Published in final edited form as:

Neuroimage. 2014 October 15; 100: 370–378. doi:10.1016/j.neuroimage.2014.06.014.

Sub-Millimeter Imaging of Brain-Free Water for Rapid Volume Assessment in Atrophic Brains

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Abstract

Introduction—Cerebral atrophy occurs in healthy aging, and in disease processes such as multiple sclerosis (MS), it correlates with disability accumulation. Imaging measurements of brain atrophy are commonly based on tissue segmentation, which is susceptible to classification errors and inconsistencies. High-resolution imaging techniques with strong contrast between brain parenchyma and cerebrospinal fluid (CSF) might allow fully automated, rapid, threshold-based determination of the free water in the brain. We hypothesized that total brain-free-water (BFW) volume and BFW volume expressed as a normalized fraction of the intracranial volume (“BFW fraction”), determined from heavily T_2 -weighted images, would be useful surrogates for cerebral atrophy and therefore would correlate with clinical measures of disability in MS.

Methods—Whole brains of 83 MS cases and 7 healthy volunteers were imaged with a 4.7-min, heavily T_2 -weighted sequence on a 3T MRI scanner, acquiring 650- μm isotropic voxels. MS cases were clinically assessed on Expanded Disability Status Scale (EDSS), Scripps Neurological Rating Scale (SNRS), Paced Auditory Serial Addition Test (PASAT), 9-Hole Peg Test (9HP), Symbol Digit Modalities Test (SDMT), and 25-Foot Walk. Twelve of the MS cases were rescanned within an average of 1.8 months to assess reproducibility. Automated calculations of BFW volume and BFW fraction were correlated with clinical measures of disability upon adjusting for age and sex. Results were compared to data from T_1 -based approaches (SIENAX and Lesion-TOADS).

Results and Discussion—BFW volume was automatically derived from heavily T_2 -weighted images with no need for separate skull stripping. BFW volume and fraction had mean scan-rescan coefficients of variation of 1.5% and 1.9%, respectively, similar to the T_1 -based approaches tested here. BFW fraction more strongly correlated with clinical measures than T_1 -derived results. Among those clinical measures, modality-specific disability scores, such as SDMT and 9HPT, were more strongly associated with BFW fraction than composite measures, such as EDSS and SNRS.

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Conclusion—The BFW method robustly estimates cerebral atrophy in an automated, fast, and reliable manner, and as such may prove a useful addition to imaging protocols for clinical practice and trials.

Keywords

Multiple sclerosis; Atrophy; Magnetic resonance imaging; Cerebrospinal fluid

1 INTRODUCTION

Cerebral atrophy commonly occurs in healthy aging, and in neurological diseases such as multiple sclerosis (MS), contributes to clinical disability. As the irreversible loss of myelin, axons, and whole neurons, atrophy is the endpoint of neurodegeneration and reflects cumulative disease burden. Atrophy may be focal or affect the entire central nervous system. The per annum atrophy rate in the general MS population is reported to be around 0.5% (1–3), whereas the rate in the general population is between 0.1% and 0.4% (2, 4, 5). Tissue loss is associated with clinical disability in MS, as cases with lower brain parenchymal fractions (BPF) – defined as the ratio of brain to intracranial volume – tend to score worse on expanded disability status scale (EDSS) and MS functional composite (MSFC) (6–10). Furthermore, large multicenter clinical trials have shown that cerebral and central atrophy at baseline are reliable predictors of long-term disability in MS cases, after adjustments for imaging protocol, baseline phenotype, and baseline EDSS (11), highlighting the important role atrophy plays in disease progression in MS.

Cerebral atrophy in advanced cases is easily identified on MRI scans as smaller volumes of brain parenchyma and enlargement of CSF spaces. Quantification of atrophy using MRI has traditionally relied on capturing these processes using manually drawn regions of interests (12, 13) or atlas-based or probabilistic tissue segmentation methods (14–16). Cross-sectional atrophy estimates may be determined from regions of interest (ROI) drawn around specific structures (12, 13, 17), such as the third ventricle or corpus callosum, but this approach does not reflect whole-brain atrophy and can be time-consuming. A common and more automated approach to atrophy assessment is to classify brain structures and calculate their volumes from T₁-weighted MRI (7, 18–23). Inversion-prepared T₁-weighted scans provide high contrast within the brain, and the images can be used as inputs to tissue segmentation and classification algorithms such as SIENAX (FMRIB, Oxford University), FreeSurfer (Harvard University), and Lesion-TOADS (IACL, Johns Hopkins University). SIENAX estimates the brain tissue volume from a single T₁-weighted scan after skull stripping and tissue classification. The volume of the tissue is normalized to a standard for cross-subject comparisons using volumetric scaling factors obtained by affine registration of the brain with a standard brain atlas derived from healthy brains (21). FreeSurfer also classifies tissue types with an atlas-based algorithm (24, 25). Different segmentation techniques provide slightly different classification results from the same healthy brains (26). However, in diseased brains, moderate to large lesion volumes as well as pronounced atrophy often cause gross errors in skull stripping and tissue classification (27).

