QUANTITATIVE SPINAL CORD MRI IN MULTIPLE SCLEROSIS

By

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Abstract

**Background:** The spinal cord (SC) is a compact structure with a functional anatomic organization that makes it an ideal substrate to study structure-function relationships in multiple sclerosis (MS). Despite these benefits, the SC has been challenging to study using conventional MRI measures due to limited clinical-radiological correlations and technical difficulties. Quantitative MRI techniques in the SC, including diffusion tensor imaging (DTI) and magnetization-transfer imaging have demonstrated increased sensitivity to underlying tissue microstructural properties in comparison to conventional MRI techniques, and have the potential to provide new insights into the extent and type of tissue damage mediating clinical disability in MS tissue.

**Methods:** 133 MS patients underwent 3-tesla cervical SC-MRI, clinical assessment, and optical coherence tomography on an annual basis over a median duration of 726 days. Quantitative SC-MRI indices, including SC-cross-sectional area (CSA), fractional anisotropy (FA), mean diffusivity (MD), perpendicular diffusivity (\(\lambda_\perp\)), parallel diffusivity (\(\lambda_\parallel\)), and magnetization-transfer ratio (MTR) were calculated across segments C3-C4. Cross-sectional relationships between clinical disability, SC-MRI indices, and retinal layers were assessed. Longitudinal change in MRI-indices over the follow-up period, and its relationship to clinical disability were assessed. The impact of SC volume normalization by subject height, SC length, and intracranial volume on the ability to detect group differences and clinical-radiological correlations were assessed.

**Results:** Quantitative SC-MRI indices demonstrate independent associations with system-specific and global clinical dysfunction beyond what can be detected by
conventional MRI. Significant correlations exist between SC-MRI and retinal layers, and both exhibit independent relationships with clinical dysfunction. There are measurable longitudinal changes in SC-MRI indices, and subject-specific trajectories of SC-MRI index change are relevant to disability at follow-up. SC normalization by SC length was consistently the best strategy to accentuate group differences and to strengthen clinical-radiological correlations.

**Interpretation:** Quantitative SC-MRI measures provide clinically relevant information in MS patients beyond that which can be gleaned from conventional, lesion-based measurements alone. Longitudinal changes in SC MRI-measures are detectable over 2 years, and subject-specific trajectories of SC-MRI index change are relevant to disability progression, warranting further investigation. Further development of quantitative SC-MRI techniques will expand their practical utility in the clinical setting.

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Introduction

Multiple Sclerosis and the Spinal Cord

The spinal cord (SC) is a common site of pathology in multiple sclerosis (MS), and lesions in this region contribute significantly to clinical disability in patients. Pathological series have demonstrated that up to 90% of patients have SC lesions, and even early in the disease course, imaging studies have consistently shown that up to 50-80% of patients already have lesions in the SC on MRI.\textsuperscript{1,2}

From an anatomical perspective, the SC is organized into discrete columns mediating specific motor and sensory functions. This compact functional organization of the SC makes it an ideal substrate to study structure-function relationships in MS.

Limitations of Conventional MRI in MS

Despite these apparent benefits, the SC has been challenging to study using conventional MRI techniques due to difficulties establishing strong clinical-radiological correlations using lesion-based measurements, and technical limitations related to the small size of this structure.

Although conventional MRI has been instrumental in developing our understanding of MS and has become an indispensable clinical tool in diagnosis and disease monitoring, there are well-established limitations of conventional MRI techniques. One well-known limitation is the phenomenon known as the “clinico-radiologic paradox” which refers to the observation that lesion-based MRI measures in the brain and SC of MS patients have poor correlations with clinical dysfunction.\textsuperscript{3} There are a variety of reasons underlying the clinico-radiologic paradox, including: the lack of
sensitivity and specificity of conventional techniques to the underlying tissue histopathology and the lack of accurate methods to quantitatively measure neurological dysfunction. Establishing stronger correlations between MRI measures and clinical dysfunction is a necessary prerequisite to further develop these techniques for use in clinical monitoring and prognostication, and as surrogate outcome measures in clinical trial settings.

The SC specifically has been difficult to study using conventional MRI techniques not only due to the clinico-radiological paradox, but also because of the small size of this structure. MRI of the SC is highly susceptible to motion artifact from vascular pulsations and respirations, and is also susceptible to partial-volume averaging from surrounding cerebrospinal fluid and vertebrae. The combination of these factors reduces the amount of accurate structural information that can be obtained using conventional MRI techniques in the SC.

**Novel MRI techniques in MS**

In recent years, a number of advanced MRI techniques have emerged which hold promise in being able to overcome many of the limitations of conventional MRI. Among these advanced techniques, one that may be of particular utility in the SC of MS patients is diffusion tensor imaging (DTI). DTI is an approach that utilizes information obtained from the movement of free water molecules in tissue. DTI indices such as fractional anisotropy (FA), mean diffusivity (MD), parallel diffusivity ($\lambda_\parallel$), and perpendicular diffusivity ($\lambda_\perp$) allow quantitative measurement of water diffusion in underlying tissue,
which provides new insights into the extent and type of microstructural damage in the structures being imaged.\textsuperscript{5}

Another advanced MRI approach that holds promise in MS is magnetization transfer imaging (MTI).\textsuperscript{5} MTI is a technique based on the transfer of magnetization from restricted water molecules in macromolecular tissues such as myelin to unrestricted free water. The degree of magnetization transfer, measured as the magnetization transfer ratio (MTR), is sensitive to myelin content. Since demyelination plays an important pathological role in MS, MTI is of value in being able to gauge, at least to some extent, the amount of myelin damage and repair in the SC in MS patients.

\textit{DTI and MTI: Applications in MS}

To date, the use of DTI and MTI in the SC of MS patients \textit{in vivo} has been limited, with only a handful of studies demonstrating correlations between DTI and MTI-derived measures in the SC and global dysfunction in relatively small numbers of MS patients.\textsuperscript{6-9} To our knowledge, longitudinal changes in DTI measures in the SC have been assessed in only three studies, all which had relatively short durations of follow-up (ranging from 3 months to 2 years).\textsuperscript{10-12} Longitudinal studies of MTI changes in the SC of MS patients are similarly limited, with one prior study assessing for correlations of baseline MTR with clinical progression.\textsuperscript{13}

Establishing strong cross-sectional and longitudinal correlations between changes in quantitative SC MRI measures and clinical change is an essential step to expand the practical use of these techniques, which include surrogate outcome measures in trial settings, and as tools for clinical monitoring and prediction in the management of MS.
patients. In addition, these techniques have the potential to provide insight into the pathological substrates of clinical disability in MS.

Based on this background discussion, the four specific aims of this dissertation are as follows:

1) **Aim 1: Characterizing the relationship between quantitative SC MRI indices with clinical measures, including global measures of disability and quantitative measures of sensorimotor function**

   We hypothesize that there will be significant correlations between individual quantitative SC MRI measures and measures of sensorimotor dysfunction, and global disability. Baseline cervical SC MRIs will be obtained in 133 MS patients and 14 healthy control subjects, and will be processed to yield DTI- and MTI-derived indices. Muscle strength and vibration sensation thresholds will be quantified in patients using a dynamometer and Vibratron II device. Global disability will be measured using the expanded disability status scale (EDSS) and multiple sclerosis functional composite (MSFC). Multivariable linear regression models will assess relationships between individual SC MRI measures and clinical disability.

2) **Aim 2: Characterizing the relationship between quantitative SC MRI indices, retinal layers, and clinical measures, including visual acuity, global measures of disability, and quantitative measures of sensorimotor function**

   We hypothesize that there will be significant correlations between SC and retinal measures, and that these measures will independently relate to clinical disability in
MS. In addition to SC MRI, MS patients underwent optical coherence tomography (OCT), with automated retinal segmentation to yield specific retinal layers, and visual assessment. Multivariable regression assessed group differences and relationships between SC, retinal, and clinical measures (including visual acuity, global disability, muscle strength, and vibration sensation threshold).

3) **Aim 3: Assessing the effect of cervical SC normalization by different factors on the detection of group differences and clarification of clinical-radiological correlations in MS.**

We hypothesize that normalization by appropriate factors will result in improved detection of group differences between MS and healthy control subjects (HC), and among MS subtypes, and will clarify clinical-radiological correlations. In addition to SC MRI and clinical assessment, self-reported height will be collected in MS patients. Multivariable regression will assess group differences and relationships between SC volume normalized by subject height, intracranial volume, and SC length and clinical measures.

4) **Aim 4: Evaluating the magnitude of longitudinal change in quantitative SC MRI measures and characterizing the relationship between longitudinal change in individual quantitative SC MRI measures with quantitative measures of sensorimotor dysfunction, and global measures of disability over the study follow-up period of 2 years**

We hypothesize that quantitative SC MRI measures will change measurably in
MS patients and that there will be significant correlations between longitudinal change in individual quantitative SC MRI measures and clinical disability progression using measures of sensorimotor dysfunction, and global disability over the study follow-up period of 2 years. Patients will undergo repeat SC MRI and clinical assessment annually. Statistical analyses using mixed-effects regression incorporating subject-specific intercepts and slopes will be utilized to determine if there are identifiable longitudinal changes in quantitative SC MRI measures over the follow-up time period, and correlations between change in SC MRI measures and clinical progression.
Chapter 1.

Quantitative MRI Correlates of Sensorimotor Function in the Spinal Cord in Multiple Sclerosis

Introduction

The spinal cord (SC) is a common site for lesions in multiple sclerosis (MS), with SC pathology likely contributing substantially to clinical disability in patients. Although the SC is a useful area to study structure-function relationships in MS, it has been difficult to evaluate by MRI due to considerable technical limitations and the “clinico-radiological paradox”.

To overcome some of the reasons underlying the clinico-radiological paradox, we sought to use advanced, quantitative MRI techniques, including DTI and MTI in the SC in MS patients. Furthermore, to accurately quantify neurological dysfunction, we obtained quantitative measures of sensory and motor dysfunction using the Vibratron II and dynamometer devices, both validated for use in MS patients.\textsuperscript{14}

We hypothesized that using DTI and MTI in conjunction with quantitative clinical measures would probe the structure-function relationships of SC pathology in MS more accurately than conventional MRI. By using complementary MRI techniques, this study expands on previous work where we demonstrated relationships between MTR abnormalities and sensorimotor impairment,\textsuperscript{15} enabling greater insight into the microstructural basis of clinical dysfunction in MS.
Methods

Study Participants

This study was approved by the institutional review boards of Johns Hopkins University and the Kennedy Krieger Institute. All participants provided written informed consent.

The study population consisted of individuals with relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), and healthy controls (HCs) (Table 1.1). To perform comparative analyses between those with high vs. low inflammatory MS, patients with RRMS were categorized as the “relapsing” subgroup, while those with SPMS and PPMS were categorized as the “progressive” subgroup. MS patients were recruited from the Johns Hopkins MS Clinic by convenience sampling. MS diagnosis was confirmed by the treating neurologist, according to the 2005 revised McDonald criteria. \(^{16}\) Expanded Disability Status Scale (EDSS) scores were determined by a Neurostatus certified examiner within 30 days of MRI. Hip strength and vibration sensation thresholds were measured within 2 weeks of MRI. Medical records were reviewed to determine disease duration and treatment status. HCs were recruited from the Johns Hopkins University community.

Magnetic Resonance Imaging

Cervical SC MRI

Cervical SC MRIs were performed on all participants using a 3-tesla Achieva scanner (Philips Medical Systems, Best, The Netherlands) using body coil excitation and two-element, phased-array, surface-coil reception.
MT-weighted images were acquired using a T2*-weighted, 3D gradient-echo sequence with a magnetization-transfer prepulse and a multi-shot echo-planar readout (EPI factor=3) with parallel imaging factor of 2 (TR/TE/\(\alpha\)=121ms/12.5ms/9\(^{\circ}\)). The scan yielded 30 contiguous 3 mm axial slices from the C2–C6 vertebral body levels, with a nominal in-plane resolution of 0.6 mm x 0.6 mm. To calculate MTR, scans were acquired with (MT\(_{on}\)) and without (MT\(_{off}\)) a 1.5 kHz off-resonance sinc-gauss-shaped radiofrequency saturation pulse. The MT\(_{on}\) was registered to the MT\(_{off}\) utilizing a 6-degree-of-freedom, rigid-body procedure implemented in FLIRT (FMRIB’s Linear Imaging Registration Tool, Oxford, UK). MTR was calculated according to the formula:(MT\(_{off}\) - MT\(_{on}\))/MT\(_{off}\).

DTI data were obtained on all participants using a multi-slice spin-echo sequence with a single-shot echo-planar readout and a parallel imaging factor of 2 (TR/TE=4727ms/63ms). Axial fat-suppressed diffusion-weighted images were obtained in 16 non-coplanar gradient directions with \(b=500\) s/mm\(^2\) and one minimally diffusion weighted acquisition (\(b_0\sim33\) s/mm\(^2\)). Slice thickness was 3 mm and the nominal in-plane resolution 1.5 x 1.5 mm\(^2\).

Additional image sequences obtained included a sagittal, multi-slice turbo spin echo (TSE factor 20, parallel imaging factor 2) short-tau inversion recovery (STIR) with FOV=250mm, acquired resolution of 1x1x2mm\(^3\) (AP, FH, RL) with TR/TE/TI=4227ms/68ms/200ms. Four averages gave a total scan time of 3 minutes. Each diffusion-weighted image was registered to the initial \(b=0\) volume using a 6-degree-of-freedom, rigid-body registration, implemented in FLIRT using JIST (Java Image Science Toolkit),\(^{17}\) which was also used to generate the diffusion tensor and maps of
fractional anisotropy (FA), mean diffusivity (MD), perpendicular diffusivity ($\lambda_\perp$), and parallel diffusivity ($\lambda_\parallel$) (Figure 1.1). The b=0 image was deformably registered to the MT and the information applied to the DW images.\textsuperscript{18,19} All DTI indices were calculated from the eigenvalues of the diffusion tensor.\textsuperscript{4}

An automated reproducible segmentation protocol was applied to the MT\textsubscript{off} images to delineate regions-of-interest (ROIs) encompassing the axial cross-section of the SC across segments C3-C4 and transferred to the DTI and MTR maps (Figure 1.2).\textsuperscript{20} SC tract-specific ROIs were not utilized due to a high degree of partial volume averaging on the DTI maps, which made it difficult to accurately localize anatomical tracts. ROIs were manually adjusted as necessary on DTI maps with residual distortion. Segments C3-C4 were chosen for analysis, as this was the region of the cervical SC with minimal image quality degradation due to motion artifact. Average values (weighted by ROI volume) of SC cross-sectional area (CSA), FA, MD, $\lambda_\perp$, $\lambda_\parallel$, and MTR were calculated within the ROIs on each MRI index map across all axial slices from C3-C4. Segments C3-C4 included an average of 11 slices per case.

Cervical SC lesions were identified and counted on the axial MT and sagittal STIR sequences by an experienced observer (JO).

**Brain MRI**

Full details of our brain MRI acquisition have been described previously.\textsuperscript{21,22} The spin echo-echo planar DTI sequence was acquired on a 3-tesla Philips scanner with 2.2 mm isotropic voxels and the following scan parameters: TE=69 ms; TR=shortest (automatically calculated); 60 slices; SENSE factor=2.5; 32 diffusion directions (Philips “overplus high” scheme); $b_0=33$ s/mm$^2$; $b=700$ s/mm$^2$; repetitions=2.
DTI images were used to calculate supratentorial brain and cerebrospinal fluid (CSF) volumes, as described previously.\textsuperscript{22} DTI-based brain volume segmentation was used as this method was found to have lower variability than alternate methods using SIENAX or lesion-TOADS software, despite susceptibility-induced distortions in the data.\textsuperscript{23} Brain parenchymal fraction (BPF) was calculated using the formula: $BPF = \frac{\text{brain volume}}{\text{brain volume} + \text{CSF volume}}$.

**Quantitative Clinical Measures**

Vibration sensation thresholds for the right and left great toes were quantified using the Vibratron II (Physitemp, Huron, NJ). For strength measurements, we averaged two maximal hip flexion efforts at each hip using a Microfet2 handheld dynamometer. Both devices have been described in detail elsewhere and have been validated for use in MS patients to reliably detect and quantify sensorimotor dysfunction.\textsuperscript{14} MS patients scanned within the 3 months after a clinical relapse were excluded.

**Statistical Analysis**

Statistical calculations were performed using STATA Version 11 (StataCorp, College Station, TX).

Student’s t-tests were used for group comparisons of SC MRI indices. Due to the exploratory nature of this study, which aims to describe relationships between MRI indices and clinical measures, adjustments for multiple comparisons were not performed.\textsuperscript{24}

Multivariable linear regression models were used to assess the relationship between clinical measures and MRI indices, with the clinical measure as the dependent variable, and the MRI index the independent variable of interest. Potentially confounding
covariates of age, gender, BPF, and SC-CSA were included in each model. In the models using quantitative clinical measurements, bilateral measurements were included for each individual. Therefore, robust standard error estimations were utilized in the regression models to account for within-subject correlations.\textsuperscript{25,26} Stepwise regression models including all quantitative MRI measures as covariates were assessed, but there was substantial instability in these models likely due to a large number of covariates. Thus, we adopted a hypothesis-driven approach and included one MRI index of interest in separate regression models. Adjusted variables plots were utilized to visually depict the adjusted relationship between a quantitative clinical measure and an individual MRI index (adjusted for all other variables in the regression model). To assess the correlation between various MRI indices, Spearman’s rank correlation coefficient was utilized. Statistical significance was defined as $p<0.05$.

