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Intensity Ratio to Improve Black Hole Assessment in Multiple Sclerosis

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Abstract

Background—Improved imaging methods are critical to assess neurodegeneration and remyelination in multiple sclerosis. Chronic hypointensities observed on T1-weighted brain MRI, “persistent black holes,” reflect severe focal tissue damage. Present measures consist of determining persistent black holes numbers and volumes, but do not quantitate severity of individual lesions.

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Objective—Develop a method to differentiate black and gray holes and estimate the severity of individual multiple sclerosis lesions using standard magnetic resonance imaging.

Methods—38 multiple sclerosis patients contributed images. Intensities of lesions on T1-weighted scans were assessed relative to cerebrospinal fluid intensity using commercial software. Magnetization transfer imaging, diffusion tensor imaging and clinical testing were performed to assess associations with T1w intensity-based measures.

Results—Intensity-based assessments of T1w hypointensities were reproducible and achieved >90% concordance with expert rater determinations of “black” and “gray” holes. Intensity ratio values correlated with magnetization transfer ratios (R = 0.473) and diffusion tensor imaging metrics (R values ranging from .283 to −.531) that have been associated with demyelination and axon loss. Intensity ratio values incorporated into T1w hypointensity volumes correlated with clinical measures of cognition.

Conclusions—This method of determining the degree of hypointensity within multiple sclerosis lesions can add information to conventional imaging.

Keywords

Axonal loss; Multiple sclerosis; Quantitative MRI; Outcome measurement; MRI; T1w hypointensity

Introduction

Hypointense areas of white matter (WM) on T1-weighted (T1w) magnetic resonance images (MRIs) persisting for at least 12 months are markers of focal tissue injury in MS known as “persistent black holes” (PBH) [1]. The pathologic correlate of a PBH is severe axon loss and matrix destruction [2–4]. Some investigators label less hypointense BHs as “gray holes” (GHs) to reflect a lower degree of axonal loss. Acute contrast enhancing lesions (CELs) may also display decreased intensity on the concurrent non-contrasted T1w images. These “acute black holes” are due primarily to inflammation and edema, as most resolve within months of contrast resolution[5].

PBHs have relevance to clinical outcomes and disease progression[6]. Several studies have shown that PBH numbers or volumes correlate with worse clinical test scores[4, 6, 7]. MS lesion “burden” has often been reported as the sum of lesion volumes, but the degree of tissue destruction may vary between lesions[8, 9]. A quantitative method to define the degree of hypointensity in individual MS lesions could improve patient monitoring, allow for better correlations to clinical measures, and may prove useful as an outcome measure in clinical trials of potential reparative therapies[7].

Our goal was to develop a simple and objective method to identify and distinguish PBHs and PGHs and to estimate the severity of the underlying tissue damage in these lesions based on degree of T1w hypointensity.

Methods

Subject Population and Clinical Testing

Human Studies Committee approval was obtained. All 38 MS subjects contributing data gave informed consent. The following clinical tests, all validated for MS, were performed at times of imaging: Expanded disability status scale (EDSS), 25 foot timed walk (25FTW), nine-hole peg test (9HPT) and the 3- and 2-second versions of the Paced Auditory Serial Addition Test (PASAT).

Image Acquisition

Human Studies Committee approval was obtained and all subjects gave informed consent. Patients were imaged on a 3.0T Siemens Trio scanner (Siemens, Erlangen, Germany). T1w was acquired using the following parameters: Repetition Time (TR) = 600ms; Echo Time (TE) = 9ms; slice thickness = 2 mm; in-plane resolution = 1×1 mm^3; total acquisition time = 4 min. T2w was acquired using the following parameters: TR = 7500ms; TE = 210ms; TI = 2500ms; slice thickness = 1 mm; in-plane resolution = 1×1 mm^3; total acquisition time = 10 min. Magnetization Transfer (MT) images were acquired with the following parameters: TR = 38ms; TE = 11 ms; Flip Angle = 15 degrees; slice thickness = 3 mm; in-plane resolution = 1×1 mm^3; total acquisition time = 8 min. Magnetization Transfer Ratio (MTR) maps were calculated pixel-by-pixel using the equation: $MTR = (S_{off} - S_{on})/S_{off} \times 100$, where S_{on} and S_{off} were signal intensities with and without saturation pulse. Axial Diffusion Weight images (DWI) covering the whole brain were acquired using a multi-b value diffusion weighting scheme (99 directions, maximum b-value 1500 s/mm^2) and the following parameters: TR = 10,000 ms; TE = 120 ms; slice thickness = 2 mm; in-plane resolution = 2×2 mm^3; total acquisition time = 16 min. Eddy current and motion artifacts of DWI were corrected, then susceptibility-induced off-resonance field was estimated and corrected. Whole brain voxel-wise DTI analyses were performed on DWI images by the in-house software developed using MATLAB[10].