Lesion-TOADS was specifically tailored to segment the MS brain by using a probability-based algorithm to account for the likelihood of lesions in the white matter (28). Based on

T₁-weighted and T₂-FLAIR intensity information as well as statistical and topological atlases, Lesion-TOADS automatically segments the brain into white matter lesions in addition to major cortical and subcortical regions. Although Lesion-TOADS-derived brain structure volumes are more strongly associated with physical impairment in MS than those derived from SIENAX's segmentation tool (18, 28), the method suffers from some of the same drawbacks as other segmentation methods, such as errors due to skull stripping and tissue classification, as well as registration errors between the T₁- and T₂-weighted scans.

Since the space left by loss of brain tissue is replaced with CSF, intracranial CSF volume, instead of brain volume, can be used as a surrogate marker of cerebral atrophy. Heavily T₂-weighted images can be tailored to provide a uniform and high CSF-to-brain contrast, from which the total volume of the free water that is not part of brain parenchyma, denoted here as brain-free water (BFW), can be determined. Recent advances in MRI techniques, such as 32-channel phased-array coils and parallel imaging, allow improved spatial resolution imaging in clinical settings, which can be used to better visualize and segment the extracerebral and sulcal CSF. The fully automated technique described here uses a simple thresholding algorithm to derive BFW volume in the intracranial compartment and then uses the scaling factors from registration to an atlas to derive a normalized BFW fraction, which can be compared among individuals. Both measures were correlated with clinical scores of disability in MS cases. Additionally, BFW assessment and its clinical correlations were compared with those from T₁-based approaches.

2 METHODS

2.1 Patient recruitment and evaluation

Seven healthy volunteers (HVs) and 83 cases on the MS spectrum were recruited for our study. The MS participants were neurologically evaluated and assessed with Expanded Disability Status Scale (EDSS), Scripps Neurological Rating Scale (SNRS), Symbol Digit Modality Test (SDMT, paper-based), Paced Auditory Symbol Addition Test (PASAT, 3 second version), 9-Hole Peg Test (9HPT), and 25-Foot (25') Timed Walk. Clinical data were obtained within 30 days of MRI acquisition. Participants gave informed consent, and the Institutional Review Board approved all protocols.

2.2 Imaging

All participants were scanned with a Siemens 3T Skyra system (software version VD11) and a 32-channel phased-array receive-only head coil. To assess scan-rescan reliability, 16 of the MS cases were rescanned within 4 months, and participants with enhancing lesions or new T₂-hyperintense lesions were excluded. Heavily T₂-weighted images were acquired three-dimensionally with a fast spin-echo sequence (FSE), prescan-normalize filter for receive coil homogeneity, TR=4800 msec, TE=752 msec, FA=100°, scan acceleration factor GRAPPA=2, echo-train-length=421, fat-saturation mode=strong, 0.65 mm isotropic resolution, bandwidth=543 Hz/Px, and acquisition time (TA)=4 min 43 sec. In addition to the heavily T₂-weighted image, 3D T₂-FLAIR (SPACE sequence with T₂-variable flip angle, TR=4800 msec, TE=354 msec, TI=1800 msec, GRAPPA=2, 1 mm isotropic resolution, bandwidth=650 Hz/Px, TA=7 min 2 sec) and T₁-MPRAGE (TR=3000 msec,

TE=3.03 msec, TI=900 msec, FA=9°, GRAPPA=3, 1 mm isotropic resolution, bandwidth=650 Hz/Px, TA=5 min) scans were also acquired.

2.3 Data analysis

The heavily T₂-weighted images from the 7 HVs were first registered (affine registration with AFNI, normalized mutual information) to each other, and the resultant images were averaged together. The individual scans were then re-registered using the same method to the average image, averaged, and Gaussian blurred with a 2 mm filter to produce a population atlas. A binary mask image was created on the atlas to identify (and tag for subsequent removal) the regions corresponding to the eyes, paranasal sinuses, and neck (below the foramen magnum).