**Results**

This study included 74 RRMS patients, 36 SPMS patients, 19 PPMS patients, and 14 HC$s$. MS patients were predominately women (65\%) and had a mean age of 45 years. Average MS disease duration was 11 years, and 69\% of patients were on disease-modifying therapies (interferon-\(\beta\): 40\%, glatiramer acetate: 30\%, natalizumab: 25\%, other medications: 5\%). In comparison to RRMS, progressive patients were significantly older, had longer disease durations, and had more severe clinical dysfunction (Table I.1). Mean vibration sensation threshold and hip flexion strength measures in MS patients were less than the 1\textsuperscript{st} percentile as compared to a normal reference population.\textsuperscript{14}
Summary statistics for all MRI measures, including MRI indices (FA, MD, $\lambda_{||}$, $\lambda_{\perp}$, MTR), SC lesion count, SC-CSA, and BPF are given in Table 1.2. DTI maps from 9 patients and the MTR map from 1 patient were not included in analyses due to inadequate image quality related to artifacts.

All MRI measures assessed were significantly different in MS vs. HCs, with the exception of FA and $\lambda_{||}$, although FA trended towards significance ($p=0.07$). When comparing MS subgroups, all MRI indices were significantly different between the progressive and relapsing subtypes, with the exception of $\lambda_{||}$ ($p=0.16$) (Table 1.2). Multivariable linear regression analyses adjusted for age, sex, SC-CSA, and BPF were performed to assess for independent relationships between functional system-specific measures of clinical disability (hip flexion strength, vibration sensation threshold) and global disability (EDSS), with MRI indices. A separate model was constructed for each individual MRI index.

In the hip flexion strength model: MD, $\lambda_{\perp}$, and $\lambda_{||}$ all demonstrated significant independent associations ($p<0.001$) with motor dysfunction, and FA showed a trend towards an independent association ($p=0.07$). In the vibration sensation threshold model: FA and MTR showed significant independent associations ($p=0.04$ and 0.05, respectively), and $\lambda_{\perp}$ showed a trend towards an independent association ($p=0.06$) (Table 1.3, Figure 1.3). In the EDSS model: all MRI indices showed significant independent associations with EDSS ($p=0.003$, $p=0.03$, $p=0.005$, $p=0.02$ for FA, MD, $\lambda_{\perp}$, and MTR respectively), with the exception of $\lambda_{||}$ ($p=0.5$) (Table 1.3). The constructed models were able to explain up to 38% of the variability in hip flexion strength, 20% of the variability in vibration sensation threshold, and 41% of the variability in EDSS score. The
percentage by which individual MRI indices that retained significant independent associations with clinical measures added to explaining clinical variability ranged from: 6.1% - 8.0% in the model of hip flexion strength, 2.0% - 2.6% in the model of vibration sensation threshold, and 2.5% - 4.8% in the EDSS model. Interestingly, although BPF was included as a covariate in each model to account for the effects of distant brain pathology, this measure did not contribute to explaining clinical variability in hip flexion strength or vibration sensation threshold in any of the models. On the other hand, SC-CSA retained significant independent relationships with clinical variables in all multivariable models (p<0.01). SC lesion count failed to significantly explain variability in either the hip flexion strength or vibration sensation threshold model (Table 1.3). Adjusted variables plots are presented in Figure 1.3 to visually depict the magnitude of the adjusted relationship between each quantitative clinical measure and each MRI index. Finally, we assessed for correlations between MRI measures of atrophy in the brain and SC. In progressive patients, there was no correlation between SC-CSA and BPF (ρ=0.017, p=0.90). In relapsing patients, there was a correlation between SC-CSA and BPF (ρ=0.24, p=0.04).

**Discussion**

Improved correlations between clinical dysfunction and imaging abnormalities are a necessary prerequisite for MRI measures to be useful for understanding the pathological substrate of clinical dysfunction. In this study, we investigated relationships between quantitative MRI indices, conventional MRI measures, and functional system-specific measures of clinical dysfunction, as well as global disability. In a cross-sectional sample
of MS patients that is large relative to other similar studies and that had adequate representation from the different MS disease subtypes, we demonstrate that quantitative MRI indices in the SC maintain significant independent relationships with system-specific measures of clinical dysfunction, as well as global disability, and that combinations of these MRI indices provide important in vivo information about the underlying tissue microstructure of the SC in MS patients.

The motor and sensory systems are particularly relevant functional systems in the SC since a large portion of the SC is comprised of ascending and descending white matter tracts mediating these functions. In a previous study, we demonstrated significant relationships between SC CSF-normalized magnetization-transfer (MTCSF) and quantitative clinical measures. Relationships between DTI indices of the SC and measures of clinical dysfunction have been explored in a few studies only. Univariate correlations between FA, $\lambda_\perp$ and clinical dysfunction have been previously reported. In multivariable models, SC FA has been shown to independently influence EDSS scores, while $\lambda_\perp$ has been found to independently predict recovery from SC relapses. Here, we expand upon previous findings by demonstrating significant relationships between a spectrum of MRI indices and both system-specific and global measures of disability. In our study, the constructed models were able to explain up to 38% of the variance in hip flexion strength, 20% of the variance in vibration sensation threshold, and 41% of the variance in EDSS score, with the contribution of individual MRI indices ranging from 2.0% – 8.0% of the clinical variance. In each model, we found a panel of MRI indices that maintained independent relationships with hip flexion strength, vibration sensation threshold, and EDSS, even after adjusting for age, sex, BPF, and SC-
CSA, which suggests that these MRI indices are capturing tissue microstructure abnormalities not reflected by the other covariates in the models. Moreover, our results expand on previous observations that measures of SC atrophy relate to clinical disability by showing that SC-CSA not only associates with global clinical dysfunction, but also with motor and sensory system-specific measures of disability.²⁸,²⁹ Taken together, our findings strongly support the utility of quantitative SC-MRI indices in the assessment of clinically relevant microstructural SC changes, and highlight the potential advantage of these indices over conventional measures of SC atrophy, which reflect tissue loss that is probably irreversible.

Comparisons of SC-MRI measures between MS and HCs, and between progressive and relapsing MS subtypes showed significant differences in all MRI indices, except $\lambda_{\|}$. This is consistent with previous studies that found differences in various DTI indices and MTR in MS patients vs. HCs,⁶⁻⁸,¹¹ and in MTR and FA in progressive vs. relapsing patients.⁷,⁹ We expand on these findings by demonstrating significant differences in FA, MD, $\lambda_{\perp}$, and MTR in progressive vs. relapsing patients. These observations suggest microstructural tissue damage in the SC may be an important factor in the evolution of disease from the relapsing to progressive phases, meriting further investigation longitudinally.⁹

Although reductions in $\lambda_{\|}$ have been shown to be sensitive to axonal damage in models of acute axonal transection,³⁰,³¹ DTI studies in ex vivo human SCs have consistently shown increased $\lambda_{\|}$.³²,³³ This may be due to a variety of factors, including partial volume effects and the dynamic nature of $\lambda_{\|}$ in chronic MS. On the other hand, $\lambda_{\perp}$ appears to be highly sensitive to microstructural changes, but not specific to particular
pathological processes in *ex vivo* human SCs,\textsuperscript{32,33} findings that are consistent with models of acute axonal transection.\textsuperscript{30,31} In practice, $\lambda_\perp$ and $\lambda_{||}$ represent water diffusion perpendicular to and along intact axonal fibers, respectively, and FA measures the extent to which water diffuses along those fibers rather than perpendicular to them. MD is an overall measure of water diffusion,\textsuperscript{34} whereas MTR is sensitive to myelin content\textsuperscript{35} and axonal density,\textsuperscript{36} as well as overall tissue water content.\textsuperscript{37} These quantitative MRI indices thus have the ability to provide complementary information on the structural integrity of SC tissue. In our study, the direction of the relationship of each MRI index (with the exception of $\lambda_{||}$) with clinical dysfunction corresponded to what is expected from models of tissue damage such as that found in MS, with positive relationships between FA and MTR and better clinical function, and negative relationships between MD and $\lambda_\perp$ and better clinical function (Table 1.3). The pathological features these MRI indices reflect are likely to be combinations of processes, including demyelination, axonal loss, inflammation, and gliosis. A longitudinal assessment of the direction and magnitude of change in these measures, and their relationship with clinical progression will be of substantial interest.

In this heterogeneous MS sample, $\lambda_\perp$ appeared to most reliably distinguish MS patients from HCs and contributed to explaining clinical variability in all three models examined. This observation is consistent with a recent study that found baseline $\lambda_\perp$ of utility in predicting recovery after SC relapses, and demonstrated dynamic changes in $\lambda_\perp$ over time.\textsuperscript{10} Our findings support the recent proposal of $\lambda_\perp$ as a useful marker of overall tissue integrity,\textsuperscript{32} and a longitudinal extension of this study will help determine the clinical utility of $\lambda_\perp$ for predicting disability progression.
Poor correlations between brain and SC measures of atrophy have been previously described,\textsuperscript{11,28} and we similarly observed this in progressive MS, with no demonstrable correlation between BPF and SC-CSA. Furthermore, BPF did not significantly contribute to explaining variability in either of the models of functional system-specific disability (vibration sensation and hip flexion strength) that are of anatomic relevance in the SC. In contrast, BPF did contribute, with most of the MRI indices, to explaining variability in EDSS, which is in keeping with existing literature of relationships between brain atrophy and EDSS.\textsuperscript{38} Taken together, we can postulate that neurodegenerative pathological processes in the brain and SC may occur semi-independently, and that this divergence may be more relevant in progressive patients. These findings underscore the need to further characterize SC-specific pathological processes, particularly in progressive subtypes, since SC pathology is a well-described contributor to disability in this subset of patients.\textsuperscript{9,39} Further study of the SC may provide a unique platform for improving our understanding of the neurodegenerative mechanisms underlying progressive disability in MS, which are relevant to all MS subtypes, but particularly the progressive forms of the disease.

There are a number of limitations to this study. First, the small number of HCs likely limited our power to compare MRI indices in patients vs. HCs. Despite this, we were able to show significant differences in MRI indices in MS patients vs. HCs, suggesting that with a larger HC sample this difference may be more pronounced. Second, we did not have clinical measures in HCs, preventing comparison of clinical-radiological correlations in MS patients vs. HCs. However, given the apparent baseline differences in MRI indices between HCs and MS patients, and the fact that HCs are
unlikely to have clinical impairment, it is improbable that this information would have changed our conclusions significantly. Third, since we only evaluated a discrete segment (C3-4) of the cervical SC, upstream and downstream pathological changes contributing to clinical dysfunction were not completely taken into account. Furthermore, the use of the ROIs encompassing the SC-CSA rather than specific columns likely resulted in a dilution of observed structure-function relationships. Notwithstanding these limitations, we were still able to demonstrate consistent and robust structure-function relationships with our employed methodology.

In conclusion, this study demonstrates significant independent relationships between quantitative SC MRI indices, particularly $\lambda_{\perp}$, and various measures of clinical dysfunction (both system-specific and global disability) after adjusting for confounding variables in a large diverse sample of MS patients. Our findings highlight the utility of quantitative SC-MRI in improving our understanding of structure-function relationships in MS, which is an important stepping-stone towards utilizing these measures in monitoring therapeutic efficacy and in developing targeted treatments. A longitudinal extension of this dataset will examine the evolution of microstructural changes in the SC, and the degree to which these changes affect clinical performance over time.
<table>
<thead>
<tr>
<th></th>
<th>All MS</th>
<th>RRMS</th>
<th>Progressive (SPMS and PPMS)</th>
<th>HCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>129</td>
<td>74</td>
<td>55</td>
<td>14</td>
</tr>
<tr>
<td>Age at MRI scan, years (SD)</td>
<td>44.7 (11.4)</td>
<td>39.3 (10.6)</td>
<td>52.0 (7.8)*‡</td>
<td>40.0 (9.3)</td>
</tr>
<tr>
<td>% Female</td>
<td>65</td>
<td>70</td>
<td>58</td>
<td>71</td>
</tr>
<tr>
<td>Disease duration, years (SD)</td>
<td>10.8 (9.2)</td>
<td>7.1 (5.6)</td>
<td>15.6 (10.7)‡</td>
<td>n/a</td>
</tr>
<tr>
<td>Median baseline EDSS (IQR)</td>
<td>3.5 (2-6)</td>
<td>2.5 (1.5-3.5)</td>
<td>6 (4-6.5)‡</td>
<td>n/a</td>
</tr>
<tr>
<td>% on disease-modifying treatment</td>
<td>69</td>
<td>88</td>
<td>44‡</td>
<td>n/a</td>
</tr>
<tr>
<td>Vibration sensation threshold, microns (SD)</td>
<td>15.1 (22.4)</td>
<td>8.1 (13.5)</td>
<td>24.8 (28.1)‡</td>
<td>n/a</td>
</tr>
<tr>
<td>Hip flexion strength, pounds (SD)</td>
<td>39.4 (18.5)</td>
<td>46.4 (15.6)</td>
<td>29.3 (18.0)‡</td>
<td>n/a</td>
</tr>
</tbody>
</table>

RRMS=relapsing-remitting multiple sclerosis, SPMS=secondary-progressive multiple sclerosis, HCs=healthy control subjects, EDSS=expanded disability status scale, SD=standard deviation, IQR=interquartile range. Disease duration was defined as the time since first symptoms attributable to MS.

*p<0.05 for comparison against HCs
‡p<0.05 for comparison against RRMS
Table 1.2: Comparison of average MRI measures between subtypes of MS patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>All MS patients</th>
<th>RRMS</th>
<th>Progressive patients (SPMS and PPMS)</th>
<th>HCs</th>
<th>All MS patients vs. HCs</th>
<th>Progressive vs. RRMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subjects</td>
<td>129</td>
<td>74</td>
<td>55</td>
<td>14</td>
<td>n/a</td>
</tr>
<tr>
<td>Cord cross-sectional area, mm² (SD)</td>
<td>76.9 (9.2)</td>
<td>79.6 (8.4)</td>
<td>73.3 (8.9)</td>
<td>83.1 (9.2)</td>
<td>-6.19</td>
<td>0.02</td>
</tr>
<tr>
<td>Lesion count, number (SD)</td>
<td>2.2 (1.5)</td>
<td>2.0 (1.5)</td>
<td>2.5 (1.3)</td>
<td>0</td>
<td>2.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Brain parenchymal fraction (SD)</td>
<td>0.86 (0.05)</td>
<td>0.88 (0.05)</td>
<td>0.83 (0.05)</td>
<td>0.90 (0.04)</td>
<td>-0.038</td>
<td>0.01</td>
</tr>
<tr>
<td>FA, (SD)</td>
<td>0.61 (0.06)</td>
<td>0.62 (0.06)</td>
<td>0.59 (0.07)</td>
<td>0.64 (0.04)</td>
<td>-0.032</td>
<td>0.07</td>
</tr>
<tr>
<td>MD [µm²/ms], (SD)</td>
<td>1.28 (0.17)</td>
<td>1.25 (0.15)</td>
<td>1.32 (0.19)</td>
<td>1.20 (0.10)</td>
<td>0.076</td>
<td>0.02</td>
</tr>
<tr>
<td>λ⊥ [µm²/ms], (SD)</td>
<td>0.77 (0.17)</td>
<td>0.74 (0.15)</td>
<td>0.81 (0.18)</td>
<td>0.67 (0.09)</td>
<td>0.097</td>
<td>0.03</td>
</tr>
<tr>
<td>λ∥ [µm²/ms], (SD)</td>
<td>2.22 (0.21)</td>
<td>2.20 (0.20)</td>
<td>2.26 (0.23)</td>
<td>2.16 (0.16)</td>
<td>0.065</td>
<td>0.27</td>
</tr>
<tr>
<td>MTR, (SD)</td>
<td>0.30 (0.05)</td>
<td>0.31 (0.04)</td>
<td>0.28 (0.05)</td>
<td>0.31 (0.02)</td>
<td>-0.014</td>
<td>0.04</td>
</tr>
</tbody>
</table>

RRMS=relapsing-remitting multiple sclerosis, SPMS=secondary-progressive multiple sclerosis, HCs=healthy control subjects, FA=fractional anisotropy, MD=mean diffusivity, λ⊥=perpendicular diffusivity, λ∥=parallel diffusivity, MTR=magnetization transfer ratio, SD=standard deviation.

p-values <0.05 are indicated in boldface.
Table 1.3: Relationships between MRI indices and clinical measures in multivariable regression models

<table>
<thead>
<tr>
<th>MRI Index</th>
<th>Hip Flexion Strength</th>
<th>Vibration Sensation</th>
<th>EDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient</td>
<td>p-value*</td>
<td>Regression coefficient</td>
</tr>
<tr>
<td>FA</td>
<td>43.43</td>
<td>0.07</td>
<td>-46.78</td>
</tr>
<tr>
<td>MD</td>
<td>-33.49</td>
<td>&lt;0.001</td>
<td>13.62</td>
</tr>
<tr>
<td>λ⊥</td>
<td>-30.92</td>
<td>&lt;0.001</td>
<td>17.79</td>
</tr>
<tr>
<td>λ∥</td>
<td>-23.46</td>
<td>&lt;0.001</td>
<td>3.57</td>
</tr>
<tr>
<td>MTR</td>
<td>56.41</td>
<td>0.12</td>
<td>-74.68</td>
</tr>
<tr>
<td>Lesion count</td>
<td>-1.43</td>
<td>0.13</td>
<td>1.27</td>
</tr>
</tbody>
</table>

EDSS=expanded disability status scale, FA=fractional anisotropy, MD=mean diffusivity, λ⊥=perpendicular diffusivity, λ∥=parallel diffusivity, MTR=magnetization transfer ratio

*p-values correspond to the regression coefficient for each MRI index generated in multivariable linear regression models adjusting for age, sex, cord spinal cross-sectional area, and brain parenchymal fraction using robust standard error estimations to account for within-subject correlations

†p-values correspond to the regression coefficient for each MRI index generated in multivariable linear regression models adjusting for age, sex, cord spinal cross-sectional area, and brain parenchymal fraction.

p-values < 0.05 are indicated in boldface.
Figure 1.1: Color-coded DTI map derived from fractional anisotropy and the principal eigenvector. Blue represents tracts running in the rostrocaudal axis; green, anteroposterior; and red, mediolateral; oblique angles are represented by a mixture of colors.

