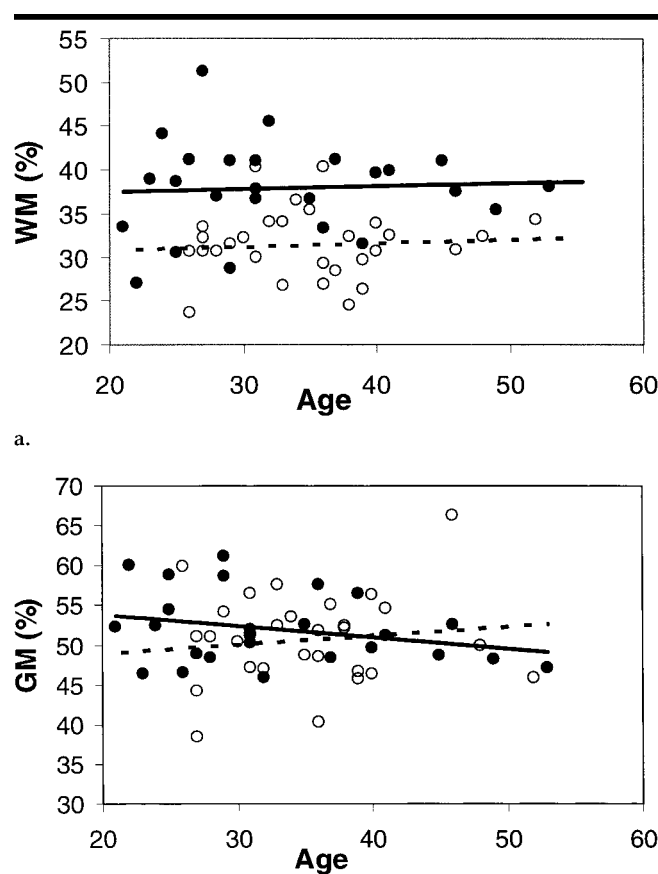


Figure 2. Scatter plots show (a) fractional white matter (WM) and (b) fractional gray matter (GM) versus age in control subjects (solid circles and solid lines) and patients with relapsing-remitting MS (open circles and dashed lines). The difference between the patients and control subjects was larger in the fractional white matter measurements than in the fractional gray matter measurements. No significant association with age was found in either fractional gray matter or fractional white matter volume with a certain age range.

white matter volume ($P < .0001$) between the patients with relapsing-remitting MS (31.4% \pm 3.9)

and the control subjects (37.9% \pm 5.3)(Fig 2a). There was a 6.4% mean difference in fractional white matter volume between the patients and control

TABLE 2
Spearman Rank Correlation Coefficients and *P* Values for the Association between Volume Measurements and EDSS Score, and Lesion Volume in Patients with Relapsing-Remitting MS

Parameter	Fractional GM		Fractional WM	
	<i>r</i>	<i>P</i> Value	<i>r</i>	<i>P</i> Value
EDSS score	-0.2	.28	-0.03	.86
Lesion volume	-0.52	<.004	-0.29	.12

Note.—Each *P* value was obtained from a two-sided test of the relevant correlation. The *P* value from the one-sided test can be obtained by dividing the *P* values in the table by 2. GM = gray matter, WM = white matter.

subjects. Neither age ($P = .65$) nor sex ($P = .21$) was significantly associated with fractional white matter volume.

There was no significant difference ($P = .37$) between the patients and control subjects in terms of either absolute (in milliliters) or fractional volume (percentage) of gray matter (Fig 2b). For fractional gray matter volume, there was no significant difference between sexes ($P = .85$) and no significant change with age ($P = .55$). However, the absolute gray matter volume in the control subjects was significantly higher ($P = .003$) in men—an average of $84.4 \text{ mL} \pm 27.4$ larger than that in women of the same age.

For patients, Spearman rank correlation coefficients were computed (Table 2) (29) to assess the relationship between volumetric measurements and clinical measurements (EDSS score), as well as T2-weighted lesion volume. However, there were no significant correlations between fractional brain tissue volumes and EDSS scores for either gray matter or white matter. Fractional gray matter volume, rather than fractional white matter volume, was significantly negatively correlated with total lesion volume ($r = -0.52$, $P < .004$) (Fig 3).

DISCUSSION

Brain atrophy is a common imaging finding in patients with MS. Quantitation of gray matter and white matter volume changes in patients with MS can be used to evaluate the specific damage and disease burden for each tissue component of the brain. The results of the current study suggest that brain atrophy in patients with relapsing-remitting MS is attributable mainly to loss of white matter. Our failure to find a similar relative loss of

gray matter in these patients suggests that brain atrophy in MS does not represent “classic” cortical atrophy. The poor correlation between EDSS score and loss of white matter may suggest that clinical measurements are not sensitive indicators of brain tissue loss. Many have advocated brain atrophy measurement in monitoring clinical trials (5–7); however, we provided further imaging insight into the tissue distribution of brain atrophy.

Most reported brain atrophy analyses (1–3) rely on subjective assessment, with visual identification of enlarged ventricles or a reduced size of corpus callosum; however, these changes may include the loss of both gray matter and white matter. We used fractional volume measurements to normalize the variability in brain size and found that, relative to the control subjects, the patients with MS had a significantly smaller fractional white matter volume ($P < .0001$) but did not have a significantly smaller fractional gray matter volume ($P = .37$) (Fig 2). This suggests that loss of white matter tissue is the major determinant of brain atrophy in relapsing-remitting MS. Such changes differ from those associated with aging (18) or other degenerative diseases such as Alzheimer disease (19,20), which is characterized by cortical atrophy, or loss of gray matter.