MS Lesion Classification

Areas of hypointensity on pre-gadolinium T1w images that met the definition of “persistent” by being present for at least 12 months were identified in the MS subjects. Amira 6.0.1 visualization and analysis software (FEI, Hillsboro, OR) was used to provide quantitative intensity values for each hypointense lesion on each scan, with lower values reflecting darker voxels. Lesion intensity assessment requires consideration of baseline intensity for each scan, to control for scan-to-scan variations. As cerebrospinal fluid (CSF) is not changed by MS pathology, CSF was used to provide a baseline for each individual scan.

Selection of voxels representative of CSF intensity consisted of starting inferiorly and moving superiorly on axial slices, until the initial appearance of both anterior horns of the lateral ventricles. The axial slice 15mm superior to this was located (typically where the anterior horns of the lateral ventricles were widest), and was used to determine the median “Baseline Intensity” for CSF for that scan (Figure 1). After initially testing 1, 5, 20 and 40 voxels, using the median of 20 ventricular voxels was found to be representative of CSF and a feasible number to select, time-wise.

To ensure exclusion of any voxels containing choroid plexus or partial volume effect from ventricle edge, the 20 lowest intensity, not necessarily contiguous, voxels within the CSF of the right and left anterior lateral ventricles at this level were selected. Voxels within one voxel distance from the ventricle edge were excluded to avoid voxels within periventricular lesions. In the rare situation of the baseline slice not containing 20 voxels unaffected by choroid plexus, the adjacent inferior axial image was also used to ensure a sample of 20 lowest intensity voxels within CSF.

For each MS lesion, the "Lesion Intensity (LI)" was defined as the median of the 5 lowest intensity voxels on all T1w slices where the lesion was visible (Figure 2). This number of voxels was chosen to be representative of the lowest intensity voxels. It took less than 1 minute to identify the median of the 5 lowest intensity voxels. Moreover, small lesions of few voxels could usually be accommodated using 5 voxels.

This value was divided by the BI for that scan to obtain an "Intensity Ratio (IR)" for the lesion relative to CSF:

$$\mathrm{IR}=\frac{\mathrm{LI}}{\mathrm{BI}}$$

Lesions were selected from throughout the brain. The only exclusion criterion for size was that a lesion have a minimum of 5 voxels, for intensity determinations. Lesion volumes were determined based on the voxel dimensions and the total number of voxels within each lesion ROI. Total WM volumes for each patient were generated using the SIENAX tool.

A single rater identified a region of interest (ROI) for a total of 181 chronic T1w hypointense MS lesions, 189 non-hypointense T2w hyperintense lesions and 113 normal appearing white matter (NAWM) areas, and determined IR for each hypointense lesion. Three of the 38 subjects had no PBH or PGH lesions.

Tests of Reproducibility

For intra-rater reproducibility determination, one examiner used the method on a single scan from each of 6 MS (2 RRMS, 2 SPMS, 2 PPMS) subjects. At two time-points one week apart, BI was determined and LI was determined for 20 lesions on the 6 scans.

For inter-rater reliability, another rater replicated the instruction protocol on 5 scans with 25 lesions from 5 subjects. We determined how potential differences in perceiving the initial appearance of the anterior lateral ventricles might affect the baseline slice chosen and the results. Thus, BI was determined on the 2 slices immediately adjacent to the baseline slice (1 inferior slice, 1 superior slice) for all 38 subjects in the study. Bland-Altman plots were used to compare the BI of the baseline slice to the BI of each of the adjacent slices[11,12].

While at least 12 months was required to designate lesions as PBH, PGH or non-hole T2w lesions (NBH), some patients with multiple scans had intervals between consecutive scans of as few as 3 weeks, ranging to 54 weeks, with an average interval of 9.2 months. Reproducibility across these scans was assessed to determine whether scan-to-scan variations and/or time might affect the lesion classification method. For this, scans from 6

MS subjects (2 RRMS, 2 SPMS, 2 PPMS) with multiple MRI scans were used. Of the 6 subjects, three had scans from 2 time points, two had scans from 3 time points, and one had scans from 6 time points spanning 112 weeks. BI was determined on T1w non-contrasted scans from each different time point in each subject. 5 BH and 5 GH lesions across the 6 subjects were selected and LI was determined at each of the time points for every lesion. IRs across scans were compared for each lesion to evaluate reproducibility over time.

Correlations of Lesion Classification using Intensity-based metrics with Diffusion tensor imaging and magnetization transfer data

Alterations in DTI parameters and MTR have been associated with the pathological severity of MS lesions[10]. Thus, IR-based classifications of PBHs, PGHs, NBH and NAWM were compared with DTI-based parameters and MTR values.

IR values obtained for each of the 181 PBH or PGH lesions from standard T1w images were examined for associations with each of four DTI imaging parameters[Apparent Diffusion Coefficient (ADC), axial diffusivity (AD), radial diffusivity (RD), and fractional anisotropy (FA)] and with MTR values, all of which have been associated with tissue damage of various types [5, 13–18]. Median values were used to represent the collective imaging parameters for each lesion ROI. Statistical modeling was used to account for intra-subject clustering of lesions.