Figure 1.2: (a) Axial section of cervical spinal cord on high-resolution magnetization-transfer (MT) sequence (b) with superimposed region-of-interest encompassing spinal cord cross-sectional area.
Figure 1.3: Graphical representation of adjusted relationships between MRI indices and quantitative clinical measures in separate multivariable models
Chapter 2

Spinal Cord Quantitative MRI Discriminates Between Disability Levels in Multiple Sclerosis

Introduction

The practicing neurologist commonly encounters the phenomenon of the clinico-radiological paradox, in which a substantial disconnect between spinal cord (SC) lesions detected with conventional MRI and physical disability in multiple sclerosis (MS) is observed.\textsuperscript{3}

This disconnect is particularly surprising in the SC, due to its compact structural organization. Anatomically, a strong correlation between MRI measures of lesion load in the SC and clinical dysfunction is expected – yet many studies confirm the lack of such a correlation.\textsuperscript{28,40} These observations, coupled with the tight anatomical organization of the SC and the propensity for SC lesions to occur in MS,\textsuperscript{1,39} make the SC an opportune structure within which to study the clinico-radiological paradox.

In order to study a real-world example of the clinico-radiological paradox, we categorized a sample of MS patients into four subgroups based on clinical disability level and MRI lesion count. Because of the sensitivity of diffusion-tensor imaging (DTI) and magnetization-transfer imaging (MTI) to microstructural tissue properties,\textsuperscript{34,35} we hypothesized that DTI and MTI indices would detect differences in individuals with differing disability levels, regardless of overall lesion count. The ability to detect clinically relevant microstructural changes in the SC with MRI is a necessary prerequisite
to more effectively utilize MRI in clinical settings and to better understand MS disease mechanisms.

**Methods**

**Standard Protocol Approvals, Registrations, and Patient Consents**

This study was approved by the institutional review boards of Johns Hopkins University and the Kennedy Krieger Institute. All participants provided written informed consent.

**Study Participants**

Patients with relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS) were recruited from the Johns Hopkins MS Clinic by convenience sampling between 2007-2009. MS diagnosis was confirmed by the treating neurologist, according to the 2005 revised McDonald criteria. Within 30-days of MRI, multiple sclerosis functional composite (MSFC) scores were obtained and expanded disability status scale (EDSS) scores were determined by a Neurostatus-certified examiner. EDSS and MSFC were chosen as clinical measures over column-specific clinical measures since EDSS and MSFC have the benefits of familiarity and clinical interpretability by most neurologists, and because regions-of-interest (ROIs) encompassing the SC cross-section rather than specific columns were utilized. For comparative analyses between patients with similar lesion loads but differing disability levels, patients were stratified into two groups according to MRI lesion count. Those with \( \leq 2 \) lesions were classified as the “low-lesion” group, while those with \( \geq 3 \) lesions were classified as the “high-lesion” group. A lesion count cut-off of 2 was
selected for this dichotomization as this was the median value across all patients and ensures adequate subgroup sample sizes. MS patients were further dichotomized according to disability level using an EDSS cut-off of 6. This particular EDSS cut-off was chosen a priori since it represents an important functional milestone with respect to clinical disability, signifying the transition from ambulating independently for 100 meters to requiring intermittent/constant unilateral assistance. Accordingly, MS patients with EDSS scores <6 were categorized as the “low-disability” subgroup, while those with EDSS ≥6 were categorized as the “high-disability” subgroup.

**Cervical SC MRI**

Cervical SC MRIs were performed on all participants using a 3-tesla Achieva scanner (Philips Medical Systems, Best, The Netherlands) using body coil excitation and two-element phased array surface coil reception. Sequences acquired included: MTI, DTI, and short-tau inversion recovery (STIR) (FOV=250mm, acquired resolution:1x1x2mm$^3$[AP,FH, RL],TR/TE/TI=4227ms/68ms/200ms), as described in detail in Chapter 1.

Post-acquisition image processing was performed, as described in Chapter 1, to generate maps of FA, MD, $\lambda_\perp$, $\lambda_\parallel$, and MTR. An automated reproducible segmentation protocol was applied to the MT$\text{off}$ images to delineate ROIs encompassing the axial cross-section of the SC across segments C3-C4 and transferred to the MRI-index maps, as described in Chapter 1.\textsuperscript{20} Average values of SC cross-sectional area(CSA), FA, MD, $\lambda_\parallel$, $\lambda_\perp$, and MTR were calculated from the ROIs of each MRI-index map across all axial slices from C3-C4.
Cervical SC lesions were counted between segments C2-C6 on the axial MT and sagittal STIR sequences by an experienced observer blinded to clinical status (JO).

**Brain MRI**

DTI images were used to calculate supratentorial brain and cerebrospinal fluid (CSF) volumes, as described in Chapter 1. Brain parenchymal fraction (BPF) was calculated using the formula: BPF = brain volume / [brain volume + CSF volume].

**Statistical Analysis**

Statistical calculations were performed using STATA Version 11 (StataCorp, College Station, TX). Student’s t-tests were used for group comparisons of SC-MRI indices. Due to the exploratory nature of this study, adjustment for multiple comparisons was not performed. Pearson’s product-moment coefficients were used to assess correlations between MRI-indices. Multivariable linear regression was used to assess differences in MRI-indices between patient subgroups, adjusting for age, sex, BPF, SC-CSA. Statistical significance was defined as p<0.05. The discriminatory capacity of combinations of MRI indices were assessed using stepwise forward selection in a logistic regression model with high/low disability as the dependent variable and MRI indices, SC-CSA, age, sex included as covariates (p<0.10 to remain in model).

**Results**

This study included a total of 124 MS patients (69 RRMS, 36 SPMS, and 19 PPMS). MS patients were predominantly female (64%) and had a mean age of 45 years. Average MS disease duration was 11 years, and 69% of patients were on disease-modifying treatments (interferon-β: 40%, glatiramer acetate:30%, natalizumab:25%,
other medications:5%)(Table 2.1). When stratified by lesion load, 69 patients fulfilled
criteria for the low-lesion subgroup, and 55 patients for the high-lesion subgroup. Within
subgroups stratified by lesion count, individuals with lower disability were younger, had
shorter disease durations, were predominantly of the relapsing subtype, and were more
likely to be on disease-modifying treatment than those with higher disability.

Comparisons of equivalent disability subgroups between patients with high vs.
low lesion counts showed remarkably similar proportions of relapsing patients and
distributions of EDSS scores (Table 2.1).

Summary statistics for all MRI measures, including quantitative MRI indices (FA,
MD, $\lambda_\perp$, $\lambda_\parallel$, MTR), SC lesion count, and SC-CSA, are given in Table 2.1. DTI maps from
8 patients and the MTR map from 1 patient were not included due to inadequate image
quality. 85% of patients had at least one SC lesion, which is consistent with prior
literature.\textsuperscript{1,2} Of all identified lesions, 93% occupied $\leq$1 vertebral level, whereas 1%
occupied $\geq$3 vertebral levels.

In patients with low lesion counts, all MRI measures were more abnormal in those
with high vs. low disability. Similarly, in patients with high lesion counts, all MRI
measures were more abnormal in those with high vs. low disability, with the exception of
$\lambda_\parallel$ and MTR(Table 2.1, Figures 2.1-2.2).

In age and sex adjusted comparisons of patients with low-lesion counts, all MRI
measures still retained a difference in comparisons of those with high vs. low disability,
with the exception of $\lambda_\parallel$. Similarly, in patients with high-lesion counts, FA, MD, and $\lambda_\perp$
remained different in comparisons of patients with high vs. low disability after adjusting
for age and sex, while SC-CSA, $\lambda_\parallel$ and MTR were not different(Table 2.2).
Additional analyses adjusting for BPF and SC-CSA, and utilizing an EDSS cut-off score of 4 and the MSFC as a measure of disability also demonstrated that MRI indices were able to discriminate between MS patients with high/low disability. Correlations between individual MRI indices are presented in Table 2.3. The clinical discriminatory capacity of combinations of MRI indices was assessed in a forward selection model (Table 2.4). In low-lesion load patients, MTR and SC-CSA were the only covariates retained in the model, while in high-lesion load patients, only FA was retained, suggesting that MRI-indices are highly correlated and that specific MRI indices have better discriminatory capacity than others in settings of different lesion loads.

**Discussion**

Weak correlations between SC lesion load, as estimated with conventional MRI, and disability status in MS patients limit the utility of lesion-based measurements in the clinical setting, and on a larger scale in clinical trials. In this study, we found consistent differences in quantitative MRI indices between patients with substantially different levels of disability, despite having similar lesion counts, even after controlling for age and sex. These findings support the concept that microstructural changes undetectable by conventional lesion count contribute substantially to clinical disability in MS, and suggest that quantitative MRI measures have the ability to provide clinically relevant information beyond that which may be gleaned from measures of MRI lesion load alone.

Regardless of lesion load, FA, MD, and $\lambda_\perp$ were consistently able to discriminate MS patients with high and low levels of disability. FA was consistently lower, MD higher, and $\lambda_\perp$ higher in individuals with high disability levels, as compared to those with
low disability levels. In practice, FA measures the proportion of water diffusion along rather than perpendicular to axonal fibers, $\lambda_{\parallel}$ represents diffusion along axonal fibers, $\lambda_{\perp}$ represents diffusion perpendicular to axonal fibers, MD is a measure of mean diffusion, and MTR is a measure sensitive to myelin content. These quantitative MRI indices provide complementary information regarding the structural integrity of SC tissue.

Our demonstration of robust differences in quantitative MRI indices of the SC in a large, diverse cohort of MS patients with high and low levels of disability, regardless of SC lesion count, expands upon prior studies that have shown correlations between FA and $\lambda_{\perp}$ and disability in MS and attests to the additive clinical value of quantitative MRI indices over conventional MRI measures. Recent DTI studies of ex vivo human SCs have demonstrated that $\lambda_{\perp}$ is highly sensitive to tissue microstructural changes but not specific to particular pathological processes, findings that are consistent with those reported in animal studies of acute axonal transection. Similarly, in the optic nerve, changes in $\lambda_{\perp}$ have been linked to both demyelination and axonal loss, based on correlational analyses with visual evoked potentials and optical coherence tomography. Overall, the observed differences in MRI indices between disability subgroups in this study are likely to reflect a combination of pathological processes, including demyelination, axonal loss, inflammation, and gliosis.

When comparing differences in quantitative MRI indices between patients with high and low levels of disability (adjusted for age and sex), all MRI measures with the exception of $\lambda_{\parallel}$ were different in patients with low-lesion counts, while only FA, MD, and $\lambda_{\perp}$ were different in patients with high-lesion counts. This finding is of interest as it suggests that in low-lesion count settings, greater perturbations in microstructural
changes, likely corresponding to a greater spectrum of pathological processes, contribute
to increased disability. On the other hand, in individuals with high-lesion counts, the
panel of quantitative MRI indices that showed a clear difference between disability levels
was more restricted, with MTR not demonstrating a difference. Since MTR is a measure
sensitive to myelin content, this finding suggests that demyelination may be less
relevant in associations with clinical disability in patients with high SC lesion counts -
with axonal degeneration likely playing a dominant role, while demyelination and axonal
pathology both appear to play a substantial role in patients with low-lesion counts.
Alternatively, this finding may suggest that in patients with high-lesion loads, the degree
of demyelination is so extensive that it can no longer distinguish between different
disability levels, or that additional pathological processes contribute to alterations in
MTR. Although the cross-sectional nature of this study precludes definitive
interpretation, our observations suggest that different pathological processes underlying
clinical disability may be at play depending on the magnitude of SC lesion load. If
substantiated in longitudinal studies, this finding may have clinical implications for
treatment selection and disease monitoring in MS patients with higher lesion loads in the
SC.

Interestingly, SC-CSA, a measure of general tissue loss, was different between the
high and low disability subgroups in patients with low-lesion loads, perhaps consistent
with early myelin and axonal loss in the more disabled patients. Importantly, some low-
lesion counts may have occurred in part due to dissolution of abnormal appearing tissue.
On the other hand, in individuals with high-lesion loads, SC-CSA was unable to
differentiate between high and low disability levels after adjusting for age and sex. These
findings suggest that in patients with high-lesion load, it is not the magnitude of tissue loss, but the structural integrity of remaining tissue that is related to disability, highlighting the utility of quantitative MRI indices in these settings.

Our findings, which demonstrate the ability of quantitative MRI indices to differentiate between modest samples sizes of MS patients with similar lesion counts, but differing disability levels, are encouraging. If confirmed prospectively, these techniques may have clinical utility in a variety of realms, including monitoring therapeutic efficacy, predicting disability progression, and as a surrogate outcome measure in clinical trials. The need for predictive measures of disease progression is apparent as the clinical practice of MS continues to evolve, and the choice of treatments with vastly different risk-benefit profiles continues to expand.44

A few prior studies have assessed the clinical predictive value of quantitative MRI indices. A recent study found that baseline cervical spine $\lambda_\perp$ was predictive of recovery from a spinal cord relapse in MS patients.10 Another study found that baseline FA was predictive of clinical progression but did not find a relationship between longitudinal change in MRI indices and disability progression.11 Finally, the ability of a “snapshot” cervical spinal cord MTR measure to predict short-term relapse rate has also been reported.13 In our study, a variety of quantitative MRI measures were able to detect differences in disability on a cross-sectional level, suggesting that these measures are sensitive to clinically relevant microstructural changes, and support the potential use of a baseline MRI scan to provide valuable information on both short-term and long-term disability progression. A longitudinal extension of this study will be of substantial interest in this regard.
This study has a number of limitations. First, the ability to distinguish between individuals with high/low disability in our study is limited by the constraints of the EDSS, and is thus heavily weighted toward ambulatory disability. In the SC, however, the motor system is a highly relevant functional system, thus the use of a scale weighted toward ambulation may be appropriate in this setting. Second, in order to dichotomize individuals into high/low disability levels, and high/low-lesion counts, EDSS and lesion count cutoffs had to be chosen. Although the designated cutoff points were carefully chosen based on a clear rationale, they may still be regarded as arbitrary given the lack of prior studies determining the most appropriate cutoff points for these measures. Third, the use of ROIs encompassing the SC-CSA resulted in the inclusion of both white- and gray-matter, likely resulting in a dilution of the observed clinico-radiological relationships. In addition, the inclusion of lesional and non-lesional tissue might have contributed to diminished specificity of quantitative MRI indices in identifying microstructural changes, however, it is also possible that important information can be derived from normal-appearing spinal cord tissue. Fourth, there was a discrepancy between SC segments included for assessment of MRI indices versus lesion counting. The segment of SC analyzed for MRI indices was limited to C3-C4 as image quality was consistently highest between these segments likely due to the least amount of distortion from motion artifact. For lesion counting, we included a larger segment of the cervical SC to promote the most accurate characterization of patients into high/low-lesion count. Notwithstanding these limitations, we were still able to demonstrate consistent and robust differences between differing levels of disability with our employed methodology.
Finally, our assessment of conventional lesion load was limited to lesion counting, which does not take into account the volume of lesioned tissue. However, lesion counting still represents a reasonable method for quantifying lesion load, since individuals with more lesions tend to have larger volumes of lesioned tissue, particularly in our study population, where 93% of lesions occupied \( \leq 1 \) vertebral level. Accordingly, it is unlikely that our interpretations would have changed substantially with a more quantitative approach of lesion quantification. Furthermore, in clinical practice, lesion counting is routinely used, making this measurement of practical use.