Heavily T₂-weighted images from individual participants were registered to the atlas (affine registration with FLIRT, normalized cross correlation cost function, trilinear interpolation), the transformation matrix was calculated, and the global scaling factor was recorded. The mask image was then transformed back to native space using the inverse of the transformation matrix and nearest-neighbor interpolation, after which it was multiplied with the heavily T₂-weighted image to obtain a participant-specific BFW image of the head. A binary BFW mask was created from the heavily T₂-weighted image by preserving only voxels with intensities higher than a threshold, which was expressed as a fraction of the 98th-percentile of signal intensity. The 98th intensity percentile was chosen instead of the maximum signal intensity in order to reduce dependence on image noise. The threshold intensity was determined once for the whole study, as described in the Results section, and was based on examination of plots of the absolute differences in BFW volumes as a function of fractional threshold in the scan-rescan cohort. BFW volumes were calculated by multiplying the total number of voxels above the threshold in BFW masks by the image resolution. Scan-rescan reproducibility of the atrophy measures was assessed with intraclass coefficients (ICC) and coefficients of variation (COV), and by Bland-Altman analysis. COVs were calculated as the average of the subject-specific COVs (standard deviation divided by mean) across the two replicate datasets in the test-retest analysis, and expressed as a percentage. Finally, BFW fraction was derived from the BFW volume by normalizing with intracranial volume of the atlas after compensating with the global scaling factor derived from the transformation matrix. The intracranial volume of the atlas was determined from a manually drawn intracranial mask of the atlas. This volume was the starting point of the BFW fraction calculation.

To compare BFW volumes with an existing method of CSF visualization, we also analyzed the T₂-FLAIR and T₁-MPRAGE scans with Lesion-TOADS as described elsewhere (28). Briefly, T₁-MPRAGE and T₂-FLAIR images were input to the SPECTRE algorithm, which performed skull stripping. The output of SPECTRE was fed into the Lesion-TOADS tissue classification algorithm to obtain tissue masks for lesions, sulcal CSF, ventricular CSF, cortical GM, WM, etc. The ventricular and sulcal CSF volume (“CSF volume”) and CSF volume normalized by intracranial volume (“CSF fraction”) were compared to BFW volume and fraction, respectively. Bland-Altman plots were used to compare the atrophy measurements from BFW and Lesion-TOADS methods. Additionally, to compare the

reproducibility of the BFW method with a more widely used cross-sectional atrophy assessment tool, T₁-MPRAGE images of the scan-rescan cohort were also analyzed with SIENAX (21), which yields a brain-volume estimate. In order to compare the reproducibility of SIENAX and the BFW method, the mean COV of brain volume estimates were calculated by both methods. BFW volume was subtracted from the intracranial volume to yield a BFW-derived estimate of subject-specific brain volume. The BFW volume COV was also converted in terms of brain volume by multiplying it by the average BFW volume and dividing by the average BFW-derived brain volume.

Correlations between clinical (EDSS, SNRS, SDMT, PASAT, 9HPT, and 25' timed walk) and atrophy (BFW volume and fraction, CSF volume and fraction from Lesion-TOADS) measures were assessed using linear regression analyses. After initial univariate analysis for covariate selection of age and sex, a stepwise selection model was used for atrophy measure and potential covariates to predict clinical disability. Additional regression analyses used lesion volume, derived from Lesion-TOADS, as a possible covariate ("full model"). All values are reported as mean $\pm$ SD unless otherwise stated, and p-values are reported directly without adjustment for multiple comparisons, as this was an exploratory study.

3 RESULTS

Eighty-three MS cases (46 women; age range: 25–70 years, 6 clinically isolated syndrome, 1 radiologically isolated syndrome, 30 relapsing-remitting MS, 17 secondary-progressive MS, and 29 primary-progressive MS) and 7 HV (4 women; age range: 24–41) were recruited for this study. Of the 16 cases rescanned to assess scan-rescan reliability and threshold analysis, 12 MS cases within an average of 1.8 months were stable clinically with no new or enhancing lesions or clinical progression (scan-rescan cohort). Table 1 summarizes the clinical assessment of the entire cohort as well as the rescanned participants.

Heavily T₂-weighted images show high signal within CSF surrounding the brain and inside the ventricles (Fig 1, third column). In addition, some lesions with high free water content (probably indicating advanced tissue destruction) could be seen, however most lesions were not visible on the heavily T₂-weighted images. Perivascular spaces could often be appreciated, particularly in the basal ganglia (not shown).