Intensity-based Lesion Burden

IR of each hypointense lesion was incorporated into lesion burden by dividing each lesion volume by its IR; the result was termed the “Hypointense Lesion Weight”. The “Weighted Hypointense Lesion Burden” (WLB) was determined by summing all “Hypointense Lesion Weights” for an individual patient. Thus, greater weight was given to a BH than a GH. Given that WM volumes vary from subject to subject based on brain size, we also normalized WLB to WM volume. Total WM volumes for each patient were generated using “SIENAX” tool in “FSLView.”[19] The volumes were normalized for subject head size. Each patient’s WLB was then divided by the total brain WM volume. This “Normalized Weighted Hypointense Lesion Burden” (NWLB) was analyzed for associations with clinical measures.

Statistical Analyses

Values of Gamma and Kendall’s tau-b were used for the assessment of concordance between subjective and IR classifications of lesion types. Bland-Altman plots were used to assess slice-to-slice reproducibility.

Multilevel linear repeated measures models were used to represent the association between IR and DTI parameters and MTR. AD, RD, ADC and MTR were approximately Gaussian on the original scale, whereas DTI FA was approximately Gaussian on a logarithmic scale. Pearson correlations and linear modeling were used to determine significance of associations between IR and each radiological marker. Clustering of lesions within patients was incorporated into the statistical models to account for dependency of multiple lesion data derived from the same individual. Correlation was assessed between the mean intensity ratio

of each person and the mean of each radiological marker for the 38 patients to determine correlation coefficients.

WLB and NWLB were analyzed for associations with 25FTW, 9HPT and PASAT results using Spearman's rho rank correlation and with EDSS using Kendall's Tau b rank correlation.

Results

Subjects

Thirty-eight MS subjects (13 RRMS, 15 PPMS, 10 SPMS) contributed brain imaging data (Table 1), of whom 35 had hypointense lesions on T1w scans that persisted at least 12 months, establishing them as "persistent".

Intensity Ratio as a semi-quantitative measure of lesion severity

Two independent raters classified 25 lesions across 3 subjects (1 RRMS, 1SPMS, 1 PPMS) as "black holes" or "gray holes." Based on this, provisional lesion type limits were set as:

Black Hole: $1.00 \leq IR \leq 1.70$

Gray Hole: $1.71 \leq IR \leq 2.60$

Non-Hole: $IR > 2.60$

An experienced rater then classified 103 lesions from 10 MS subjects as BH, GH or NBH, blinded to IR values. Subjective classifications were compared with IR-determined classifications (Table 2). Over 91 percent of the time the two methods were in accord. Values of Gamma and Kendall's tau-b were 0.99 and 0.91, respectively, showing very strong concordance (Table 2).

There were no cases of extreme discordance in which a BH by one method was classified as a NBH by the other method. For each method, one lesion was called a GH but was classified as NBH by the other method, both lesions were in IR range of 2.55 – 2.65. Seven discordantly classified lesions occurred for BH vs. GH. Each had IR in the 1.67 – 1.73 range, near the border between BHs and GHs. The kappa statistic was 0.87, indicating a strong agreement between methods, with no evidence for systematic discordance.

Time to apply method

After determination of the IR cutoffs for classification, the method was applied across 35 subjects to 181 T1w hypointense MS lesions. With practice, time to select the CSF baseline 20 voxels was 5 minutes. The time to select the 5 lowest intensity lesion voxels and determine median for a lesion was 1 minute.

Reproducibility

Intra-rater—One rater determined BI and LI in 20 MS lesions on 6 MR scans from 6 MS subjects at two time-points, one week apart. Exact concordance of BI and LI values was observed when the same rater re-examined the 20 MS lesions later.

Inter-rater—The two raters chose the same axial section for BI determination for three of the five subjects, with exact concordance in those cases. For the other two subjects' scans, different but adjacent axial images were chosen for determination of BI. This resulted in BIs of 203.5 versus 201.5 in one subject, and 152 versus 150.2 in the other. No lesions changed in classification as a result of these BI differences. Both raters chose the exact same 5 lowest intensity voxels to determine median value in the lesions, obtaining identical LI values in all 25 lesions.

Bland-Altman plots were then used to assess differences when adjacent but different axial images were selected to determine BI (Figure 3a, 3b). Over 97% of points fell within the limits of agreement. The mean difference for the −1 scan was below 0, whereas the mean difference of baseline and +1mm scan was above 0, depicting a slight increase in intensity from −1 scan to the +1 scan.

Reproducibility over time—Ten individual PBH or PGH lesions from 6 subjects were examined longitudinally over as long as 112 weeks. Some lesions were examined on as many as 6 different scans. The mean interval between scans was 9.2 months (range 3 weeks to 54 weeks). No lesion changed in classification when evaluated across scans. PGHs displayed more variability than PBHs. Standard deviations ranged from 1% to 4% of mean IR (Table 3).