Our findings illustrate that microstructural changes undetectable by conventional MRI contribute to observed differences in clinical disability in MS patients. These findings promote not only the utility of quantitative MRI indices in being able to provide important insight into the microstructural changes contributing to disability, but also suggest that with further development, these measures could be of clinical utility in both the management of individual patients, and as a surrogate outcome measure in clinical trials. Further studies are needed to assess the relationships of lesion counts, quantitative MRI indices, and clinical disability progression longitudinally, and to evaluate the relationships of these indices with more detailed characterizations of clinical disability in MS.
Table 2.1: Comparisons of clinical characteristics and MRI measures in patient subgroups, stratified by lesion load

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>Low Lesion</th>
<th>High Lesion</th>
<th>p-value</th>
<th>Low Lesion</th>
<th>High Lesion</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>124</td>
<td>55</td>
<td>14</td>
<td>n/a</td>
<td>31</td>
<td>24</td>
<td>n/a</td>
</tr>
<tr>
<td>Age at MRI scan, years (SD)</td>
<td>45.2 (11.1)</td>
<td>42.2 (11.9)</td>
<td>51.3 (6.5)</td>
<td>0.008</td>
<td>43.1 (10.3)</td>
<td>51.2 (8.6)</td>
<td>0.003</td>
</tr>
<tr>
<td>% Female</td>
<td>63.7</td>
<td>78.2</td>
<td>64.3</td>
<td>0.28</td>
<td>45.2</td>
<td>54.2</td>
<td>0.50</td>
</tr>
<tr>
<td>Disease Duration, years (SD)</td>
<td>10.9 (9.3)</td>
<td>8.4 (9.0)</td>
<td>19.4 (10.0)</td>
<td>0.0002</td>
<td>8.7 (6.6)</td>
<td>14.7 (8.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>Median EDSS (IQR)</td>
<td>3.5 (2-6)</td>
<td>2.5 (2.0 - 3.5)</td>
<td>6.5 (6.0 - 6.5)</td>
<td>&lt;0.001</td>
<td>2.5 (1.4 - 4.0)</td>
<td>6.5 (6.0 - 6.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% on disease-modifying treatment</td>
<td>69.4</td>
<td>81.8</td>
<td>50.0</td>
<td>0.01</td>
<td>76.7</td>
<td>41.7</td>
<td>0.008</td>
</tr>
<tr>
<td>% RRMS</td>
<td>56.5</td>
<td>78.2</td>
<td>7.1</td>
<td>&lt;0.001</td>
<td>71.0</td>
<td>12.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lesion count (SD)</td>
<td>2.2 (1.5)</td>
<td>1.1 (0.9)</td>
<td>1.4 (0.6)</td>
<td>0.18</td>
<td>3.5 (0.7)</td>
<td>3.8 (0.9)</td>
<td>0.20</td>
</tr>
<tr>
<td>Spinal cord cross-sectional area, mm$^2$ (SD)</td>
<td>76.9 (9.3)</td>
<td>80.1 (8.5)</td>
<td>67.4 (9.4)</td>
<td>&lt;0.001</td>
<td>78.2 (8.0)</td>
<td>73.5 (7.7)</td>
<td>0.03</td>
</tr>
<tr>
<td>FA, (SD)</td>
<td>0.61 (0.06)</td>
<td>0.61 (0.06)</td>
<td>0.57 (0.07)</td>
<td>0.03</td>
<td>0.63 (0.06)</td>
<td>0.59 (0.06)</td>
<td>0.02</td>
</tr>
<tr>
<td>MD [μm$^2$/ms], (SD)</td>
<td>1.27 (0.16)</td>
<td>1.25 (0.15)</td>
<td>1.41 (0.23)</td>
<td>0.003</td>
<td>1.22 (0.12)</td>
<td>1.31 (0.17)</td>
<td>0.02</td>
</tr>
<tr>
<td>$\lambda_\perp$ [μm$^2$/ms], (SD)</td>
<td>0.76 (0.16)</td>
<td>0.74 (0.14)</td>
<td>0.90 (0.23)</td>
<td>0.003</td>
<td>0.70 (0.13)</td>
<td>0.81 (0.16)</td>
<td>0.01</td>
</tr>
<tr>
<td>$\lambda_\parallel$ [μm$^2$/ms],</td>
<td>2.22 (0.21)</td>
<td>2.19 (0.19)</td>
<td>2.34 (0.26)</td>
<td>0.02</td>
<td>2.19 (0.21)</td>
<td>2.24 (0.21)</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>MTR, (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>0.30 (0.04)</td>
<td>0.31 (0.04)</td>
<td>0.27 (0.05)</td>
<td><strong>0.003</strong></td>
<td>0.30 (0.03)</td>
<td>0.28 (0.06)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

SD=standard deviation, EDSS=expanded disability status scale, IQR=interquartile range, RRMS=relapsing-remitting multiple sclerosis, FA=fractional anisotropy, MD=mean diffusivity, $\lambda_\perp$=perpendicular diffusivity, $\lambda_\parallel$=parallel diffusivity, MTR=magnetization transfer ratio

*p-values are derived from comparisons of low vs. high EDSS in subgroups stratified by lesion count. p-values <0.05 are indicated in boldface.
Table 2.2: Age- and sex-adjusted differences in MRI measures and effect size in patient subgroups, stratified by lesion load

<table>
<thead>
<tr>
<th></th>
<th>Low lesion</th>
<th></th>
<th>High lesion</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted difference</td>
<td>Effect Size</td>
<td>p-value</td>
<td>Adjusted difference</td>
</tr>
<tr>
<td></td>
<td>between low/high</td>
<td></td>
<td></td>
<td>between low/high</td>
</tr>
<tr>
<td></td>
<td>EDSS subgroups</td>
<td></td>
<td></td>
<td>EDSS subgroups</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>12.11</td>
<td>0.20</td>
<td>&lt;0.001</td>
<td>3.18</td>
</tr>
<tr>
<td>cross-sectional area (mm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>0.046</td>
<td>0.073</td>
<td>0.03</td>
<td>0.046</td>
</tr>
<tr>
<td>MD [µm²/ms]</td>
<td>-0.15</td>
<td>0.11</td>
<td>0.008</td>
<td>-0.10</td>
</tr>
<tr>
<td>λ⊥ [µm²/ms]</td>
<td>-0.16</td>
<td>0.12</td>
<td>0.005</td>
<td>-0.11</td>
</tr>
<tr>
<td>λ∥ [µm²/ms]</td>
<td>-0.13</td>
<td>0.053</td>
<td>0.07</td>
<td>-0.055</td>
</tr>
<tr>
<td>MTR</td>
<td>0.033</td>
<td>0.078</td>
<td>0.02</td>
<td>0.011</td>
</tr>
</tbody>
</table>

FA=fractional anisotropy, MD=mean diffusivity, λ⊥=perpendicular diffusivity, λ∥=parallel diffusivity, MTR=magnetization transfer ratio, EDSS=expanded disability status scale
*p-values are derived from comparisons of low vs. high EDSS in subgroups stratified by lesion count; p-values <0.05 are indicated in boldface; adjusted differences calculated using the following formula: [MRI measure from low disability subgroup] – [MRI measure from high disability subgroup]; effect sizes estimate the proportion of variance in MRI indices attributable to disability categorization
Table 2.3: Pearson’s product-moment correlation coefficients between pairs of MRI indices

| Coefficient (p-value) | FA       | MD       | $\lambda_\perp$ | $\lambda_{||}$ | MTR      | SC-CSA    |
|-----------------------|----------|----------|-----------------|----------------|----------|-----------|
| FA                    | 1.0000   |          |                 |                |          |           |
| MD                    | -0.6860  (<0.001) | 1.0000   |                 |                |          |           |
| $\lambda_\perp$      | -0.8810  (<0.001) | 0.9369   (<0.001) | 1.0000          |            |          |           |
| $\lambda_{||}$       | -0.1786  (0.06)  | 0.8237   (<0.001) | 0.5735          (<0.001) | 1.0000   |          |           |
| MTR                   | 0.2121   (0.02)  | -0.2384  (0.01)  | -0.2272         (0.01) | -0.1905  (0.04) | 1.0000   |           |
| SC-CSA                | 0.3114   (<0.001) | -0.3083  (<0.001) | -0.3513         (<0.001) | -0.1529  (0.10) | 0.2559   (0.004) | 1.0000 |

FA=fractional anisotropy, MD=mean diffusivity, $\lambda_\perp$=perpendicular diffusivity, $\lambda_{||}$=parallel diffusivity, MTR=magnetization transfer ratio, SC-CSA=spinal cord cross-sectional area

Table 2.4: Forward selection logistic regression model including all MRI indices

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>High/low disability categorization using EDSS cut-off of 6.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covariates included in forward selection model</td>
<td>FA, MD, $\lambda_\perp$, $\lambda_{</td>
</tr>
<tr>
<td>Retained covariates in patients with low lesion load</td>
<td>MTR, SC-CSA</td>
</tr>
<tr>
<td>Retained covariates in patients with high lesion load</td>
<td>FA, age</td>
</tr>
</tbody>
</table>

EDSS=expanded disability status scale, FA=fractional anisotropy, MD=mean diffusivity, $\lambda_\perp$=perpendicular diffusivity, $\lambda_{||}$=parallel diffusivity, MTR=magnetization transfer ratio, SC-CSA=spinal cord cross-sectional area
*covariates with p-values < 0.10 retained in forward selection model
Figure 2.1: Comparisons of MRI measures in low vs. high disability subgroups in patients with low lesion load

*Boxplots depict median, inter-quartile range, and upper and lower fences of each MRI index*
Figure 2.2: Comparisons of MRI measures in low vs. high disability subgroups in patients with high lesion load

*Boxplots depict median, inter-quartile range, and upper and lower fences of each MRI index*
Chapter 3

Relationships between Quantitative Spinal Cord MRI and Retinal Layers in Multiple Sclerosis

Introduction

Multiple sclerosis (MS) is an immune-mediated disorder of the central nervous system (CNS) that has a spectrum of clinical manifestations due to lesions involving various regions of the CNS. Clinical symptoms attributable to lesions in the optic nerve and spinal cord (SC) are two of the most common ways in which MS patients present.\textsuperscript{39,46} Even in patients without a clinical history of optic neuritis or myelopathic symptoms, pathological changes are seen within the optic nerve and SC, making both structures important regions to study in MS.\textsuperscript{47,48}

The retina and SC are also distinct, compact anatomic regions of the CNS where lesions would be expected to cause specific neurological deficits, making them optimal substrates to study structure-function relationships. In recent years, high-definition optical coherence tomography (OCT) has enabled the quantification of both axonal and neuronal layers of the retina. Prior studies have found correlations between specific retinal layers and clinical and radiological disease activity, suggesting that retinal layers reflect both regional and global disease processes in MS.\textsuperscript{49-51} More recently, relationships between specific retinal layers and brain substructure volumes in MS have been reported, suggesting that pathological processes in specific layers of the retina may reflect distinct global pathological processes in MS.\textsuperscript{52}
The importance of assessing the SC using quantitative MRI to better understand microstructural changes mediating clinical disability in MS patients has been demonstrated in a number of prior studies. In addition, these studies have observed semi-independent pathological processes in the brain and SC, further highlighting the importance of including SC-MRI measures to allow a comprehensive assessment of MS-related clinical disability.

Several observations raise the possibility of a specific relationship between the retina and SC in MS patients. There are clinical variants of demyelinating disease that predominately affect the optic nerves and SC, including opticospinal MS, Devic’s Disease, and myelin-oligodendrocyte (MOG)-induced optico-spinal experimental autoimmune encephalitis (EAE) in mice. From a pathophysiologic standpoint, the overexpression of MOG in the SC and optic nerves, and evidence of MOG cross-reactivity with neuronal proteins further raise the possibility of a link between these two anatomic regions of the CNS. To date, relationships between retinal and SC measures in MS have not been assessed, and the degree to which these measures independently relate to clinical disability in MS are not yet known.

The principal aim of this study was to assess relationships between SC-MRI measures, retinal layers, and clinical dysfunction in MS patients. We hypothesized that correlations between SC and retinal measures would exist, and that these measures would independently relate to clinical disability in MS. Understanding relationships between tissue damage in distinct CNS compartments is essential to gain a comprehensive assessment of the pathological processes in MS, and to better understand how these disease processes contribute to mediating both regional and global clinical disability.
Methods

This study was approved by the institutional review board of Johns Hopkins University. All participants provided written informed consent.

Study Participants

Patients with relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS) were recruited between 2007-2009 from the Johns Hopkins MS Clinic by convenience sampling. MS diagnosis was confirmed by the treating neurologist, according to the 2005 McDonald criteria.16

Clinical Measures

Within 30 days of MRI, multiple sclerosis functional composite (MSFC) scores were obtained and expanded disability status scale (EDSS) scores were determined by a Neurostatus-certified examiner. Vibration sensation thresholds (VST) for the right and left great toes were quantified using the Vibratron II (Physitemp, Huron, NJ) and hip-flexion strength (HFS) was measured at the right hip using Microfet2 handheld-dynamometer (Hoggan Industries, Jordan, UT), as described in Chapter 1.

Optical Coherence Tomography

Retinal imaging was performed using spectral-domain Cirrus HD-OCT (model 4000;version 5.0;Carl Zeiss Meditec), described in detail elsewhere.57,58 Briefly, peripapillary and macular data were obtained with the Optic Disc Cube 200x200 protocol and Macular Cube 512x128 protocol, respectively. OCT scanning was performed by experienced technicians, and scans monitored to ensure reliable fixation. Scans with artifact or signal strength less than 7/10 were excluded.
Macular cube scans were analyzed in a masked fashion using segmentation software, described in detail elsewhere.\textsuperscript{59,60} Briefly, segmentation performed in 3-dimension identifies the inner limiting membrane, the outer boundaries of the macular retinal nerve fiber layer (mRFNL), the inner plexiform layer (IPL), outer plexiform layer, and the inner boundary of the retinal pigment epithelium. Following identification of these boundaries, thicknesses of the mRNFL, ganglion-cell layer (GCL)+IPL, INL+outer plexiform layer, and outer nuclear layer (ONL, including inner and outer photoreceptor segments) were calculated in an annulus of an inner radius of 0.54 mm, outer radius of 2.4 mm, centered on the fovea. This segmentation protocol has been shown to be reproducible in MS patients and healthy control subjects (HCs).\textsuperscript{60}

Bilateral retinal measures were obtained in all subjects. The OCT in closest temporal proximity to the SC-MRI was selected for analysis. The median time difference between SC-MRI and OCT was 179 days.

**Cervical SC MRI**

Cervical SC MRIs were performed on all participants using a 3-tesla Achieva scanner (Philips Medical Systems, Best, The Netherlands) using body coil excitation and two-element phased array surface coil reception. Sequences acquired included: MTI, DTI, and short-tau inversion recovery (STIR) (FOV=250mm, acquired resolution:1x1x2mm\(^3\)[AP,FH, RL], TR/TE/TI=4227ms/68ms/200ms), as described in detail in Chapter 1.

Post-acquisition image processing was performed, as described in Chapter 1, to generate maps of FA, MD, \(\lambda_\perp\), \(\lambda_{||}\), and MTR.
An automated reproducible segmentation protocol was applied to the MT\textsubscript{off} images to delineate ROIs encompassing the axial cross-section of the SC across segments C3-C4 and transferred to the MRI-index maps, as described in Chapter 1.\textsuperscript{20} Average values of SC cross-sectional area (CSA), FA, MD, $\lambda_{||}$, $\lambda_{\perp}$, and MTR were calculated from the ROIs of each MRI-index map across all axial slices from C3-C4.

**Brain MRI**

Full details of our brain MRI acquisition have been described previously.\textsuperscript{21,22} For this dataset, DTI-based brain volume segmentation had lower scan-to-scan variability than alternate T1-based methods and was used to calculate supratentorial brain and CSF volumes.\textsuperscript{22,23} Brain parenchymal fraction (BPF) was calculated using the formula: $\text{BPF} = \frac{\text{brain volume}}{\text{brain volume} + \text{CSF volume}}$.

**Statistical Analysis**

Statistical calculations were performed using STATA Version 11 (StataCorp, College Station, TX). Student t-tests were used for group comparisons of SC-MRI. Multivariable linear regression (MLR) using robust standard error estimation (RSEE) to account for within-subject, inter-eye correlations assessed group differences in retinal measures. Due to the exploratory nature of this study, adjustment for multiple comparisons was not performed.\textsuperscript{24} Relationships between retinal and SC-MRI measures were assessed using MLR adjusting for age, sex, BPF, and history of optic neuritis using RSEE. Partial correlations (which do not account of inter-eye correlations) were calculated for illustrative purposes. Relationships between retinal and SC-MRI measures with clinical disability were assessed using MLR with each clinical measure as the dependent variable, and SC-CSA and RNFL included as covariates. These two measures
were chosen as representative SC and retinal measures as they demonstrated the strongest correlations with one another. Age, sex, BPF, and history of optic neuritis were included as covariates in the clinical models, and RSEE was utilized. Statistical significance was defined as \( p<0.05 \).

**Results**

This study included 102 MS patients (66 RRMS, 24 SPMS, 12 PPMS) and 11 HCs. MS patients were 69% female and had a mean age of 43 years, and an average MS disease duration of 9.6 years. 71% of MS patients were on disease-modifying treatments: interferon-\( \beta \), 40%; glatiramer acetate, 35%; natalizumab, 24%; and other medications, 1% (Table 3.1).

On assessment of SC-MRI measures: SC-CSA, SC-FA, and SC-\( \lambda_\perp \) were different between MS vs. HCs \( (p<0.10) \) and between MS subtypes \( (p<0.05) \). SC-MTR was different between MS subtypes \( (p=0.004) \), but not between MS vs. HCs \( (p=0.31) \). For retinal layers: peripapillary-RNFL (pRNFL), GCL+IPL, and mRNFL were different between MS and HCs \( (p<0.05) \), while there was no significant difference in these layers between MS subtypes (Table 3.1).