Higher thresholds of the heavily T₂-weighted images, and thus increased selectivity for hyperintense voxels, excluded darker voxels on the outer edges of CSF spaces and resulted in lower BFW volumes. Plotting BFW volume as a function of intensity threshold (i.e., fraction of the 98th percentile of overall signal intensity) showed regions of rapid change at low threshold values (0–0.3). However, at thresholds greater than 0.3, BFW volume decreased much more slowly and linearly, and the absolute value of the scan-rescan differences stabilized at just under 2% (Fig 2A). Based on these data and visual inspection of heavily T₂-weighted images at various thresholds, 0.5 was selected as the best threshold for BFW volume calculation.

Examples of BFW masks generated with the selected threshold are shown in the right-most column of Fig 1. The signal from areas of CSF more than one voxel away from tissue was about 10 times higher than the brain parenchyma in the heavily T₂-weighted images,

providing sufficient contrast for easy CSF segmentation in the masks. Skull was completely absent from BFW masks, which depicted CSF regions without any need for dedicated skull stripping. Heavily T₂-weighted images had adequate resolution and signal for small features, such as sulcal convolutions and choroid plexus, to be consistently segmented on the masks. In rare cases, portions of lesions were sufficiently hyperintense to be included in the BFW mask. Perivascular spaces were in most cases not bright enough to appear on the masks. All 106 cases (7 HV and 83 MS, 16 with repeated measurements, of which 12 were used for reproducibility analysis) were successfully analyzed with the BFW method.

Bland-Altman analysis of scan-rescan BFW volume showed no bias or trend, with an ICC of 0.99 and scan-rescan COV of 1.5% (Fig 2B). By comparison, Lesion-TOADS segmentation of CSF volume had a scan-rescan COV of 0.63%. Estimates of brain volume from the BFW method and SIENAX had scan-rescan COVs of 0.99% and 1.5%, respectively. When expressed in terms of brain volume using the average BFW and intracranial volumes, the COV of 1.5% translated to 0.3%. Bland-Altman analysis of intracranial volume, derived from the intracranial mask of the atlas and the scaling values from atlas alignment, also showed no bias or trend, with an ICC of 0.99 and a COV of 0.67% (Fig 2C). Similarly, BFW fraction showed no bias with repeated measurements and had an ICC of 0.97 and a COV of 1.9% (Fig 2D). CSF fraction derived from Lesion-TOADS had a scan-rescan COV of 0.64%.

Both BFW method and Lesion-TOADS classified CSF, as can be seen in a representative MS case in Fig. 3A. However, BFW masks more tightly followed CSF contours on T₂-FLAIR and T₁-MPRAGE than those from Lesion-TOADS and had fewer obvious errors in tissue classification. Compared to Lesion-TOADS, the BFW method more consistently removed choroid plexus and the septum pellucidum from CSF, as well as vessels traversing the CSF space and brain. On average, BFW volume was lower than CSF volume from Lesion-TOADS by $15.0 \pm 8.9\%$, $p < 0.005$ (Fig. 3B), and the intracranial volume derived from the BFW method was lower than that derived from Lesion-TOADS by $3.4 \pm 3.9\%$, $p < 0.005$ (data not shown).

Figures 4A and B show the group-averaged BFW volume (in mm³) and BFW fraction among the various clinical subtypes of subjects scanned. BFW volume correlated more strongly and significantly with SDMT, 9HPT, and PASAT, whereas CSF volume estimated by Lesion-TOADS was associated only with SDMT (Fig 4C, Table 2). Normalizing BFW volume by intracranial volume strengthened the associations between atrophy and clinical outcome: the semi-partial correlation coefficients of all clinical assessments increased, and all clinical scores but 25' timed walk were significantly correlated with BFW fraction (Fig 4D, Table 2). Likewise, Lesion-TOADS correlations with PASAT and 9HPT became stronger after adjusting for intracranial volume. BFW fraction was the most predictive measure to estimate the clinical outcome among all those tested, accounting for up to 17% more variance in clinical outcome compared to the estimates of CSF fraction from Lesion-TOADS.

Age, sex, lesion volume, and BFW volume accounted for 17–30% of the variance in clinical measures in the full model tested here (data not shown). Clinical measures were consistently

better-predicted with BFW fraction than with BFW volume (except for the case of 25' timed walk, where the predictions were similar): simply by normalizing BFW volume with intracranial volume, 8–19% more of clinical outcome could be explained. Furthermore, the full model using BFW fraction explained more of the variation (21% increase on average) in all of the clinical measures, except 25' timed walk, compared to similar models that used CSF fraction.

4 DISCUSSION

In this study, a high-resolution, heavily T₂-weighted scan that provides high contrast between brain parenchyma and free water in the ventricles and sulci was used to quantify the total volume of brain-free water, a surrogate marker for cerebral atrophy. Such images essentially silhouette all soft tissue within the intracranial compartment, so that the only residually bright voxels primarily contain free water. Once a coarse atlas (used for registration) and tissue mask are generated, the BFW method is completely automated, yielding BFW volume and BFW fraction for each case.