Comparisons of Intensity-Based Lesion Classification with MTR and Fractional anisotropy from T1w images

PBHs have reduced MTR and DTI-derived fractional anisotropy (FA) compared to mildly hypointense lesions, non-hypointense T2w lesions and NAWM[5,20, 21]. Thus, correlations of IR with MTR measures and DTI-derived FA of individual lesions were assessed. MTR and FA each progressively decreased as lesion classification moved from NAWM to PGH to PBH ($p < 0.0001$ for each pair of adjacent categories for both measures) (Figures 4a and 4b).

Correlations of Intensity-Based Classification and Lesion Metrics with Diffusion Tensor Imaging Parameters from T1 images

Lesion IRs values for the 181 PBH and PGH lesions from 35 MS patients were examined for correlations with MTR (Figure 5a), as well as DTI –derived parameters of AD, RD, and ADC (Figures 5b – 5d). MTR values directly correlated with IR values in the 181 PBHs and PGHs (Table 4; Figure 5a, $p < 0.0001$). DTI-derived AD, RD, and ADC were each inversely associated with IR (Table 4, $p < 0.0001$ for each). Log DTI FA values changed in the same direction associated with IR, increasing as IR increased, but associations did not reach statistical significance ($p = 0.099$; Table 4).

Normalized Weighted Lesion Burden

"Normalized WLB" correlated with cognition components of the MSFC, the 3 second PASAT ($R = −0.572$, $p < 0.01$) and 2 second PASAT ($R = −0.428$, $p < 0.05$)(Figures 6a and 6b).

Significant associations were not found for NWLB with motor tests such as 25 FTW, 9HPT or EDSS. However, associations of NWLB with 25FTW (R = 0.188, Spearman's rho), 9HPT (0.071 and 0.107, Spearman's rho) and EDSS (R=0.121, Kendall's tau-b rank) were directionally valid (Table 5). Unweighted LB and non-normalized WLB also correlated significantly with 3 second (−.52 and −.54, respectively) and 2 second (−.39 and −.39, respectively) PASAT scores, but to a lesser extent than for NWLB.

Discussion

MRI plays an important role in diagnosis and management of MS. However, conventional T1w and T2w brain imaging techniques do not correlate well with MS disability[22]. Lesion-specific factors such as pathologic heterogeneity and different degrees of functional eloquence of varying CNS locations, coupled with suboptimal methods for measuring clinical disability contribute to a lack of strong correlations. Additionally, conventional T1w and T2w imaging contrasts are scan-specific and depend not only on the MR characteristics of tissue but also the pulse sequences and magnet strength, making them difficult to quantitate. MS lesions that are persistently hypointense on T1w images, so-called "persistent black holes," contain more axon loss neuropathologically[3]. Numbers and volumes of T1w BHs have demonstrated better correlations with disability than T2w lesion measures[6, 23–25]. In a study that compared several imaging modalities performed at baseline in RRMS for correlations with worsening clinical disability over 10 years, PBH number and increasing PBH volumes performed best[24]. Comparisons of imaging and neuropathology in over 100 MS lesions found that degree of hypointensity was strongly associated with axonal density ($r = 0.74$, $p < 0.0001$).[7]

The development of anti-inflammatory therapies that reduce MS relapse rate has transformed the lives of many MS patients, and was aided greatly by the ability of gadolinium-enhancing lesions to serve as surrogates of relapses[26]. However, a comparable biomarker of neurodegeneration has not surfaced. Strong evidence supports the potential for using PBHs as a marker of neurodegeneration which might be exploited in individual patients and as an outcome measure in trials of potential neuroprotective agents.

Importantly, numbers and volumes of PBH change in concert with worsening in clinical disability[24]. A 2008 meeting of international experts in MS imaging established five criteria that imaging outcomes should fulfill to be used as surrogate markers for proof of concept neuroprotection studies in MS[27, 28]. These were pathological specificity, reproducibility, sensitivity to change, clinical relevance and response to treatment. PBH observed on T1w images were considered to be among the four most promising measures identified at that meeting (others were brain volumetrics, ocular coherence tomography, and MTR)[28]. The present study was performed because the value of PBHs as potential surrogate markers of neurodegeneration could be enhanced by more objective and quantitative measurements.

Another quantitative method of calculating BH intensity relative to NAWM and CSF intensities on the scan has been described[29]. This method depends on subjective identification of an area of NAWM, which may not be truly normal in an MS patients[22,30]. The reliability of this method has been questioned due to scan-to-scan variations in intensity

and inclusion of a changing weight variable “k” in the intensity calculations[31]. Thus, we developed a simpler and more reproducible methodology to identify and assess level of hypointensity within MS lesions on standard T1w scans, using intensity of ventricular CSF as a benchmark on each scan. Metrics derived with the new method proposed herein were reproducible, stable in established lesions over time, and had face validity when compared to expert rater identifications of black and gray holes. While selecting the slice based upon the initial appearance of the anterior ventricles has some subjectivity and 40% of the time varied by an axial slice between two examiners, this did not change lesion categorizations. New T1w quantitative imaging techniques have been reported, and have improved clinical correlation compared to T2w lesion counts/volumes. [31,32]. Notably, the hypointensity metrics we describe are standard imaging measures in clinical practice, and do not require extra sequences with longer scan times. Importantly, T1w hypointensity burdens that incorporated IR correlated with cognitive assessments.