In MS, there were consistently significant correlations between SC-CSA, SC-FA, SC-\( \lambda_\perp \) and pRNFL \( (p=0.01,p=0.002,p=0.001, \) respectively), mRNFL \( (p=0.007, p=0.03, p=0.04) \), and GCL+IPL \( (p=0.003,p=0.003,p=0.01) \) after adjusting for age, sex, prior optic neuritis, and BPF. There were no significant correlations between SC-MRI measures and INL or ONL, nor were there relationships between SC-MRI (CSA, FA, \( \lambda_\perp \)) and BPF \( (p>0.05 \) for all comparisons). In HCs, there were no correlations between SC measures.
(CSA, FA, λ⊥) and pRNFL, mRNFL, or GCL+IPL. There were observed correlations between SC-MTR and GCL+IPL and mRNFL (p=0.04, p=0.008) in HCs, but these observations are unlikely to be of significance given the small number of HCs. When correlations between SC and retinal measures were assessed based on MS-subtype, they were generally stronger in progressive patients in comparison to relapsing MS patients. (Table 3.2)

In multivariable models of clinical dysfunction, when representative SC, retinal, and brain atrophy measures (SC-CSA, pRNFL, and BPF) were included simultaneously with other covariates (age, sex, prior optic neuritis, BPF): SC-CSA and pRNFL retained independent relationships with low-contrast VA (p=0.04, p=0.002, respectively), high-contrast VA (p=0.06, p=0.008) and VST (p=0.01, p=0.05). SC-CSA alone retained an independent relationship with EDSS (p=0.001) and HFS (p=0.001), while SC-CSA and BPF retained independent relationships with MSFC (Figure 3.1, Table 3.3). Notably, BPF only contributed significantly to explaining clinical variance in MSFC.

When clinical measures were assessed by MS patient subgroup, distinct patterns of relationships between SC and retinal measures with clinical dysfunction emerged. Both SC-CSA and pRNFL independently contributed towards explaining variance in vision and VST in relapsing patients while in progressive MS, both SC-CSA and pRNFL showed trends towards independently contributing to explaining disability in EDSS and HFS, but did not independently contribute to explaining variance in vision and VST (Tables 3.4a and 3.4b).
Discussion

In this study, we found significant correlations between quantitative SC-MRI and specific retinal layers in MS patients, suggesting that pathological changes in two functionally and spatially distinct CNS compartments occur to an extent in parallel, and reflect global pathological processes that are distinct from those captured by brain atrophy measures. Furthermore, when relationships between SC, retinal, and brain atrophy were assessed with various clinical measures: both retinal and SC-MRI measures simultaneously contributed to explaining the variance in functional system-specific clinical disability of relevance to the visual system and SC, suggesting that MS-related changes in the retina and SC are capturing clinically-relevant global pathological processes that are not adequately captured by either measure alone, or by a measure of brain atrophy.

Disease mechanisms in MS are complex and incompletely understood. Although inflammation is likely a significant driving force behind MS-related pathology in all stages of disease, accumulating evidence suggests that immunopathological mechanisms of tissue injury and region-specific pathology may be distinct in different disease stages. In early MS, inflammation is associated with brief episodes of blood-brain-barrier opening, resulting in an influx of peripheral mediators of inflammation. In contrast, inflammation in progressive stages of MS is frequently observed around vessels with intact blood-brain-barriers, and is characterized by fewer lymphocytes, but more activated macrophages and microglia. Furthermore, while oxidative damage in early MS is closely associated with inflammation, in progressive MS, despite dampened inflammation, there is pronounced oxidative stress due to a number of additional
mechanisms of radical generation, including sodium-calcium channel dysregulation.\textsuperscript{65} Considering region-specific pathological differences: prior studies have found limited correlations between measures of SC and brain atrophy, suggesting that pathogenic mechanisms in MS evolve semi-independently in the brain and SC, particularly in progressive phases of disease, supporting the idea that MS disease mechanisms may differentially affect specific regions of the CNS.\textsuperscript{11,28,54} Our observations of strong correlations between SC and retinal measures, but absent correlations between measures of SC and brain atrophy support the notion that MS disease pathology is non-uniform in distinct CNS compartments,\textsuperscript{28,52} and suggest that there are clinically-relevant pathological processes occurring in the SC and retina that are distinct from those in the brain. Moreover, our observations of stronger SC and retinal correlations in progressive patients supports the view that immunopathological and tissue injury mechanisms of MS differs by disease stage, and may be indicative of more uniform pathological changes occurring in later MS stages. On the other hand, it is possible that alternate explanations could account for these observations, including the possibility that measures of atrophy in the brain and SC may not be reflective of equivalent disease processes.

Noteworthy is the observation that both retinal and SC atrophy measures independently contributed to explaining variance in visual acuity and VST, both which are system-specific clinical measures of relevance in the retina and SC, respectively. Our findings expand upon a prior study which demonstrated that retinal layers reflect global CNS processes in MS, and that specific retinal layers may reflect differential pathological processes, including neurodegeneration and inflammation.\textsuperscript{52} Our findings suggest that not only do retinal and SC-MRI measures reflect global pathological processes in MS that are
not adequately captured by brain atrophy alone, but that regional and global pathological processes that SC-MRI reflect are distinct from those that are captured by retinal measures, and that these processes independently contribute to mediating clinical disability in various functional systems. These findings highlight the importance of including an assessment of multiple compartments of the CNS affected by MS to better understand both global and regional disease processes, their interplay, and how they contribute to clinical disability in various functional systems.

Amongst clinical measures, we found that with the inclusion of SC and retinal measures, brain atrophy retained a significant relationship with only MSFC. Brain atrophy is a well-established measure of clinical relevance in MS,\textsuperscript{38} which our findings do not undermine. However, the ability of retinal and SC measures to better reflect pathological changes of relevance to disability is likely due to the compact anatomic organization of both regions, resulting in less clinically “silent” tissue in comparison to the whole brain, allowing enhanced detection of microstructural changes that are of clinical relevance both locally, and on a global level. Future studies assessing the relative contribution of measures of gray matter atrophy in the brain with retinal and SC measures will be of interest.

Depending on the clinical measure, we found differential contributions of SC and retinal measures to explaining clinical variance. Both SC and retinal measures contributed to explaining clinical variance with measures of visual acuity and VST, while SC-CSA alone contributed to explaining clinical variance with EDSS, HFS, and MSFC. This observation may be due, at least in part, to the differential sensitivity of the SC and retinal measures chosen (SC-CSA and pRNFL) to picking up tissue loss relevant to
clinical disability in specific functional systems. For instance, SC-CSA may be a measure that is more adept at picking up tissue loss in the corticospinal tracts, rather than the dorsal columns of the SC, which may partially explain the dominant correlation between measures of lower limb strength and SC-CSA. In addition, differential vulnerabilities of clinically-relevant tissue to the particular clinical measure may also account for this observed difference. Prior studies assessing relationships between axonal density and plaque load have demonstrated good correlations in the corpus callosum and cerebral hemispheres, but absent correlations in the SC, suggesting that there may be differential tissue vulnerability to axonal injury in different CNS compartments. In addition, a comprehensive histopathological assessment of the SC not only found differential tract-specific axonal vulnerabilities in the SC, but even found different vulnerabilities of specific axons within tracts. Since the SC and retinal measures we chose (SC-CSA and pRNFL) in this study specifically assessed for tissue loss, depending on tissue vulnerability to axonal loss of clinically-relevant regions in specific clinical systems, the relative contribution of these individual measures to clinical disability may differ substantially. Another factor contributing to these findings may be that the major functional pathways mediating vision and sensation route through the thalamus, which is known to be highly atrophic even in early MS. Thus, SC and retinal atrophy may be relevant to vision and sensation, as they reflect anterograde and retrograde degeneration from primary thalamic pathology. Taken together, these findings further highlight the heterogeneity and complexity of MS disease pathogenesis, and underscore the importance of sampling different regions of the CNS to better understand MS pathology,
since measures of tissue destruction in specific compartments may be of more or less relevance, depending on the clinical system under consideration.

This study has a number of limitations. First, our number of HCs was limited, and many of our subgroup analyses were performed using a relatively small sample size. Notwithstanding this limitation, we were still able to identify consistent relationships between a variety of retinal and SC measures in MS patients, which lends further credence to our findings. However, the small number of HCs likely resulted in the observation of spurious correlations between SC and retinal measures that are of questionable significance. Second, we did not control for multiple comparisons, as this was an exploratory study; however, all analyses were performed with established a priori hypotheses. Finally, SC-MRIs and OCTs were not performed simultaneously for many participants, since there was a lag between the time point when SC-MRIs were obtained on patients, and when OCTs with segmented retinal layers were available. Notwithstanding this time lag, that we were able to demonstrate consistent correlations suggest that these findings are robust, and worthy of further exploration.

Our findings illustrate that SC and retinal measures reflect on-going global pathological processes of relevance to a spectrum of disability measures in MS patients that measures of brain atrophy do not adequately capture. These findings highlight the importance of combining measures from unique compartments of the CNS to facilitate a more thorough examination of regional and global disease processes that contribute to clinical disability in MS. Further studies in prospective, larger sample sizes are needed to confirm these findings, and to assess how the combination of SC, retinal, and brain measures relate to clinical disability progression. With prospective confirmation, this
approach not only has the potential to be of significant clinical utility, but may substantially enhance our understanding of the evolution of disease processes in MS.
Figure 3.1: Graphical representation of adjusted relationships between retinal Measures, spinal cord MRI measures, and clinical measures (all retinal/clinical relationships adjusted for age, sex, brain parenchymal fraction, SC-CSA, all SC/clinical relationships adjusted for age, sex, brain parenchymal fraction, p-RNFL)
<table>
<thead>
<tr>
<th></th>
<th>All MS (n=102, 204 eyes)</th>
<th>RRMS (n=66, 132 eyes)</th>
<th>Progressive (SPMS and PPMS) (n=36, 72 eyes)</th>
<th>HCs (n=11, 22 eyes)</th>
<th>p-value (MS vs. HC)</th>
<th>p-value (RRMS vs. PMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subjects, n</strong></td>
<td>102</td>
<td>66</td>
<td>36</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age at MRI scan, years (SD)</strong></td>
<td>43.0 (11.5)</td>
<td>37.9 (10.2)</td>
<td>52.3 (7.4)</td>
<td>38.5 (8.9)</td>
<td>0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>% Female</strong></td>
<td>68.6</td>
<td>72.7</td>
<td>61.1</td>
<td>72.7</td>
<td>0.78</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>Disease duration, years (SD)</strong></td>
<td>9.6 (8.6)</td>
<td>6.5 (5.2)</td>
<td>15.0 (10.6)</td>
<td>n/a</td>
<td>n/a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Median baseline EDSS (IQR)</strong></td>
<td>3.0 (2-6)</td>
<td>2.5 (1.5 – 3.5)</td>
<td>6.0 (4.0 – 6.5)</td>
<td>n/a</td>
<td>n/a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>% on disease-modifying treatment</strong></td>
<td>71.3</td>
<td>84.8</td>
<td>47.2</td>
<td>n/a</td>
<td>n/a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Vibration sensation threshold, microns (SD)</strong></td>
<td>14.1 (21.1)</td>
<td>8.0 (13.8)</td>
<td>25.2 (26.9)</td>
<td>n/a</td>
<td>n/a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Hip flexion strength, pounds (SD)</strong></td>
<td>39.3 (18.2)</td>
<td>45.1 (15.7)</td>
<td>28.2 (17.9)</td>
<td>n/a</td>
<td>n/a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>% Eyes with history of optic neuritis</strong></td>
<td>24.4</td>
<td>26.0</td>
<td>21.3</td>
<td>n/a</td>
<td>n/a</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Cord cross-sectional area, mm² (SD)</strong></td>
<td>77.3 (8.6)</td>
<td>79.8 (8.0)</td>
<td>72.7 (7.9)</td>
<td>83.3 (9.5)</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>SC Lesion count, number (SD)</strong></td>
<td>2.1 (1.5)</td>
<td>1.8 (1.5)</td>
<td>2.6 (1.3)</td>
<td>0</td>
<td>&lt;0.001</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Brain parenchymal fraction (SD)</strong></td>
<td>0.87 (0.05)</td>
<td>0.89 (0.05)</td>
<td>0.84 (0.04)</td>
<td>0.90 (0.05)</td>
<td>0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>FA, (SD)</strong></td>
<td>0.61 (0.06)</td>
<td>0.62 (0.06)</td>
<td>0.60 (0.06)</td>
<td>0.64 (0.05)</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>MD [µm²/ms], (SD)</strong></td>
<td>1.28 (0.16)</td>
<td>1.25 (0.15)</td>
<td>1.33 (0.17)</td>
<td>1.20 (0.11)</td>
<td>0.13</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>λ⊥ [µm²/ms], (SD)</strong></td>
<td>0.76 (0.16)</td>
<td>0.74 (0.15)</td>
<td>0.81 (0.17)</td>
<td>0.67 (0.10)</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>(\lambda_{</td>
<td></td>
<td>}[\mu\text{m}^2/\text{ms}],)</td>
<td>(\text{SD}))</td>
<td>(\lambda_{</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>---------------------------------</td>
<td>----------------</td>
<td>---------------------------------</td>
<td>----------------</td>
<td>---------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td><strong>MTR, (SD)</strong></td>
<td>0.30 (0.05)</td>
<td>0.31 (0.04)</td>
<td>0.28 (0.05)</td>
<td>0.31 (0.02)</td>
<td>0.31 (0.05)</td>
<td>0.31 (0.05)</td>
</tr>
<tr>
<td><strong>pRNFL[\mu\text{m}], (SD)</strong></td>
<td>82.2 (12.2)</td>
<td>83.2 (12.6)</td>
<td>80.5 (11.4)</td>
<td>92.9 (10.3)</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>GCL + IPL[\mu\text{m}], (SD)</strong></td>
<td>69.8 (10.1)</td>
<td>70.6 (10.0)</td>
<td>68.4 (10.1)</td>
<td>83.0 (4.7)</td>
<td>&lt;0.001</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>mRNFL[\mu\text{m}], (SD)</strong></td>
<td>28.4 (5.4)</td>
<td>28.6 (5.6)</td>
<td>28.0 (5.0)</td>
<td>34.4 (3.4)</td>
<td>&lt;0.001</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>INL + OPL [\mu\text{m}], (SD)</strong></td>
<td>65.2 (4.9)</td>
<td>65.6 (4.8)</td>
<td>64.4 (5.1)</td>
<td>65.4 (5.0)</td>
<td>0.88</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>ONL [\mu\text{m}], (SD)</strong></td>
<td>119.8 (8.2)</td>
<td>119.1 (7.0)</td>
<td>121.0 (9.8)</td>
<td>120.9 (6.0)</td>
<td>0.56</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*OCT comparisons adjusted for between-eye correlation
Table 3.2: Relationships between OCT Measures and SC MRI Measures in MS (p-values displayed and partial correlation coefficients displayed)

<table>
<thead>
<tr>
<th></th>
<th>SC-CSA</th>
<th>SC-FA</th>
<th>SC- $\lambda_{\perp}$</th>
<th>SC-MTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Patients (n=102): p-values (partial correlation coefficients)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRNFL</td>
<td>0.01 (0.23)</td>
<td>0.002 (0.28)</td>
<td>0.001 (-0.25)</td>
<td>0.34 (0.08)</td>
</tr>
<tr>
<td>GCL +IPL</td>
<td><strong>0.005</strong> (0.24)</td>
<td><strong>0.002</strong> (0.29)</td>
<td><strong>0.02</strong> (-0.23)</td>
<td><strong>0.06</strong> (0.17)</td>
</tr>
<tr>
<td>mRNFL</td>
<td>0.007 (0.22)</td>
<td>0.03 (0.22)</td>
<td>0.04 (-0.20)</td>
<td>0.24 (0.11)</td>
</tr>
<tr>
<td>INL</td>
<td>0.55 (0.06)</td>
<td>0.85 (0.02)</td>
<td>0.94 (-0.007)</td>
<td>0.45 (0.06)</td>
</tr>
<tr>
<td>ONL</td>
<td>0.80 (-0.03)</td>
<td>0.19 (0.14)</td>
<td><strong>0.08</strong> (-0.16)</td>
<td>0.34 (0.10)</td>
</tr>
<tr>
<td>Relapsing MS (n=66): p-values (partial correlation coefficients)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRNFL</td>
<td>0.43 (0.09)</td>
<td>0.03 (0.25)</td>
<td>0.007 (-0.28)</td>
<td>0.43 (-0.08)</td>
</tr>
<tr>
<td>GCL +IPL</td>
<td>0.10 (0.19)</td>
<td>0.03 (0.28)</td>
<td>0.03 (-0.28)</td>
<td>0.67 (0.05)</td>
</tr>
<tr>
<td>mRNFL</td>
<td><strong>0.03</strong> (0.23)</td>
<td><strong>0.09</strong> (0.21)</td>
<td><strong>0.07</strong> (-0.23)</td>
<td>0.97 (0.005)</td>
</tr>
<tr>
<td>INL</td>
<td>0.65 (0.06)</td>
<td>0.51 (-0.07)</td>
<td>0.67 (0.05)</td>
<td>0.26 (0.12)</td>
</tr>
<tr>
<td>ONL</td>
<td>0.90 (-0.02)</td>
<td>0.06 (0.20)</td>
<td><strong>0.06</strong> (-0.23)</td>
<td>0.19 (0.19)</td>
</tr>
<tr>
<td>Progressive MS (n=36): p-values (partial correlation coefficients)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRNFL</td>
<td><strong>0.002</strong> (0.44)</td>
<td><strong>0.005</strong> (0.39)</td>
<td><strong>0.02</strong> (-0.31)</td>
<td>0.15 (0.21)</td>
</tr>
<tr>
<td>GCL +IPL</td>
<td><strong>0.03</strong> (0.29)</td>
<td><strong>0.003</strong> (0.40)</td>
<td><strong>0.04</strong> (-0.28)</td>
<td><strong>0.02</strong> (0.31)</td>
</tr>
<tr>
<td>mRNFL</td>
<td>0.21 (0.14)</td>
<td>0.09 (0.26)</td>
<td>0.12 (-0.24)</td>
<td>0.39 (0.14)</td>
</tr>
<tr>
<td>INL</td>
<td>0.84 (0.03)</td>
<td>0.15 (0.20)</td>
<td>0.41 (-0.12)</td>
<td>0.78 (0.03)</td>
</tr>
<tr>
<td>ONL</td>
<td>0.91 (-0.01)</td>
<td>0.98 (-0.004)</td>
<td>0.98 (-0.003)</td>
<td>0.45 (-0.08)</td>
</tr>
<tr>
<td>HCs (n=11): p-values (partial correlation coefficients)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRNFL</td>
<td>0.45 (0.26)</td>
<td>0.19 (0.41)</td>
<td>0.07 (-0.58)</td>
<td>0.5 (0.21)</td>
</tr>
<tr>
<td>GCL +IPL</td>
<td>0.09 (0.43)</td>
<td>0.81 (-0.10)</td>
<td>0.72 (-0.14)</td>
<td><strong>0.04</strong> (0.42)</td>
</tr>
<tr>
<td>mRNFL</td>
<td>0.13 (-0.34)</td>
<td>0.91 (0.02)</td>
<td>0.67 (-0.06)</td>
<td><strong>0.008</strong> (0.48)</td>
</tr>
<tr>
<td>INL</td>
<td>0.68 (-0.16)</td>
<td>0.18 (-0.43)</td>
<td>0.13 (0.53)</td>
<td>0.06 (0.49)</td>
</tr>
<tr>
<td>ONL</td>
<td>0.87 (-0.06)</td>
<td>0.57 (0.16)</td>
<td>0.35 (-0.32)</td>
<td>0.13 (0.44)</td>
</tr>
</tbody>
</table>

*p-values are derived from linear regression using robust standard error estimations to account for intereye correlations, adjusting for age, sex, brain parenchymal fraction, and history of optic neuritis.