To account for variation in head size in the population, BFW volume was normalized with the scaling factors used to register the heavily T₂-weighted image to a population atlas and the volume of the intracranial space in the atlas, thereby deriving a BFW fraction. In MS cases, heavily T₂-weighted image-derived volume measures outperformed similar markers derived from Lesion-TOADS, an atlas-based, topology-corrected segmentation algorithm specifically designed to account for the effects of brain lesions on tissue classification (28). As these data are generated from a modified but still conventional imaging approach (a T₂-weighted fast-spin-echo sequence), the results reported here suggest that, at least to some degree, the relatively poor association between conventional imaging and clinical measures may be explained by suboptimal imaging and image analysis approaches.

The basic premise of measuring atrophy with BFW fraction is that the CSF compartment expands to occupy the space created by the atrophying brain. While measures of the total CSF space have been used both postmortem (29) and in vivo by quantitative or pseudo-quantitative MRI (30, 31), the MRI method proposed here offers a fast and accurate surrogate for brain volume in vivo. However, variability in the BFW fraction among healthy brains, related to factors other than head size per se, might act to confound this imaging marker in cross-sectional studies. In our cohort, the standard deviation in BFW fraction among HVs was 0.01, smaller than the standard deviation in the MS cohort (0.03). Furthermore, cerebral atrophy may be nonlinearly related to disease duration, where the propensity for accelerated atrophy is reported to increase with disease severity and perhaps age (2). Changes to the statistical models could be made in larger clinical trials to account for this nonlinearity. Additionally, atrophy (32) and lesion volume (33) measurements from the spinal cord, the upper part of which is captured on our sagittally acquired images, could in principle be included in the full model to more completely account for clinical disability; this is a subject of ongoing study.

Numerous studies have established positive correlations between atrophy and clinical impairment in MS; these results have been reviewed (6, 10). In a study of 40 MS cases,

increased third ventricle width and volume were associated with worse EDSS score ($sr=0.36$, $p=0.02$) (34). Similarly, BPF was correlated with concurrent physical disability in an 8-year study of 134 cases with relapsing-remitting MS (9). To get a more comprehensive and precise understanding of the clinical manifestations of atrophy, measures of cognitive function (PASAT and SDMT), upper and lower extremity function (9HPT and 25' timed walk), and general impairment (EDSS and SNRS) were obtained in this study. After adjusting for any significant effects of age and sex, BFW volume significantly correlated with SDMT, PASAT, and 9HPT. When BFW volume was normalized by intracranial volume, the correlations strengthened, and significant associations with EDSS and SNRS emerged. These findings are consistent with those of Fisher et al (9), who noted a stronger correlation between BPF and MSFC (a composite score generated from PASAT, 9HPT, and 25' timed walk) than between BPF and EDSS. 25' timed walk was not predicted by any of the atrophy measurements, perhaps because of the extensive spinal cord dependence of leg function and mobility. The extent of observed correlation is in part a function of the study population, which in the current study had a wide range of disability. However, a wide disability range by itself does not induce higher correlations, as Shiee et al. (18) reported stronger correlations than those seen here despite the larger disability range in the current study.

Interestingly, the three tests most correlated with BFW (SDMT, PASAT, and 9HPT) involve very specific tasks that require attention, concentration, and fine motor control (including the SDMT, which, as implemented here, required participants to note their answers on paper rather than verbally), as opposed to EDSS and SNRS, which are composite indicators of general neurologic impairment (35, 36). Brain atrophy is an imaging indicator of cumulative tissue damage, and clinical outcomes that similarly reflect underlying pathology are necessary to decipher disease progression. The strong correlations between atrophy and assessments requiring specific cognitive and behavioral tasks suggest that SDMT, PASAT, and 9HPT, rather than more global disability scores, are better suited as clinical outcome measures in clinical trials, although additional studies are required to fully characterize their potential responsiveness to treatment.

Where atrophy predicted clinical scores, BFW method outperformed Lesion-TOADS, as evidenced by higher semi-partial correlation coefficients. This suggests that CSF volumes estimated from our 650- μm isotropic CSF-weighted scan more accurately reflect extent of disease than those obtained from 1 mm³ scans and T₁-based segmentation approaches. The improved performance of BFW fraction over Lesion-TOADS may be attributed to its sensitivity to expanding sulci in addition to the ventricles and to the exclusion of blood vessels and intraventricular soft tissue (e.g., choroid plexus), thus providing an objective and accurate measure of global atrophy. Even at such high resolution, the heavily T₂-weighted image is acquired in less than 5 minutes. Furthermore, the BFW method relies on relatively simple image registration and threshold operations for its atrophy calculation and uses an atlas only for removal of hyperintense extracranial structures such as the eyes and neck. Thus, our method does not require atlas-based or probabilistic segmentation of intracranial tissues, which is known to be a major source of error, especially in diseased brains (37).