In summary, we report upon an efficient method to estimate the degree of hypointensity within PBH that can be performed using standard T1w images and commercial imaging software. This method has potential for semi-automated integration into clinical trials and practice, as a marker of lesion severity and possibly lesion recovery. Next steps would include additional longitudinal studies with larger cohorts, different scanners, and multiple centers. This method may be especially useful for trials of therapeutics with potential to impact axonal degeneration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- A method to classify and estimate the severity of T1-weighted hypointensities ("persistent black and gray holes") is presented
- This severity quantification method correlated with diffusion tensor imaging and magnetization transfer ratios, methods that have been previously shown to associate with MS pathology
- Incorporating lesion severity into calculations of T1-weighted hypointensity lesion burden improved correlations with tests of cognition

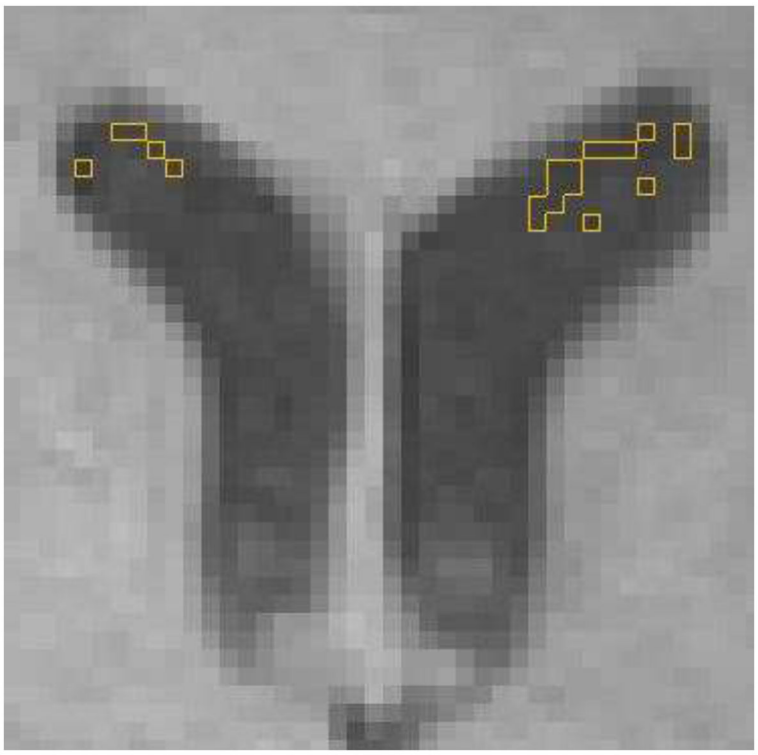

Figure 1. Illustration of determining "baseline intensity"

The median value for the 20 voxels of lowest intensity (identified by yellow lines) within the lateral ventricles at an axial slice 15mm superior to the initial appearance of the lateral ventricles determined BI. Only voxels that were more than one voxel away from the ventricle edge were used.

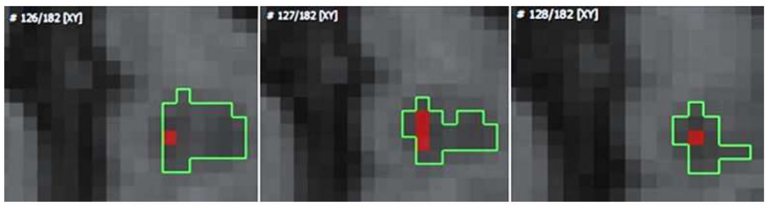

Figure 2. Illustration of determining "lesion intensity"

The five voxels of lowest intensity within a lesion were identified. The median intensity of these 5 voxels determined LI. Notice that voxels on three different slices were used for this particular lesion.