** partial correlation coefficients are presented for illustrative purposes only and represent the magnitude of the correlation between retinal and spinal cord measures adjusted for age, sex, brain parenchymal fraction, and history of optic neuritis, but not accounting for within-subject inter-eye correlations.

***p-values < 0.10 bolded
Table 3.3: Beta-coefficients, p-values, and $r^2$ values of multivariable models of clinical dysfunction in all MS patients, n=102

<table>
<thead>
<tr>
<th>Clinical Models</th>
<th>EDSS ($r^2 = 0.40$)</th>
<th>VA ($r^2 = 0.18$)</th>
<th>VA 1.25 ($r^2 = 0.18$)</th>
<th>Vibratron ($r^2 = 0.24$)</th>
<th>Dynamometry ($r^2 = 0.36$)</th>
<th>MSFC ($r^2 = 0.31$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>p-value</td>
<td>$\beta$</td>
<td>p-value</td>
<td>$\beta$</td>
<td>p-value</td>
</tr>
<tr>
<td>SC-CSA</td>
<td>-0.075</td>
<td><strong>0.001</strong></td>
<td>0.21</td>
<td><strong>0.06</strong></td>
<td>-0.65</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td>RNFL</td>
<td>-0.13</td>
<td>0.35</td>
<td><strong>0.008</strong></td>
<td>0.26</td>
<td><strong>0.002</strong></td>
<td>0.05</td>
</tr>
<tr>
<td>Age</td>
<td>0.074</td>
<td><strong>&lt;0.001</strong></td>
<td>0.12</td>
<td>0.23</td>
<td>0.0016</td>
<td>0.98</td>
</tr>
<tr>
<td>Sex</td>
<td>0.59</td>
<td>0.09</td>
<td>1.67</td>
<td>0.40</td>
<td>-1.16</td>
<td>0.54</td>
</tr>
<tr>
<td>BPF</td>
<td>-2.70</td>
<td>0.49</td>
<td>41.5</td>
<td><strong>0.09</strong></td>
<td>-14.8</td>
<td>0.44</td>
</tr>
<tr>
<td>Hx ON</td>
<td>-0.46</td>
<td>0.20</td>
<td>-2.81</td>
<td>0.33</td>
<td>-2.75</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*r^2* values represent percent of total clinical variance explained by all covariates in model; coefficients are beta coefficients of each covariate in multivariable clinical model.
Table 3.4a: Beta-coefficients, p-values, and \( r^2 \) values of multivariable models of clinical dysfunction in relapsing MS Patients, n=66

<table>
<thead>
<tr>
<th>Clinical Models</th>
<th>EDSS (( r^2 = 0.12 ))</th>
<th>VA (( r^2 = 0.22 ))</th>
<th>VA 1.25 (( r^2 = 0.16 ))</th>
<th>Vibratron (( r^2 = 0.20 ))</th>
<th>Dynamometry (( r^2 = 0.29 ))</th>
<th>MSFC (( r^2 = 0.24 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta )</td>
<td>p-value</td>
<td>( \beta )</td>
<td>p-value</td>
<td>( \beta )</td>
<td>p-value</td>
</tr>
<tr>
<td>SC-CSA</td>
<td>-0.042</td>
<td>0.16</td>
<td>0.18</td>
<td>0.11</td>
<td>-0.42</td>
<td>0.05</td>
</tr>
<tr>
<td>RNFL</td>
<td>-0.0010</td>
<td>0.96</td>
<td>0.23</td>
<td>0.01</td>
<td>0.25</td>
<td>0.02</td>
</tr>
<tr>
<td>Age</td>
<td>0.029</td>
<td>0.16</td>
<td>0.23</td>
<td>\textbf{0.05}</td>
<td>0.13</td>
<td>0.22</td>
</tr>
<tr>
<td>Sex</td>
<td>0.048</td>
<td>0.30</td>
<td>2.97</td>
<td>0.24</td>
<td>-0.63</td>
<td>0.82</td>
</tr>
<tr>
<td>BPF</td>
<td>-1.67</td>
<td>0.71</td>
<td>36.0</td>
<td>0.33</td>
<td>-32.7</td>
<td>0.17</td>
</tr>
<tr>
<td>Hx ON</td>
<td>-0.38</td>
<td>0.36</td>
<td>-3.11</td>
<td>0.45</td>
<td>-2.44</td>
<td>0.30</td>
</tr>
</tbody>
</table>

\( *r^2 \) values represent percent of total clinical variance explained by all covariates in model; coefficients are beta coefficients of each covariate in multivariable clinical model
Table 3.4b: Beta-coefficients, p-values, and $r^2$ values of multivariable models of clinical dysfunction in progressive MS Patients, n=36

<table>
<thead>
<tr>
<th>Clinical Models</th>
<th>EDSS ($r^2 = 0.28$)</th>
<th>VA ($r^2 = 0.11$)</th>
<th>VA 1.25 ($r^2 = 0.33$)</th>
<th>Vibratron ($r^2 = 0.20$)</th>
<th>Dynamometry ($r^2 = 0.37$)</th>
<th>MSFC ($r^2 = 0.26$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β p-value</td>
<td>β p-value</td>
<td>β p-value</td>
<td>β p-value</td>
<td>β p-value</td>
<td>β p-value</td>
<td>β p-value</td>
</tr>
<tr>
<td>SC-CSA</td>
<td>-0.066 0.05</td>
<td>0.13 0.61</td>
<td>0.083 0.63</td>
<td>-0.65 0.28</td>
<td>0.64 0.07</td>
<td>0.0075 0.36</td>
</tr>
<tr>
<td>RNFL</td>
<td>-0.04 0.13</td>
<td>0.16 0.19</td>
<td>0.37 &lt;0.001</td>
<td>-0.81 0.17</td>
<td>0.42 0.06</td>
<td>0.0044 0.54</td>
</tr>
<tr>
<td>Age</td>
<td>0.051 0.06</td>
<td>0.18 0.62</td>
<td>-0.06 0.59</td>
<td>0.072 0.88</td>
<td>-0.59 0.09</td>
<td>-0.0083 0.38</td>
</tr>
<tr>
<td>Sex</td>
<td>0.39 0.49</td>
<td>0.74 0.82</td>
<td>-1.89 0.41</td>
<td>-0.45 0.95</td>
<td>7.52 0.15</td>
<td>-0.051 0.71</td>
</tr>
<tr>
<td>BPF</td>
<td>2.00 0.76</td>
<td>27.4 0.38</td>
<td>3.72 0.85</td>
<td>-24.5 0.77</td>
<td>-53.7 0.27</td>
<td>4.85 0.004</td>
</tr>
<tr>
<td>Hx ON</td>
<td>-0.50 0.30</td>
<td>-2.31 0.36</td>
<td>-3.12 0.20</td>
<td>-7.83 0.53</td>
<td>-1.11 0.83</td>
<td>0.058 0.75</td>
</tr>
</tbody>
</table>

*β=beta coefficients from regression models; $r^2$ values represent percent of total clinical variance explained by all covariates in model; coefficients are beta coefficients of each covariate in multivariable clinical model.
Chapter 4

Spinal Cord Normalization in Multiple Sclerosis

Background and Purpose

Spinal cord (SC) pathology is common in multiple sclerosis (MS), and the importance of utilizing SC-based MRI measures in clinical investigation is increasingly recognized.\(^{39,54}\) An important unresolved issue is the optimal normalization factor for SC atrophy measures in cross-sectional studies. Normalization reduces the biological variation of structural measurements unrelated to disease effects; in the brain, this is typically accomplished by measuring intracranial volume (ICV).\(^{70,71}\) Elimination of variation unrelated to MS maximizes the statistical power to detect group differences, enabling more effective assessment of differences between MS cases and healthy control subjects (HC).

Prior studies have assessed a variety of normalization factors for SC volume (SCV), including ICV,\(^ {72-74}\) thecal-sac volume,\(^ {75}\) and SC length (yielding average cross-sectional area).\(^ {76}\) These studies in relatively small cohorts have resulted in differing conclusions regarding appropriate normalization factors. One compared raw cervical SCV to normalization by thecal-sac and ICV, concluding that raw SCV showed the largest group differences and the strongest correlations with EDSS.\(^ {77}\) Another study found that normalization provided only limited improvement over raw SCV, but among the factors assessed (which included ICV, SC length, body surface area, and body-mass index), normalization by SC length best accentuated group differences and improved clinical-radiological correlations.\(^ {76}\)
The aim of our study was to assess the effect of cervical SC normalization by three different factors (subject height, ICV, and SC length) on the detection of group differences and clarification of clinical-radiological correlations in MS. To expand on existing work, we assessed a relatively large MS cohort (n=133) with adequate representation from both progressive and relapsing MS, enabling a thorough evaluation of normalization effects across the MS spectrum. Furthermore, to better assess clinical-radiological correlations, we utilized a variety of global and system-specific clinical measures, relevant to SC function, including EDSS, vibration sensation threshold, motor strength, and MSFC. We hypothesized that normalization by appropriate factors would result in improved detection of group differences between MS and HC, and among MS subtypes, and would also clarify clinical-radiological correlations.

Methods

Study Participants

This study was approved by the institutional review board; all participants provided informed consent.

The study sample consisted of individuals with clinically-isolated syndrome, relapsing-remitting MS, secondary-progressive MS, primary-progressive MS, and HC (Table 4.1). To perform comparative analyses between high and low inflammatory MS, patients with clinically-isolated syndrome and relapsing-remitting MS were together categorized as “relapsing” and those with secondary-progressive and primary-progressive MS as “progressive.” MS cases were recruited from the MS clinic by convenience sampling. Diagnosis was confirmed by the treating neurologist, according to 2005
EDSS was determined by a Neurostatus-certified examiner within 30 days of MRI. Hip strength and vibration sensation thresholds were measured within 2 weeks of MRI. Medical records were reviewed to determine disease duration and treatment status. MS cases scanned within 3 months after a clinical relapse were excluded. HC were recruited from the community. Subject heights were self-reported.

**Magnetic Resonance Imaging**

Cervical SC MRI was performed on all participants using a 3T Philips Achieva scanner with body-coil excitation and two-element surface-coil reception. Axial images were acquired using a 3D gradient-echo sequence with multishot echo-planar readout (3 lines-per-shot) with parallel imaging factor of 2 (TR/TE/flip angle=121ms/12.5ms/9°). The scan yielded 30 contiguous 3mm slices between C2 and C6, with nominal in-plane resolution 0.6x0.6mm. An automated, reproducible segmentation protocol delineated SC cross-sectional area between C3 and C4, as described in Chapter 1.²⁰ All regions-of-interest generated using automated segmentation were manually inspected, and corrected if necessary. SC length was measured between segments C3 and C4 (number of slices spanning C3-C4 multiplied by slice thickness).

DTI images of the brain were also acquired, as described in Chapter 1, and used to calculate supratentorial brain and cerebrospinal fluid volumes, as described previously.²² ICV was calculated as the sum of brain and cerebrospinal fluid volume.

**Quantitative Clinical Measures**

Vibration sensation threshold of the right great toe was quantified using Vibratron II (Physitemp, Huron, NJ), and hip flexion strength at the right hip was quantified using a Microfet2 handheld dynamometer, as described in Chapter 1.
**Statistical Analysis**

Statistical calculations were performed using STATA Version 11 (StataCorp, College Station, TX). Multivariable linear regression was used to compare group outcomes adjusted for age. Spearman’s rank method assessed correlations between outcomes and clinical measures. Statistical significance was set at p<0.05, and due to the exploratory nature of this study, there was no adjustment for multiple comparisons. Proportional normalization was performed by dividing raw SCV by the normalization factor of interest. Residual normalization was performed by including the normalization factor of interest as a covariate in the multivariable regression models. Normalization factors were selected based on existing literature and the identification of moderate correlations between each factor and SCV. Likelihood ratio tests of nested models assessed the value of multiple normalization factors in detecting group differences and improving clinical correlations.

**Results**

This study included 133 MS cases (4 clinically-isolated syndrome, 74 relapsing-remitting, 36 secondary-progressive, 19 primary-progressive; 78 “relapsing”, 55 “progressive”) and 11 HC. MS cases were predominantly women (65%) and had a mean age of 44 years. Average disease duration was 10 years, and 67% of patients were on disease-modifying therapies (interferon-β: 40%, glatiramer acetate: 30%, natalizumab: 25%, other medications: 5%). Relapsing cases were younger, had shorter disease durations, and were less disabled than progressive cases (Table 4.1).
Raw SCVs, without normalization, demonstrated moderate correlations with all normalization factors \( (r=0.39 \text{ with subject height}, r=0.55 \text{ with SC length}, r=0.40 \text{ with ICV}) \). In age-adjusted comparisons of MS vs. HC, raw SCV and normalizations by height and SC length, but not by ICV, were significantly different between MS and HC. SCV normalized by height and SC length, but not raw SCV or normalization by ICV, showed differences between relapsing and progressive MS (Tables 4.2). In age-adjusted group comparisons of primary-progressive MS vs. HC and primary-progressive vs. secondary-progressive MS, normalization by SC length or height showed differences \( (p<0.05) \), but raw SCV did not \( (p>0.10) \).

Age-adjusted group comparisons were performed utilizing both proportional and residual normalization, yielding similar results. To facilitate interpretation, the proportional method was used for clinical-radiological correlation analyses (Tables 4.3a, 4.3b, 4.3c and Figures 4.1a and 4.1b). Overall, normalization by SC length consistently increased the strength of the correlations (Figures 4.1a and 4.1b, Table 4.3a). Normalization by height generally increased correlation strength, whereas normalization by ICV was erratic, increasing correlations with EDSS and vibration, but decreasing correlations with MSFC and strength. There were no correlations with raw SCV in relapsing MS, but normalization by SC length suggested correlations with EDSS, strength, and vibration (Table 4.3b). In progressive MS, raw SCV and normalization by SC length demonstrated robust correlation strengths, whereas normalization by height and ICV diminished these correlations (Table 4.3c). Residual normalization by length also yielded increased correlations, but normalization by ICV diminished correlations with MSFC and strength.
Finally, additional normalization to ICV after normalization by length slightly accentuated differences between MS and HC (p<0.01 for likelihood-ratio test) and between relapsing and progressive MS (p<0.01). On the other hand, in the assessment of clinical-radiological relationships, additional normalization by ICV contributed only to the vibration model (p<0.01) but not to the other clinical measures, including EDSS, MSFC, and strength (p>0.10).

**Discussion**

We assessed the effect of cervical SCV normalization by a number of factors on the ability to detect group differences, and to strengthen clinical-radiological correlations using a spectrum of clinical measures, in MS. We found that normalization by SC length – essentially, resulting in a measurement of mean cross-sectional area – was consistently the best strategy to accentuate group differences between MS and HC, and among MS subtypes, and to strengthen clinical-radiological correlations. Normalization appears to be particularly relevant in settings where there is subtle disease-related SC atrophy.

Our findings are in keeping with a recent study that found normalization by length to most robustly accentuate group differences and strengthen clinical-radiological correlations.\textsuperscript{76} As in this prior study, which also assessed normalization by body-mass index and body surface area, we included a variable related to subject size (specifically, height) as a normalization factor, as the SC is generally longer in taller people. Although height performed relatively well as a normalization factor in our analyses, SC length more consistently accentuated group differences and clinical-radiological correlations. However, in settings where it is difficult or time-consuming to obtain SC length, subject
height would nonetheless be a useful normalization factor. Interestingly, in contrast to this prior study that found normalization by length resulted in only a small improvement over raw SCV, we found that normalization by length substantially improved our ability to detect group differences and magnify clinical correlations. This difference may result from the nearly fourfold larger sample size in the current study (133 vs. 34 MS cases). As our study patients did not routinely undergo lumbar MRI, we were unable to normalize by the lumbar-enlargement area, which performed relatively well in another study.\textsuperscript{75} Of note, any comparisons between our results and those from prior studies must be made taking into account methodological differences.