The reduced white matter volume in patients with MS may result from loss of axons (21), loss of myelin (22), and gliosis (23,24). However, because MS is a demyelinating disease, myelin loss might contribute more to the volume loss of white matter. The relative sulcal enlarge-

ment in patients with MS might be due to the loss of white matter rather than of gray matter within the gyral cores and subarcuate fibers. However, in the current study, the total white matter volumes were determined with the composition of normal and abnormal white matter tissues in patients with MS.

Our results regarding gray matter volume demonstrated no statistically significant difference between the patient and control groups. Although investigators in previous studies (25,26) have shown a significant decrease in *N*-acetylaspartate, a specific neuronal marker for axon and cell bodies, in MS, results of the current study suggest no significant volume loss of gray matter in patients with relapsing-remitting MS. Therefore, *N*-acetylaspartate loss in normal-appearing white matter and lesions (25,26) in patients with MS could have resulted from axonal damage in white matter that resulted from volume (myelin and axon) loss, which is consistent with the white matter volume loss in our study.

Investigators in a recent study of gray matter magnetization transfer ratio (10) found a statistical difference in gray matter magnetization transfer ratio histograms between patients with relapsing-remitting MS and control subjects, which suggests the existence of subtle abnormality in gray matter as well. However, these abnormalities were measured microscopically and may not cause volumetric changes of gray matter during the relapsing-remitting course of disease.

An important issue is the relationship

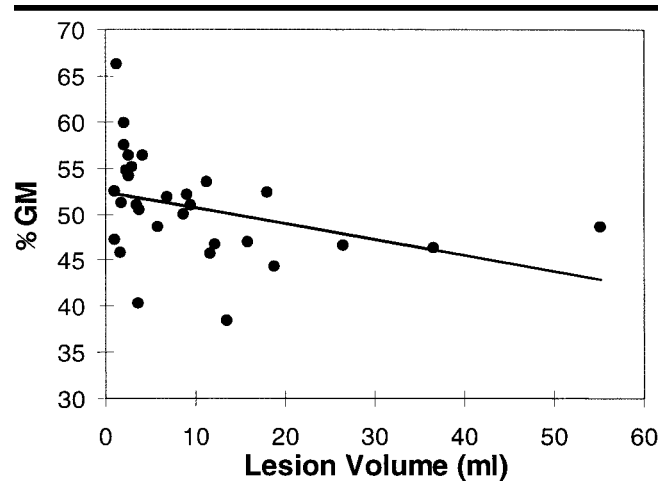


Figure 3. Scatterplot shows the relationship between fractional gray matter (%GM) volumes in 30 patients with relapsing-remitting MS and their lesion volume, measured on the T2-weighted images. Solid line is least squares regression line to predict fractional gray matter volume. The Spearman rank correlation coefficient was used for the association ($P < .004$).

between atrophy and clinical functioning. Our data indicate a lack of correlation between loss of white matter and EDSS score and are consistent with data from previous studies (5,27), which have shown no correlation between whole-brain atrophy and EDSS score in patients with relapsing-remitting MS. Others (28,29), however, have shown weak relationships between brain atrophy and EDSS score. This indicates that the EDSS score may not be a sensitive marker for brain tissue loss in MS.

However, there may be several other explanations for such inconsistencies. First, in the two previously mentioned studies (28,29) no normalized volume measurements accounting for brain-size variations were used. When variability between individuals was corrected with the fractional volume measurement, no correlation between brain atrophy and EDSS score was found (5,27). Second, techniques of brain atrophy measurement in the literature (28,29) are based on regional or smaller brain sections that may not accurately represent overall clinical status. Third, our analysis was based exclusively on patients with relapsing-remitting MS and might therefore be expected to produce different results than would studies of patients with general MS (28,29). This is an important consideration because the observation may be different in different clinical groups. For example, there was a negative correlation between brain atrophy and clinical measurement in only secondary progressive MS (27).

Although the contributions to brain atrophy in MS were thought to come from loss of myelin and axons, we found a poor correlation ($r = -0.29$) between lesion volume and loss of white matter in patients with relapsing-remitting MS. We postulate that white matter volume loss occurs independently of T2-weighted abnormalities, which represent a broader abnormal spectrum, including inflammation, edema, demyelination, gliosis, myelin, and axonal loss. This is in agreement with the findings of some previously published studies (5,27-29), which suggest that brain atrophy in MS may progress with only some detectable MR imaging abnormalities such as "black holes," but not with others such as edema. Interestingly, we found a negative correlation between fractional gray matter volume and T2 lesion load in patients. This suggests that lesion load in white matter affects gray matter, a process of Wallerian degeneration by upstream effect on neuronal cell bodies.

In conclusion, loss of white matter tissue accounts for the bulk of brain atrophy in patients with relapsing-remitting MS. This is unlike the cause of atrophy in other degenerative diseases such as Alzheimer disease. However, a significant loss of white matter was poorly associated with clinical disability, as measured with the EDSS score. Our results provide data on the separate tissue net effect of disease burden in MS and suggest that gray matter and white matter component analysis may add specificity to the interpretation of atrophy data.

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