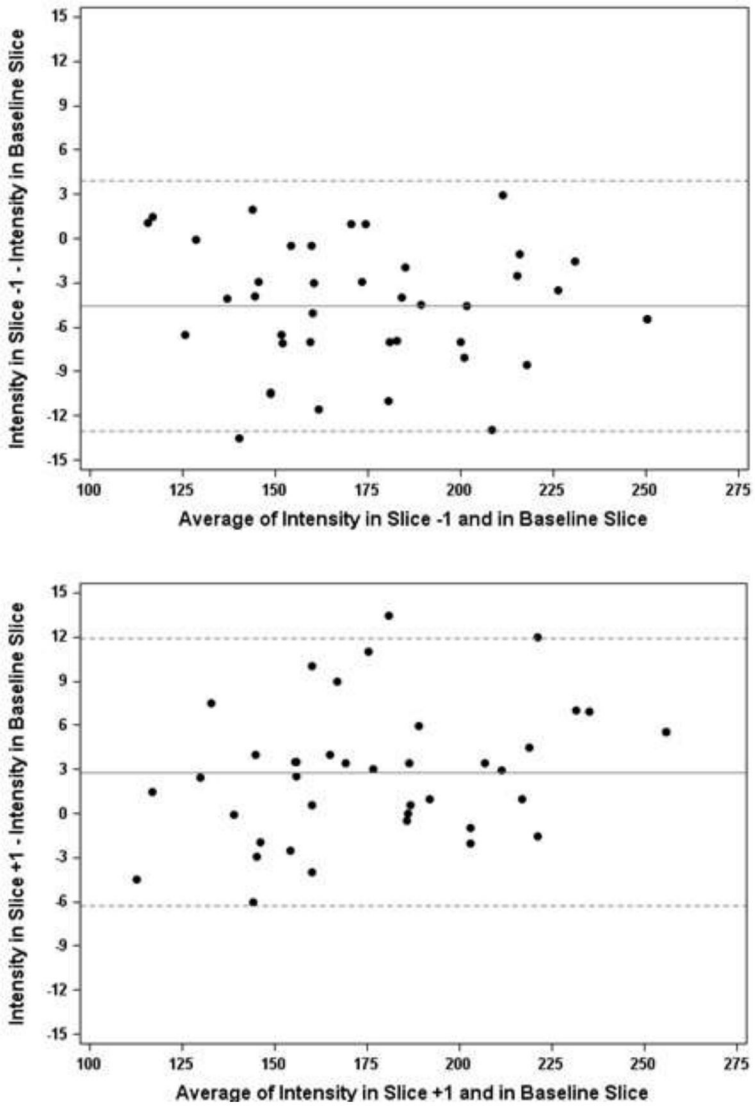

Figure 3. Level of agreement between baseline intensity determinations for adjacent axial slices

Bland-Altman plots were used to determine the limits of agreement (dotted lines represent ± 2 SD of the difference) when CSF intensity (BI) was assessed on adjacent axial images. 3a) compares BI obtained for the slice at 15mm above initial perception of lateral ventricles to the immediately inferior slice; 3b) compares BI obtained for the slice superior to and the slice at 15mm above initial perception of lateral ventricles. More than 97% of the points fell within the limits of agreement.

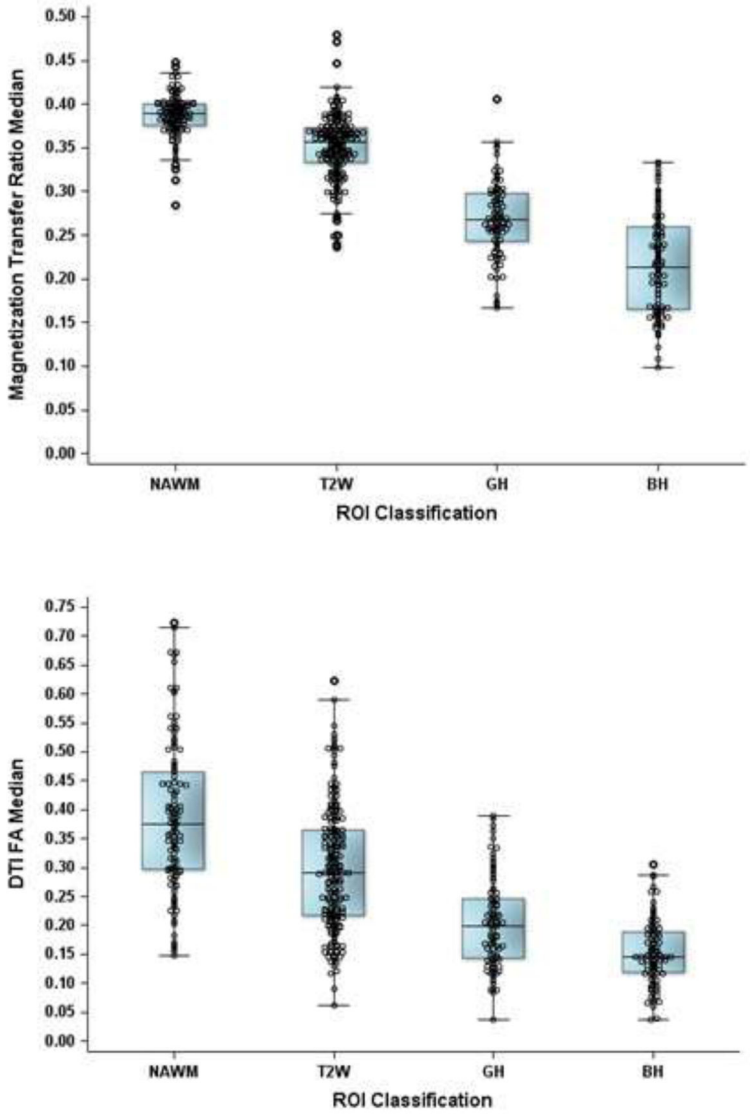

Figure 4. Associations of Magnetization Transfer Ratios and Fractional anisotropy values with IR-based classifications