As seen in prior studies, normalization by ICV was generally of limited utility.\textsuperscript{76,77} This observation underscores the importance of utilizing relevant and appropriate factors, since normalization by irrelevant factors such as ICV can paradoxically hinder the ability to detect group differences and clinical-radiological correlations. Interestingly, normalization by ICV consistently increased clinical correlations with vibration, in contrast to all other clinical measures. Furthermore, additional normalization by ICV, after normalization by length, contributed to explaining variance in vibration, but not any of the other clinical measures. These findings suggest that vibration may be uniquely correlated with ICV, such that individuals with larger heads have higher vibration sensation thresholds, perhaps due to the increased length of sensory fibers in the dorsal columns.

Our relatively large sample size allowed more detailed assessment of normalization effects by MS subtype and showed that normalization by SC length and subject height accentuated group differences. Normalization by SC length most increased
the magnitude of clinical-radiological correlations in relapsing MS, where the correlation was not apparent with raw SCV but only emerged with normalization by SC length (EDSS, strength, vibration). In progressive MS, raw SCV and SCV normalized by SC length demonstrated robust correlations, whereas normalization by height and ICV actually diminished correlations. This observation expands on a prior study in relapsing MS patients that found a relationship between SCV and EDSS only after normalization by lumbar-enlargement area.\textsuperscript{75} Taken together, these findings suggest that normalization is particularly relevant for identifying subtle differences (which is the case when comparing MS subtypes) and clinical correlations in cases where there is less intrinsic SC damage (especially relapsing MS). This is also a highly pertinent issue in longitudinal studies where subtle changes in SC measurements and their relation to disability progression are important to identify.

In this study, we comprehensively assessed clinical-radiological correlations using a variety of clinical measures, including two quantitative measures that are highly relevant in the SC (motor strength and vibration sensation threshold) as well as two measures of global disability (EDSS and MSFC). Although EDSS is a global measure, it is heavily weighted towards ambulatory function,\textsuperscript{41} while MSFC is a more multidimensional measure of disability, since it includes measures of lower-limb function (25-foot timed walk), upper-limb function (9-hole peg test), and cognition (paced auditory serial addition test).\textsuperscript{78} As expected, with both raw and normalized SCV, we consistently observed stronger correlations with strength, vibration, and EDSS in comparison to MSFC, likely a reflection of the specificity of structure-function relationships in the SC. Regardless of the clinical measure, we found that normalization
by SC length consistently accentuated the observed correlations, whereas ICV generally
diminished correlations. These observations further support the notion that only relevant
normalization factors should be applied to identify clinical-radiological correlations.
Prior studies compared the residual and proportional methods of normalization in the
brain, with each method harboring different relative strengths and weaknesses. In order
to ensure the stability of our observations, we compared normalization using both
methods, finding no substantial differences. Therefore, for ease of interpretation, we
preferentially utilized the proportional method to assess clinical-radiological correlations.
In keeping with a prior study, we found that normalization by multiple factors
simultaneously (SC length and ICV) diminished the ability to detect clinical correlations.
Although there was suggestion of an improvement in the detection of group differences
with normalization by both length and ICV, after taking into account the lack of an
improvement in detecting clinical correlations, we believe that including multiple
normalization factors simultaneously is generally not useful.

There are a number of limitations to this study. First, since there was no “gold
standard” against which we could compare normalized volumes, we utilized the
combination of accentuation of group differences and strengthening of clinical-
radiological correlations to indicate an appropriate normalization factor. Taking into
account pathological and imaging literature demonstrating group differences and clinical
correlations with SCV in MS, this approach seems reasonable. Second, we
measured the volume of a limited segment of the cervical SC (C3-C4), as this was the
segment with the best image quality. We therefore did not perform the same analyses
utilizing the entire cervical SC; however, as demonstrated in a previous study, it is
unlikely that including a larger portion of the cervical SC would have changed our
general conclusions. It is worth noting that the portion of the SC we analyzed is typically
accessible for volumetric measurement using head coils, and could thus be easily
combined with routine imaging of the brain. Finally, subject height was self-reported,
making information bias a potential issue. However, self-reported height is generally
reported with a systematic tendency towards overestimation. If most study participants
overestimated their height to a similar extent, such a bias would not change the
magnitude of the group differences or correlations observed.\textsuperscript{81,82}

In conclusion, using a number of normalization factors and clinical measures,
normalization of SCV by SC length – essentially, measuring the average cross-sectional
area – consistently improves the ability to detect group differences and clinical-
radiological correlations in MS. This is particularly important in settings where subtle
differences in SC atrophy need to be detected. If substantiated in larger, prospective
studies, these findings have important implications for the use of SC measurements in
both clinical practice and trial settings.
Table 4.1: Clinical characteristics and MRI measures in patient subgroups

<table>
<thead>
<tr>
<th></th>
<th>All MS</th>
<th>Relapsing MS</th>
<th>Progressive MS</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>133</td>
<td>78</td>
<td>55</td>
<td>11</td>
</tr>
<tr>
<td>Age at MRI scan, [years] (SD)</td>
<td>44 (12)</td>
<td>39 (11)**</td>
<td>52 (8)</td>
<td>40 (9)</td>
</tr>
<tr>
<td>% Female</td>
<td>65</td>
<td>69</td>
<td>58</td>
<td>71</td>
</tr>
<tr>
<td>Disease duration, [years] (SD)</td>
<td>10 (9)</td>
<td>7 (6)**</td>
<td>16 (11)</td>
<td>n/a</td>
</tr>
<tr>
<td>Median baseline EDSS (IQR)</td>
<td>3.5 (2 - 6)</td>
<td>2.5 (1.5 – 3.5)**</td>
<td>6.0 (4.0 – 6.5)</td>
<td>n/a</td>
</tr>
<tr>
<td>% on disease-modifying treatment</td>
<td>67</td>
<td>83**</td>
<td>44</td>
<td>n/a</td>
</tr>
<tr>
<td>MSFC, Z-score</td>
<td>0.022 (0.68)</td>
<td>0.27 (0.51)**</td>
<td>-0.33 (0.75)</td>
<td>n/a</td>
</tr>
<tr>
<td>Vibration sensation threshold, [microns] (SD)</td>
<td>14.6 (22.1)</td>
<td>7.7 (13.2)**</td>
<td>24.8 (28.1)</td>
<td>n/a</td>
</tr>
<tr>
<td>Hip flexion strength, [pounds] (SD)</td>
<td>39.8 (18.6)</td>
<td>46.7 (15.5)**</td>
<td>29.3 (18.0)</td>
<td>n/a</td>
</tr>
<tr>
<td>Height [m]</td>
<td>1.71 (0.09)</td>
<td>1.70 (0.09)</td>
<td>1.72 (0.09)</td>
<td>1.67 (0.09)</td>
</tr>
<tr>
<td>Intracranial volume [mL]</td>
<td>887 (87)</td>
<td>880 (86)</td>
<td>896 (88)</td>
<td>903 (94)</td>
</tr>
<tr>
<td>Spinal cord length [mm] (SD)</td>
<td>33.5 (3.1)</td>
<td>33.0 (3.2)</td>
<td>34.1 (3.0)</td>
<td>34.3 (2.6)</td>
</tr>
</tbody>
</table>

*p < 0.05 in comparison vs. HC
**p < 0.05 vs. progressive MS
Table 4.2: Age-adjusted group comparisons of spinal cord volume with normalization by the proportional method

<table>
<thead>
<tr>
<th>Spinal cord volume (C3-C4)</th>
<th>MS Mean (SD)</th>
<th>HC Mean (SD)</th>
<th>Mean difference</th>
<th>p-value</th>
<th>Relapsing Mean (SD)</th>
<th>Progressive Mean (SD)</th>
<th>Mean difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw [mm$^3$], (SD)</td>
<td>2576.94 (388.17)</td>
<td>2838.46 (288.76)</td>
<td>-261.51</td>
<td>0.03</td>
<td>2632.95 (392.22)</td>
<td>2497.52 (371.50)</td>
<td>-135.43</td>
<td>0.32</td>
</tr>
<tr>
<td>Normalized to subject height [mm$^2$], (SD)</td>
<td>1.52 (0.20)</td>
<td>1.68 (0.15)</td>
<td>-0.16</td>
<td>0.02</td>
<td>1.56 (0.18)</td>
<td>1.46 (0.21)</td>
<td>-0.097</td>
<td>0.04</td>
</tr>
<tr>
<td>Normalized to spinal cord length [mm$^2$], (SD)</td>
<td>77.0 (9.2)</td>
<td>83.1 (9.2)</td>
<td>-6.1</td>
<td>0.04</td>
<td>79.6 (8.5)</td>
<td>73.3 (8.9)</td>
<td>-6.4</td>
<td>0.008</td>
</tr>
<tr>
<td>Normalized to intracranial volume* (x10$^3$), (SD)</td>
<td>2.92 (0.43)</td>
<td>3.16 (0.28)</td>
<td>-0.24</td>
<td>0.08</td>
<td>3.00 (0.40)</td>
<td>2.80 (0.44)</td>
<td>-0.20</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*unitless measure
Table 4.3a: Spearman’s correlation coefficients using proportional method (all MS cases, n=133)

<table>
<thead>
<tr>
<th></th>
<th>Spearman’s rank correlation coefficient (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EDSS</td>
</tr>
<tr>
<td>Raw spinal cord volume</td>
<td>-0.20 (0.02)</td>
</tr>
<tr>
<td>Normalized to height</td>
<td>-0.26 (0.006)</td>
</tr>
<tr>
<td>Normalized to spinal cord length</td>
<td>-0.43 (&lt;0.001)</td>
</tr>
<tr>
<td>Normalized to intracranial volume</td>
<td>-0.23 (0.01)</td>
</tr>
</tbody>
</table>

*EDSS = expanded disability status scale, MSFC = multiple sclerosis functional composite.
Table 4.3b: Correlations between clinical measures and spinal cord volume with normalization by the proportional method (relapsing cases, n=78)

<table>
<thead>
<tr>
<th></th>
<th>EDSS</th>
<th>MSFC</th>
<th>Hip flexion strength</th>
<th>Vibration sensation threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw spinal cord volume</td>
<td>-0.0082</td>
<td>0.0065</td>
<td>0.28 (0.02)</td>
<td>-0.082 (0.48)</td>
</tr>
<tr>
<td>Normalized to height</td>
<td>-0.087</td>
<td>0.089</td>
<td>0.15 (0.23)</td>
<td>-0.09 (0.45)</td>
</tr>
<tr>
<td>Normalized to spinal cord length</td>
<td>-0.19</td>
<td>0.13</td>
<td>0.25 (0.04)</td>
<td>-0.25 (0.03)</td>
</tr>
<tr>
<td>Normalized to intracranial volume</td>
<td>-0.039</td>
<td>-0.092</td>
<td>0.077 (0.52)</td>
<td>-0.21 (0.07)</td>
</tr>
</tbody>
</table>

*EDSS = expanded disability status scale, MSFC = multiple sclerosis functional composite.
Table 4.3c: Correlations between clinical measures and spinal cord volume with normalization by the proportional method (progressive cases, n=55)

<table>
<thead>
<tr>
<th></th>
<th>EDSS</th>
<th>MSFC</th>
<th>Hip flexion strength</th>
<th>Vibration sensation threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw spinal cord volume</td>
<td>-0.38 (0.005)</td>
<td>0.22 (0.10)</td>
<td>0.45 (0.001)</td>
<td>-0.27 (0.05)</td>
</tr>
<tr>
<td>Normalized to height</td>
<td>-0.22 (0.15)</td>
<td>0.31 (0.04)</td>
<td>0.21 (0.18)</td>
<td>-0.37 (0.01)</td>
</tr>
<tr>
<td>Normalized to spinal cord length</td>
<td>-0.45 (0.0007)</td>
<td>0.29 (0.03)</td>
<td>0.41 (0.004)</td>
<td>-0.30 (0.03)</td>
</tr>
<tr>
<td>Normalized to intracranial volume</td>
<td>-0.15 (0.29)</td>
<td>-0.0099 (0.94)</td>
<td>0.28 (0.06)</td>
<td>-0.40 (0.004)</td>
</tr>
</tbody>
</table>

*EDSS = expanded disability status scale, MSFC = multiple sclerosis functional composite.
Figure 4.1a: Scatter plots of clinical-MRI correlations (raw C3-C4 spinal cord volume)
Figure 4.1b: Scatter plots of clinical-MRI correlations (C3-C4 spinal cord volume normalized to spinal cord length by the proportional method (to yield mean cross-sectional area)
Chapter 5

Longitudinal Changes in Quantitative Spinal Cord MRI Measures

Introduction

In previous chapters, we demonstrated consistent cross-sectional relationships between DTI and MTI indices and quantitative measures of sensorimotor dysfunction and global measures of disability in a cohort of MS patients. We found that quantitative SC MRI measures in MS patients were strongly correlated with disability and had the ability to provide clinically relevant information on microstructural tissue integrity beyond that which could be obtained from conventional MRI measures alone. Furthermore, we demonstrated that quantitative SC MRI measures had the ability to reliably distinguish between MS patients with significantly different disability levels, when lesion-based measurements could not.

To date, longitudinal changes in DTI measures in the SC have been assessed in only three studies, all which had relatively short durations of follow-up (ranging from 3 months to 2 years). Longitudinal studies of MTI changes in the SC of MS patients are similarly limited, with one prior study assessing for correlations of baseline MTR with clinical progression.

Establishing strong longitudinal correlations between changes in quantitative SC MRI measures and clinical change is an essential step to further develop the practical clinical utility of these techniques.

To this end, we sought to: evaluate the magnitude of longitudinal change in quantitative SC MRI measures over the study follow-up period of 2 years, and to
characterize the relationship between longitudinal change in quantitative SC MRI with clinical disability at follow-up, and clinical progression.

We hypothesize that quantitative MRI measures in the SC will change measurably in MS patients over the study follow-up period of 2 years, and that there will be significant correlations between longitudinal change in individual quantitative SC MRI measures and measures of sensorimotor dysfunction, and global disability at follow-up.

Methods

This study was approved by the institutional review board of Johns Hopkins University. All participants provided written informed consent.

Study Participants

MS patients in the baseline cohort (described in detail in prior chapters) with a minimum of one follow-up study visit who underwent SC MRI and clinical examination were included in the study population. The baseline cohort consisted of patients with relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS), who were recruited between 2007-2009 from the Johns Hopkins MS Clinic by convenience sampling. MS diagnosis was confirmed by the treating neurologist, according to the 2005 McDonald criteria.¹⁶

Clinical Measures

At follow-up study visits, subjects underwent clinical assessment within 30-days of MRI. Expanded disability status scale (EDSS) scores were determined by a
Neurostatus-certified examiner. Vibration sensation thresholds for the right and left great toes were quantified using the Vibratron II (Physitemp, Huron, NJ). For strength measurements, two maximal hip-flexion efforts were averaged at each hip using a Microfet2 handheld-dynamometer (Hoggan Industries, Jordan, UT). Both devices have been described elsewhere and have been validated for use in MS patients to reliably detect sensorimotor dysfunction.\textsuperscript{14} MS patients scanned within the 3-months after a clinical relapse were excluded.

**Cervical SC MRI**

At follow-up study visits, MS patients underwent a cervical SC MRI, described in detail in Chapter 1.

**Statistical Analysis**

Mixed-effects regression incorporating subject-specific intercepts and slopes was utilized to assess longitudinal change in individual quantitative SC MRI measures. The model utilized was: Predicted MRI index = B0 + B1*age + B2*sex + b_{oi} + b_{1i}*age, where b_{oi} represents the random-effect of subject-specific intercept, and b_{1i} represents the random-effect of subject-specific slope. Age (in months) at scan date was chosen as the time-variable, as MRI measures are known to change with age, even in healthy people. Thus, using this measure allowed adjustment for age, while simultaneously acting as a time variable.

Correlations between subject-specific slopes and follow-up clinical measures were assessed using Pearson’s product moment and Spearman’s rank correlation.
coefficients. Pearson’s product moment correlation coefficient was chosen for clinical measures with normal distributions, while Spearman’s rank correlation coefficient was chosen for clinical measures with skewed distributions. Relationships between subjects with faster/slower rates of SC MRI index change (the upper and lower tertiles of subjects, according to subject-specific slopes) and clinical progression (based on EDSS, dynamometry, and vibration sensation threshold) were assessed using the chi-squared test. Clinical progression was defined as: follow-up EDSS ≥ 1.5 if baseline EDSS=0; EDSS increase of ≥ 1.0 if baseline EDSS < 6.0; EDSS increase of ≥ 0.5 if EDSS ≥ 6.0; dynamometry deterioration of ≥ 20% of baseline value, vibration sensation threshold deterioration of ≥ 20% of baseline value. Finally, correlations between follow-up clinical measures, and subjects with faster and slower rates of SC MRI index change (the upper and lower tertiles of subjects according to subject-specific slopes) were assessed using spearman’s rank correlation coefficient.

**Results**

This was a preliminary analysis because of a significant proportion of missing data that were unavailable for analysis at the time of writing. The complete dataset will be available for analysis in the upcoming months.

Of 133 MS patients in the baseline cohort, 74 subjects with a minimum of 1 follow-up study visit were identified. The median follow-up duration was 726 days (inter-quartile range = 372-754 days). 52 subjects had a single follow-up study visit, 18 subjects had 2 follow-up visits, while 4 subjects had 3 follow-up visits. Subject-specific
trajectories of MRI index change are displayed in Figure 5.1. Clinical characteristics of this study population are described in Table 5.1.