The BFW method also eliminates the need for dedicated skull stripping. Oftentimes, in our experience, the skull-stripping step of automated segmentation methods is faulty, either removing parts of the brain (e.g. cerebellum) or leaving bits of skull, and the errors must be corrected manually (27, 38). Inaccurate skull stripping leads to the miscalculation of extracerebral CSF volumes, so the absence of a requirement for separate skull stripping is a highly attractive aspect of our method, as it is truly a completely automated process. While the skull does not need to be removed from heavily T₂-weighted images, orbits and spinal cord are hyperintense on the raw images and need to be masked out via registration to an atlas. However, since these structures are localized in specific areas of the head, instead of surrounding the entire brain, removing them is far simpler than skull stripping. The ability of heavily T₂-weighted images to natively discriminate the intracranial compartment, with no more required than a simple masking step, could in principle be extended to other applications that require skull stripping.

Lesion volume is a structural reflection of pathology that is distinct from diffuse tissue atrophy (39, 40). The results from several studies suggest that total lesion volume correlates to some extent with brain atrophy but is not enough on its own to explain brain tissue loss (7, 9, 15, 19, 41). Thus, we examined how much of clinical outcome could be explained with age, sex, and lesion volume, along with cerebral atrophy, in the “full model.” On average, this model accounted for 23% of the variance in clinical outcome when BFW volume was used as an atrophy marker. With BFW fraction, the variance explained increased to 26%. Compared with those with Lesion-TOADS-derived measurements, models with BFW-derived atrophy measurements explained a higher proportion of all the clinical assessments except 25' timed walk, where no associations could be detected for either method.

The high CSF-to-brain contrast is achieved in this study using a fast-spin-echo sequence with long echo times on the order of 750 ms. As CSF has a much longer T₂ than does brain tissue (42, 43), such a pulse sequence achieves signal nulling from brain parenchyma. Long-echo-time sequences have been previously described (44) for MR cholangiopancreatography (45) and for imaging the inner ear (46, 47). Structures with T₂ values similar to CSF were also seen in and around the spinal canal and orbits, and inflammatory changes within the paranasal sinuses and mastoid air cells were also hyperintense in the images, but all of these could be easily removed using a mask derived from the simple atlas we created from 7 HV scans. In addition, since segmentation requires uniform signal intensities from CSF throughout the brain, the sequence was modified with adiabatic pulses, and images were corrected for receive-coil profiles. High contrast between CSF and brain could also be achieved by other mechanisms such as heavily T₁-weighted scans. However, several other regions such as sinuses, skull, and image background would appear dark along with the CSF in such images. As these structures are more proximal and enclose the CSF space, BFW volumes would be more difficult to extract.

In this paper, we distinguish between BFW and CSF to acknowledge the fact that some chronic and highly destructive lesions are hyperintense on the heavily T₂-weighted images, and as such portions of their volumes were included in the BFW volume and fractions. Such findings, albeit from a small fraction of all lesions, may introduce slight additional

variability in the full model, where lesion volume was used as a covariate in the analysis, but this effect is probably minor. On the other hand, CSF volume and fraction from Lesion-TOADS simply refers to the CSF class of tissue as determined by the algorithm. However, imprecise segmentation of the ventricles and sulci along with the inclusion of choroid plexus, septum pellucidum, and subarachnoid-space vessels into the CSF class by Lesion-TOADS caused the volumes to be overestimated by an average of 15% relative to BFW method. Full-model regression analysis of the Lesion-TOADS-based CSF fraction underperformed compared to BFW fraction for almost all disability measures. The fact that BFW measures' correlations with neurologic impairment were compared only with those derived from Lesion-TOADS limits the generalizability of the conclusion that the BFW method improves clinical correlations. That said, Lesion-TOADS-derived brain-volume estimates have shown improved correlations with clinical disability scores relative to SIENAX in the past (18).