4a) IR-based lesion classifications in relation to MTR values and 4b) Fractional anisotropy (FA) values. Regions were classified as NAWM, or as non-black/gray hole, PGH and PBH using IR and showed decreasing trends from NAWM to PBH ($p < 0.001$, for each using model clustering lesions within patients).

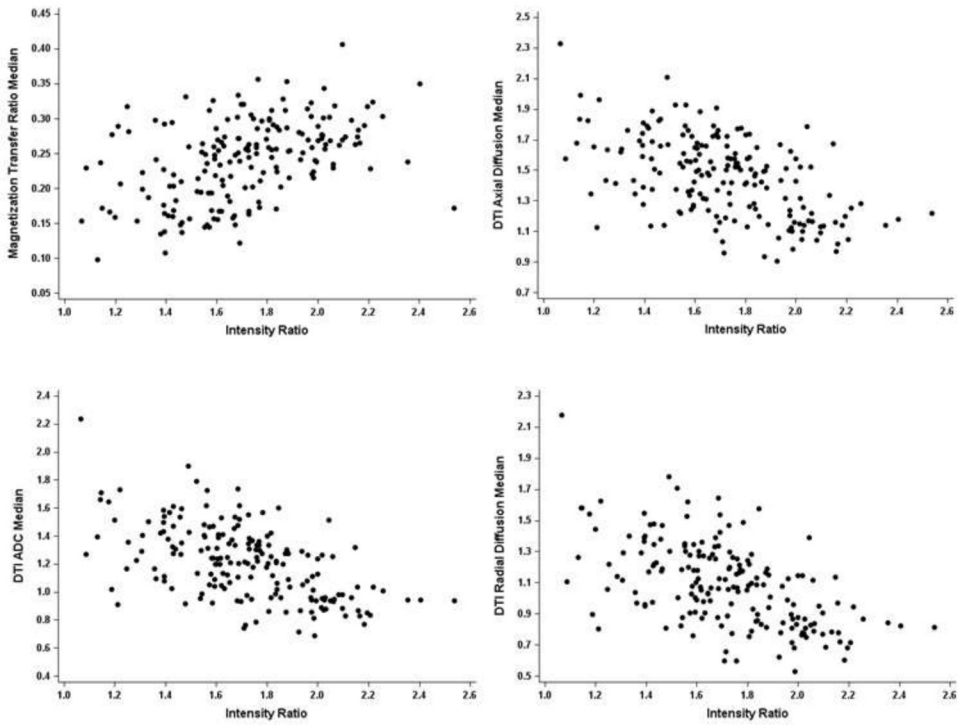

Figure 5. Associations of IR values with MTR and DTI-derived AD, RD, and ADC

Associations of IR values of PGHs and PBHs with 5a) MTR (R= .473, p=.004), 5b) AD (R= −.515, p < .002), 5c) ADC (R= −.531, p=.001), and 5d) RD (R= −.530, p =.001) are shown. Pearson correlations and linear modeling were used to determine the strength of linear association between IR and each radiological marker. Correlation coefficients are based on the mean values per patient over all lesions to account for clustering.