Of the baseline cohort, when subjects excluded from the analysis due to missing data were compared to those included in the longitudinal analysis: there were no detectable differences in clinical characteristics (age, sex, disease duration, disease subtype, EDSS, proportion on disease-modifying therapy) or quantitative SC MRI measures between the two groups.

When the utility of including a random intercept and slope in the mixed effects model was assessed controlling for age and sex, the model fit improved with the addition of these random effects (LRT p<0.001). Over the study follow-up time period, FA decreased (p=0.16), MD increased (p=0.08), $\lambda_\perp$ (p=0.21) increased, $\lambda_\parallel$ increased (p=0.27), MTR decreased (p=0.001), and SC-CSA (p=0.06) decreased.

When correlations between subject-specific slopes ($b_{i1}$) for each individual quantitative SC MRI index and follow-up clinical measures (EDSS, vibrotactile, dynamometer) were assessed, there were moderate-strong significant correlations observed (Table 5.2). When relationships between individuals with faster/slower trajectories of SC MRI-index change vs. the study population mean (based on the upper and lower tertiles of $b_{i1}$) with clinical progression were assessed, a handful of associations between subject-specific slopes and clinical progression were identified (Table 5.3), although most relationships were not significant.

On the other hand, when correlations between individuals with faster/slower trajectories of SC MRI index change vs. the study population mean with follow-up clinical measures were assessed, there were stronger correlations observed with follow-
up clinical measures in those with accelerated SC MRI index change for a number of the MRI indices, including FA, MD, $\lambda_{o}$, and MTR (Table 5.4), suggesting that the individual rates of change of MRI measures is relevant to clinical disability at follow-up.

**Discussion**

In this preliminary analysis of 74 MS patients, a number of quantitative SC MRI indices change over the follow-up study period, in the appropriate “direction” that one would expect to represent the combinations of pathological processes typically seen in chronic disease, including demyelination, axonal loss, gliosis. Noteworthy is the observation that not only do quantitative SC MRI indices change measurably over 2 years in the study population, but that individual, subject-specific trajectories of change in quantitative SC MRI measures seem to be of relevance with regards to clinical progression.

The idea of individual, subject-specific trajectories of MRI index change being relevant to clinical progression is intriguing and warrants further investigation in a larger study population, and over a longer duration of follow-up. These findings emphasize the importance of utilizing more complex statistical modelling techniques that can incorporate subject-specific components in the analysis and interpretation of longitudinal MRI data. With further study, a more accurate estimate of the expected change of quantitative SC MRI measures in a chronic MS population could be established, which would allow quantitative SC MRI techniques to be utilized in the clinical setting as prediction tools for individual patients. The development of such techniques would enable a more “personalized” approach to the clinical care of MS patients, which is
becoming increasingly relevant in the clinical practice of MS, where treatment options with vastly different risk:benefit profiles are available. In addition, with further confirmation, these techniques have the potential to be used as surrogate outcome measures in clinical trial settings, and potentially as a biomarker of disease in MS. The need for an accurate surrogate outcome measure and biomarker of disease continues to be one of the greatest unmet needs in MS. Although it is unlikely that a single metric will be able to fulfill this role, these findings raise the possibility the quantitative SC MRI measures may contribute to this role.

This preliminary analysis is limited by a significant proportion of missing data, which prevents the ability to form any definitive conclusions. However, it is reassuring that the missing subjects did not differ in clinical characteristics or MRI measures in comparison to those subjects included in the analysis. Repeating these analyses once the full dataset is available will be necessary to confirm these preliminary findings. In addition, the assessment of clinical progression based on the upper and lower tertiles of subject-specific slope is limited by the small numbers of subjects in each tertile, which likely contributed to the lack of consistency of findings across all MRI and clinical measures.

**Conclusions**

In MS, quantitative SC-MRI indices change over a median follow-up period of 2 years, likely reflecting ongoing pathological processes. Of clinical relevance is that subject-specific trajectories of SC-MRI index change are relevant to disability at follow-
up, suggesting that individual dynamics of change should be assessed in the interpretation of longitudinal SC-MRI measures, and to expand the practical utility of these techniques.
Table 5.1: Demographics and clinical characteristics at baseline

<table>
<thead>
<tr>
<th></th>
<th>All MS</th>
<th>RRMS</th>
<th>Progressive (SPMS and PPMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>74</td>
<td>43</td>
<td>31</td>
</tr>
<tr>
<td>Age at MRI scan, years (SD)</td>
<td>45 (12)</td>
<td>38 (11)</td>
<td>54 (7)†</td>
</tr>
<tr>
<td>% Female</td>
<td>73</td>
<td>77</td>
<td>68</td>
</tr>
<tr>
<td>Disease duration, years (SD)</td>
<td>10 (9)</td>
<td>6.3 (5.2)</td>
<td>16 (11)†</td>
</tr>
<tr>
<td>Median EDSS (IQR)</td>
<td>3.5 (2.0 – 6.0)</td>
<td>2.0 (1.0 – 3.0)</td>
<td>6.0 (4.0 – 6.5)†</td>
</tr>
<tr>
<td>% on disease-modifying treatment</td>
<td>75</td>
<td>86</td>
<td>58†</td>
</tr>
<tr>
<td>Vibration sensation threshold, microns (SD)</td>
<td>10 (17)</td>
<td>4.8 (8.2)</td>
<td>18 (23)†</td>
</tr>
<tr>
<td>Hip flexion strength, pounds (SD)</td>
<td>40 (18)</td>
<td>46 (16)</td>
<td>30 (17)†</td>
</tr>
</tbody>
</table>

RRMS=relapsing-remitting multiple sclerosis, SPMS=secondary-progressive multiple sclerosis, HCs=healthy control subjects, EDSS=expanded disability status scale, SD=standard deviation, IQR=interquartile range. Disease duration was defined as the time since first symptoms attributable to MS. †p<0.05 for comparison against RRMS
Table 5.2: Correlations (Pearson’s and Spearman’s) between subject-specific slopes and Clinical Measures for individual MRI indices

<table>
<thead>
<tr>
<th>$b_{1i}$ for MRI Index</th>
<th>Correlation Coefficients (p-values)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EDSS*</td>
<td>Dynamometer*</td>
</tr>
<tr>
<td>FA</td>
<td>-0.31 (&lt;0.001)</td>
<td>0.30 (&lt;0.001)</td>
</tr>
<tr>
<td>MD</td>
<td>0.32 (&lt;0.001)</td>
<td>-0.36 (&lt;0.001)</td>
</tr>
<tr>
<td>$\lambda_\perp$</td>
<td>0.34 (&lt;0.001)</td>
<td>-0.31 (&lt;0.001)</td>
</tr>
<tr>
<td>$\lambda_\parallel$</td>
<td>0.15 (0.06)</td>
<td>-0.21 (0.009)</td>
</tr>
<tr>
<td>MTR</td>
<td>-0.27 (&lt;0.001)</td>
<td>0.30 (&lt;0.001)</td>
</tr>
<tr>
<td>C3-C4 Cord Area</td>
<td>-0.44 (&lt;0.001)</td>
<td>0.42 (&lt;0.001)</td>
</tr>
</tbody>
</table>

* = Pearson’s product-moment correlation coefficient, † = Spearman’s correlation coefficient, $b_{1i}$ = random-effect of subject-specific slope

Table 5.3: Relationships between clinical progression and individuals in the upper and lower tertiles of subject-specific slopes (p-values of chi-squared test)

<table>
<thead>
<tr>
<th>Individuals in upper and lower tertiles of subject-specific slopes for individual MRI measures</th>
<th>Chi-squared p-values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EDSS progression</td>
<td>Hip-flexion Strength Progression</td>
</tr>
<tr>
<td>FA</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>MD</td>
<td>0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>$\lambda_\perp$</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>$\lambda_\parallel$</td>
<td>0.8</td>
<td>0.03</td>
</tr>
<tr>
<td>MTR</td>
<td>0.08</td>
<td>0.002</td>
</tr>
<tr>
<td>C3-C4 Cord Area</td>
<td>0.5</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Table 5.4: Comparisons of Correlations (Pearson’s and Spearman’s) between individuals in the upper and lower tertiles of subject-specific slopes and follow-up clinical measures

<table>
<thead>
<tr>
<th>Individuals in upper and lower tertiles of subject-specific slopes for individual MRI measures</th>
<th>Subject-Specific Slope Tertiles</th>
<th>Correlation Coefficients (p-values)</th>
<th>Follow-up EDSS *</th>
<th>Follow-up Hip-flexion Strength*</th>
<th>Follow-up Vibration Sensation Threshold †</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>Upper</td>
<td>-0.08 (0.6)</td>
<td>0.16 (0.3)</td>
<td>-0.15 (0.26)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lower</td>
<td>-0.23 (0.09)</td>
<td>0.08 (0.6)</td>
<td>-0.05 (0.7)</td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>Upper</td>
<td>0.40 (0.002)</td>
<td>-0.58 (&lt;0.001)</td>
<td>0.38 (0.003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lower</td>
<td>0.20 (0.1)</td>
<td>-0.03 (0.8)</td>
<td>0.42 (0.001)</td>
<td></td>
</tr>
<tr>
<td>λ⊥</td>
<td>Upper</td>
<td>0.23 (0.09)</td>
<td>-0.16 (0.3)</td>
<td>0.11 (0.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lower</td>
<td>0.25 (0.07)</td>
<td>-0.21 (0.13)</td>
<td>0.39 (0.004)</td>
<td></td>
</tr>
<tr>
<td>λ∥</td>
<td>Upper</td>
<td>0.45 (0.004)</td>
<td>-0.44 (0.001)</td>
<td>0.21 (0.11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lower</td>
<td>-0.11 (0.4)</td>
<td>0.11 (0.42)</td>
<td>-0.19 (0.17)</td>
<td></td>
</tr>
<tr>
<td>MTR</td>
<td>Upper</td>
<td>0.15 (0.3)</td>
<td>0.27 (0.05)</td>
<td>0.15 (0.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lower</td>
<td>-0.56 (&lt;0.001)</td>
<td>0.61 (&lt;0.001)</td>
<td>-0.54 (&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>Cord Area</td>
<td>Upper</td>
<td>-0.41 (0.001)</td>
<td>0.18 (0.22)</td>
<td>-0.008 (0.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lower</td>
<td>-0.33 (0.01)</td>
<td>-0.02 (0.9)</td>
<td>0.02 (0.9)</td>
<td></td>
</tr>
</tbody>
</table>

*=Pearson’s product-moment correlation coefficient, †Spearman’s correlation coefficient
Figure 5.1: Quantitative SC MRI measures in MS patients (individual lines represent individual subjects)
CONCLUSIONS

Completion of the specific aims of this dissertation collectively provide evidence that quantitative SC MRI measures are important to assess, and are of utility in MS patients for a number of reasons.

First, quantitative SC MRI measures demonstrate robust correlations with various clinical measures of relevance in MS patients, including global disability and system-specific measures of clinical disability. These measures independently contribute to explaining clinical disability, even after controlling for other confounding variables. These findings support the notion that quantitative SC MRI measures have the ability to provide clinically relevant information on tissue microstructural integrity relevant to clinical disability, above and beyond that which may be gleaned from measures of MRI lesion load alone, and highlight the importance of utilizing these measures to better understand factors in the SC that mediate disability in MS.

Second, there are significant correlations between quantitative SC-MRI and specific retinal layers in MS patients, suggesting that pathological changes in two functionally and spatially distinct CNS compartments occur to an extent in parallel, and reflect global pathological processes that are distinct from those captured by brain atrophy measures alone. Furthermore, both retinal and SC MRI measures simultaneously contribute to explaining the variance in functional system-specific clinical disability of relevance to the visual system and SC, suggesting that MS-related changes in the retina and SC are capturing clinically relevant global pathological processes that are not adequately captured by either measure alone, or by a measure of brain atrophy. These findings highlight the importance of combining measures from unique compartments of
the CNS to facilitate a more thorough examination of regional and global disease processes that contribute to clinical disability in MS.

Third, when considering normalization factors in the SC, normalization of SCV by SC length – which is essentially a measure of the average cross-sectional area - consistently improves the ability to detect group differences and clinical-radiological correlations in MS. This is particularly important in settings where subtle differences in SC atrophy need to be detected. This finding underscores the importance of selecting and utilizing appropriate normalization factors when assessing SC atrophy.

Finally, quantitative SC MRI measures change measurably in MS patients over a 2 year follow-up period. Although these results are preliminary, noteworthy is the observation that subject-specific trajectories of SC MRI index change are relevant to clinical disability at follow-up, suggesting that individual dynamics of change should be assessed in the interpretation of longitudinal SC-MRI measures, and are worthy of further exploration.

Future directions in this line of investigation include: assessing longitudinal change of quantitative SC MRI measures in this MS study population once the full dataset is complete, performing similar analyses in larger samples of MS patients, and confirming these findings in prospective studies. In addition, it will be of interest to assess these specific aims in a cohort consisting exclusively of specific MS subtypes, including primary progressive and secondary progressive MS patients.

In conclusion, this dissertation provides evidence that quantitative, SC MRI measures are relevant to both global, and system-specific measures of clinical disability in MS patients, in both cross-sectional and longitudinal settings. With prospective confirmation in a
larger cohort of MS patients, these techniques have the potential for significant clinical utility, not only as a predictive tool and biomarker of disease in practice settings, but also potentially in clinical trial settings as a surrogate outcome measure. Moreover, quantitative SC MRI may contribute to providing insight into the elusive underlying mechanisms of disease in MS, which could set the stage for the development of more efficacious treatment strategies.
References


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72. Mann RS, Constantinescu CS, Tench CR. Upper cervical spinal cord cross-sectional area in relapsing remitting multiple sclerosis: application of a new


EDUCATION
1998 - 2001 Bachelor of Science (BSc) with High Distinction, Human Biology University of Toronto
2001 - 2005 Doctor of Medicine (MD) Queen’s University
2005 - 2010 Neurology Residency Division of Neurology, University of Toronto
2010 - current Neuroimmunology Fellowship Department of Neurology, Johns Hopkins University
2010 – current PhD Candidate, Graduate Training Program in Clinical Investigation Johns Hopkins Bloomberg School of Public Health Johns Hopkins School of Medicine

MEDICAL LICENSES AND CERTIFICATIONS
2010 - current Fellow of the Royal College of Physicians and Surgeons of Canada (FRCPC) Neurology
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*joint first authorship

Hyduk SJ, **Oh J**, Xiao H, Chen M, Cybulsky MI. Paxillin selectively associates with constitutive and chemoattractant-induced high affinity α4β1 integrins: implications for integrin signaling.
**SELECTED ABSTRACTS**


BOOK CHAPTERS


PEER REVIEW


ADMINISTRATIVE & COMMITTEES

2009 - 2010 Chief Resident, Neurology Residency Program, University of Toronto

2007 - 2008 Resident Representative, Neurology Education Committee, Neurology Residency Program, University of Toronto.

2008, 2010 Resident Representative, Neurology Residency Program Interview Committee, University of Toronto

2002 – 2005 Founder and President, Student Interest Group in Neurology, Queen’s University

TEACHING EXPERIENCE:

2010 Neurology Clinical Skills Preceptor, Johns Hopkins School of Medicine

2007 - 2009 Brain and Behaviour Lecture Series for Medical Students: Vision Week Seminar Lecturer, Headache Seminar Lecturer, University of Toronto

2000 - 2001 MCAT Course Instructor, The Princeton Review

AWARDS AND HONOURS (Highlights):

2012 - current Multiple Sclerosis Society Transitional Career Development Award
2012 Sommer Scholarship, Johns Hopkins Bloomberg School of Public Health
2012 American Academy of Neurology Fellows Scholarship to Annual Meeting
2010 - 2011 Multiple Sclerosis Society of Canada Postdoctoral Fellowship Award
2010 PAIRO Trust Fund Resident Teaching Award
2010 J.T. Marotta Teaching Award, University of Toronto
2009 John Wherett Research Award, University of Toronto
2009 American Academy of Neurology Scholarship to Annual Meeting
2008 Sabiha Al-Hassan Neurology Award, University of Toronto
2003 J.D Hatcher Research Award, Queen’s University
2001 - 2003 Robert Bruce Scholarship, Queen’s University
2001 Edgar Forrester Medical School Entrance Scholarship, Queen’s University
2001 The Chancellor’s Medal, University of Toronto
<table>
<thead>
<tr>
<th>Year</th>
<th>Scholarship/Award</th>
<th>Institution/University</th>
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<tbody>
<tr>
<td>1998-2000</td>
<td>J.D. Schulz Research Scholarship Heart and Stroke Foundation</td>
<td>University of Toronto Scholar, University of Toronto</td>
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<td></td>
<td></td>
<td>Dean’s List, University of Toronto</td>
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<tr>
<td>2000</td>
<td>George Gray Falle Scholarship, University of Toronto</td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td>Dr. John Knowles Collins Scholarship, University of Toronto</td>
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<td>1998</td>
<td>Peter Larkin Entrance Scholarship, University of Toronto</td>
<td></td>
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<tr>
<td>1998</td>
<td>The Governor-General’s Award, The Woodlands School</td>
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**MEDICAL SOCIETY MEMBERSHIPS**
Current member, Ontario Medical Association and Canadian Medical Association

**OTHER SKILLS AND CERTIFICATIONS**
- Advanced Cardiac Life Support (ACLS), 2005
- Languages: Fluent in English and Korean, functional Japanese and French