The reproducibility of the measured BFW fraction depends largely on the selected threshold and registration to the atlas. The average CSF volume varied linearly between the thresholds of 0.3 and 0.8, with the calculated volume of 363 ± 60 ml at a threshold of 0.3 and 184 ± 25 ml at a threshold of 0.8. Most of the variation occurred at the CSF-brain and CSF-extracranial tissue interfaces and was determined to be due to signal drop in voxels containing both CSF and tissue (i.e., partial volume averaging). Although 0.65-mm isotropic voxels have less partial volume averaging than standard 1 mm^3 voxels, partial volumes are still present in heavily T_2 -weighted images due to very small sulcal CSF spaces, and the BFW method does not account for them. In the absence of accurate knowledge of the true CSF volume, we chose the threshold at 0.5, safely within the linear region. Additionally, in order to reduce threshold dependence on pixels with artifactually high intensities, the threshold was calculated based upon the 98th percentile of signal intensities rather than the maximum.

BFW method is less reproducible than CSF segmentation by Lesion-TOADS (COV of 1.5% vs. 0.63%), perhaps because the BFW method is far simpler than the sophisticated Lesion-TOADS algorithm, which uses precise registrations to statistical and topological atlases. However, a better reproducibility did not directly translate to improved accuracy in Lesion-TOADS, as there were several regions such as choroid plexus and sulcal CSF that were consistently misclassified by Lesion-TOADS but correctly classified by BFW method (Fig 3A), and Lesion-TOADS had weaker correlations to neurological status. This may be due to the use of priors for tissue classification in Lesion-TOADS. Lesion-TOADS consistently overestimated the CSF volume by 10–20% compared to BFW method (Fig 3B). The correlations of BFW fraction with clinical disability were consistently better than those from Lesion-TOADS (Fig 4 C and D), suggesting that the BFW method may more accurately capture disability-related brain volume changes.

To compare the reproducibility of the BFW method to a popular cross-sectional measure of brain volume, BFW volume had to be converted to brain volume. There is no direct way to measure brain volume using BFW imaging, as dark pixels include not only brain but additional structures, such as blood vessels, meninges, choroid plexus, skull, scalp, and background, etc. Nevertheless, brain volume was estimated from BFW images as the difference between ICV (using atlas and scaling factors) and BFW volume. The COV of this

BFW-derived brain volume, 0.99%, is superior to SIENAX's variability calculated from the same subjects (1.5%). When the COV of BFW volume is normalized to the volume of the non-BFW intracranial contents (brain and additional structures), the COV drops to 0.3%. To detect intra-individual changes in time frames on the order of 1 year, the COV of BFW-derived brain volume must be smaller than the brain atrophy rate, which is typically 0.5%/year in MS (1–3). A longitudinal extension of the BFW method that does not require normalization by ICV may achieve this goal.

5 CONCLUSION

Total BFW volume and BFW fraction are robustly estimated from heavily T₂-weighted images and are shown here to be reliable and easily translatable measures of brain atrophy in MS. In particular, BFW fraction strengthens correlations between imaging and clinical data and, for cross-sectional applications, strikes a reasonable balance between accuracy and reproducibility. Although the required heavily T₂-weighted sequence is not currently acquired in most MRI protocols, in our judgment, its high performance in assessing atrophy is worth the less than 5 minutes of extra scan time required to obtain a reasonable single-time-point estimate of brain atrophy, a prevalent finding in many neurological diseases and in normal aging. As heavily T₂-weighted images use a common manufacturer-provided pulse sequence, the BFW method could easily be implemented on clinical scanners.

Acknowledgments

This study was supported by the Intramural Research Program of the National Institute of Neurologic Disorders and Stroke (NINDS), National Institutes of Health. We are grateful to the Neuroimmunology Clinic (especially Joan Ohayon and Kaylan Fenton) for performing neurological exams on our participants, the National Institute of Mental Health (NIMH)/NINDS Functional Magnetic Resonance Facility, Tianxia Wu (NINDS), Colin Shea (NINDS), John Ostuni (NINDS), and Souheil Inati (NIMH).

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Highlights

- High-resolution, heavily T2-weighted scan shows brain-free water (BFW)
- BFW-volume, derived from intensity thresholding these scans, used as atrophy marker
- Method circumvents skull stripping and brain tissue classification
- BFW-volume improves clinical correlations of a conventional T1-based measurement

Fig. 1.