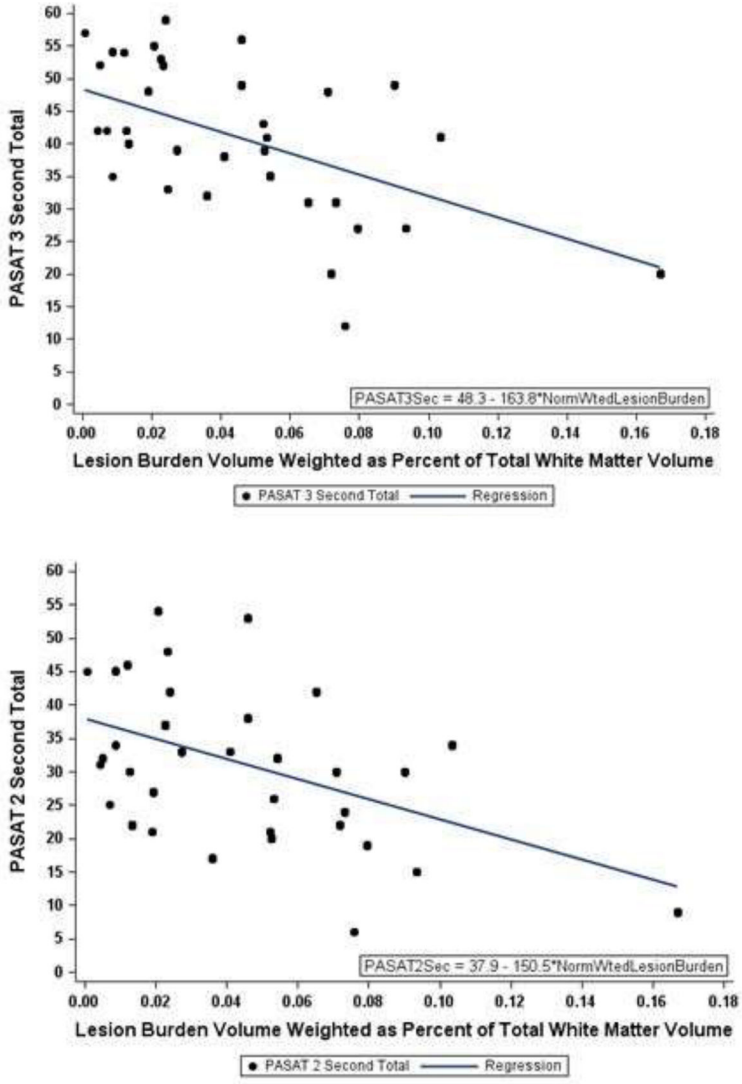

Figure 6. Associations between IR and cognitive test scores

6a) Normalized weighted lesion burden was inversely associated with 3 sec PASAT scores (Table 5: R= −.572, $p < 0.01$). 6b) Normalized weighted lesion burden was inversely associated with 2-sec PASAT scores (Table 5: R = − .428, $p < 0.05$).

Table 1

Demographic profile of patients.

	Mean	SD	Range (Median)
Age (years)	51.5	10.6	25 – 72 (55)
Disease Duration (years)	16.5	11.9	1.5 – 43.3 (13.7)
EDSS	4.8	1.7	1.5 – 6.5 (6.0)
Gender	26 females, 12 males		
MS Subtype[a]	13 RRMS, 15 PPMS, 10 SPMS		

[a]RRMS = relapsing-remitting multiple sclerosis, PPMS = primary-progressive multiple sclerosis, SPMS = secondary-progressive multiple sclerosis

Table 2

Comparison of subjective lesion classification to classification based on Intensity Ratio.

		Intensity Ratio based categorizations			
		BH	**GH**	**NBH**	**total**
Neurologist categorizations	BH	27	3	0	30
	GH	4	36	1	41
	NBH	0	1	31	32
	Total	31	40	32	103

Table 3

Reproducibility of intensity ratios over time.

Subject	MS Subtype	Lesion Type	Number of Scans	Time Interval[a]	Mean IR ± SD	IR Range/Values[b]	Mean Volume mm^3 ± SD
1	RRMS	PGH	3	2 months	2.177 ± 0.043	2.127 – 2.203	32 ± 5
2	RRMS	PBH	6	26 months	1.617 ± 0.018	1.594 – 1.636	108 ± 6
		PGH	6	26 months	2.151 ± 0.072	2.038 – 2.227	24 ± 5
3	SPMS	PGH	3	24 months	1.811 ± 0.066	1.736 – 1.861	79 ± 10
4	SPMS	PBH	2	12 months	1.418 ± 0.021	1.403, 1.433	140 ± 6
		PBH	2	12 months	1.630 ± 0.022	1.614, 1.646	233 ± 14
5	PPMS	PBH	2	11 months	1.570 ± 0.006	1.566, 1.574	22 ± 0
		PBH	2	11 months	1.600 ± 0.005	1.592, 1.600	91 ± 2
6	PPMS	PGH	2	13 months	1.738 ± 0.022	1.722, 1.753	21 ± 2
		PGH	2	13 months	2.398 ± 0.046	2.365, 2.430	17 ± 1
Mean GH SD: 0.050							
Mean BH SD: 0.015							

[a] Time interval between the first scan and last scan

[b] IR values were listed instead of range if the subject only had 2 scans

Table 4

Coefficients of correlation between IR and MTR and DTI parameters.

	Correlation Coefficient[a]	P-Value[b]
MTR	.473	.0041
DTI AD	−.515	.0015
DTI RD	−.530	.0011
DTI ADC	−.531	.0010
Log DTI FA	.283	.0990

[a]Correlation coefficients were weighted by the number of lesions per patient, to account for within-patient clustering.

[b]Pearson correlations and linear modeling were used to determine significance of associations.

Table 5

Associations of Normalized Weighted Lesion Burden with clinical measures.

	Spearman's rho
PASAT 3 sec raw score	−0.572**
PASAT 2 sec raw score	−0.428*
25 Foot Timed Walk – average of 2 trials	0.188
9HPT Dominant Hand – average of 2 trials	0.071
9HPT Non-dominant Hand – average of 2 trials	0.107
	Kendall's Tau b
EDSS	0.121

** $p < 0.01$;

* $p < 0.05$;

N = 38