T_2 -weighted fluid attenuated inversion recovery (FLAIR, first column), T_1 -weighted magnetization-prepared rapid acquisition of gradient echoes (MPRAGE, second column), heavily T_2 -weighted images (third column), and thresholded brain-free water (BFW) masks (fourth column) from three representative multiple sclerosis cases (top: 53-year-old woman with primary-progressive multiple sclerosis, expanded disability status scale (EDSS) = 6.5; middle: 37-year-old man with relapsing-remitting multiple sclerosis, EDSS = 0; bottom: 53-year old man with relapsing-remitting multiple sclerosis, EDSS = 2). Although the vitreous humor in the eye appears bright on heavily T_2 -weighted images, the orbits are automatically removed from the BFW mask (top row). As the skull and fat have low signal on these heavily T_2 -weighted images, no dedicated skull-stripping step is needed to generate BFW masks. Arrows in the middle row point to adjacent lesions that are visible on FLAIR and MPRAGE, but not on heavily T_2 -weighted images. The bottom row shows examples of lesions that can be seen on the heavily T_2 -weighted image but are nearly completely removed on the BFW mask; in general, MS lesions are not visualized on the BFW mask.

Fig. 2.

(A) Mean percent absolute scan-rescan difference in brain-free water (BFW) volumes derived from the BFW images in 12 participants, as a function of intensity threshold (fraction of the 98th percentile of overall signal intensity in the BFW images; error bars represent SD). Reproducibility stabilizes at thresholds greater than 0.3, and the threshold for BFW volume calculation was set at 0.5. Bland-Altman plots of the scan-rescan differences in BFW volumes (B) and fractions (C) versus average values do not show bias or trend (dotted lines represents 95% confidence interval).

Fig. 3.

(A) Heavily T₂-weighted image-derived brain-free water (BFW)-mask (top left) and corresponding lesion-TOADS classification of cerebrospinal fluid (CSF; bottom left, brick red) in a representative case (33-year-old man with primary progressive multiple sclerosis, Expanded Disability Status Scale = 3.5). Ventricle borders from both segmentation methods are superimposed onto the co-registered T₂-weighted fluid attenuated inversion recovery image (right).

(B) Bland-Altman plot showing difference in BFW volume and CSF volume derived from lesion-TOADS in the full multiple sclerosis cohort (83 participants, dotted lines represent 95% confidence interval).

Figure 4.

(A) Brain-free water (BFW) volume and (B) BFW fraction from various clinical subtypes of the subjects investigated (CIS: clinically isolated syndrome, RIS: radiological isolated syndrome, PPMS: primary-progressive; RRMS: relapsing-remitting; and SPMS: secondary-progressive multiple sclerosis). Magnitudes of semi-partial correlation coefficients of various clinical scores for (C) BFW and CSF volumes (from Lesion-TOADS), and (D) BFW and CSF fractions. Coefficients with absolute values greater than 0.2 (i.e. above dashed line) had p-values less than 0.05, whereas those greater than 0.3 (i.e. above dotted line) had p-values less than 0.005.

Table 1

Clinical assessments of multiple sclerosis cases, including Expanded Disability Status Scale (EDSS), Scripps Neurological Rating Scale (SNRS), Symbol Digit Modality Test (SDMT, paper-based), and Paced Auditory Serial Addition Test (PASAT, 3 second version) scores, as well as 9-Hole Peg Test (9HPT) and 25-foot (25') walk times.

	EDSS	SNRS	SDMT	PASAT	9HPT	25' walk
All subjects (n=83)						
Median (25 th , 75 th)	3.5 (2, 6)	78 (63, 90)	45.5 (37, 56)	51 (36, 56)	24 (20, 30)	45.5 (37.3–55.8)
Range	0–7.5	45–100	12–82	16–60	16.1–77.2	2.9–57.8
Rescanned subjects (n=12)						
Median (25 th , 75 th)	2 (2, 6)	78 (66, 89)	46 (39, 58)	51 (42, 60)	24 (19, 27)	10 (4.4–11.2)
Range	1–6.5	50–100	33–82	16–60	16.6–30.65	3.2–42.4

Table 2

Semi-partial correlation coefficients (sr) of various clinical scores with brain-free water (BFW) volume and BFW-fraction (derived from the heavily T₂-weighted images), as well as cerebrospinal fluid (CSF) volume and CSF-fraction (derived from Lesion-TOADS based tissue classification algorithm), adjusted for significant effects of age and sex.

	Volume		Fraction	
	<u>BFW</u>	<u>Lesion-TOADS</u>	<u>BFW</u>	<u>Lesion-TOADS</u>
EDSS	0.15	0.12	0.25 [*]	0.12
SNRS	-0.15	-0.04	-0.27 [*]	-0.16
SDMT	-0.30 ^{**}	-0.25 [*]	-0.52 ^{**}	-0.41 ^{**}
PASAT	-0.23 [*]	-0.09	-0.38 ^{**}	-0.31 ^{**}
9HPT	0.42 ^{**}	0.08	0.39 ^{**}	0.23 [*]
25' walk	0.05	0.08	0.15	0.00

^{*}
 $p < 0.05$

^{**}
 $p < 0.005$