BRIEF COMMUNICATION

Diffusion basis spectrum imaging for identifying pathologies in MS subtypes

Afsaneh Shirani1,2, Peng Sun3, Kathryn Trinkaus4, Dana C. Perantie1, Ajit George3, Robert T. Naismith1, Robert E. Schmidt5, Sheng-Kwei Song3 & Anne H. Cross1

1The John L. Trotter Multiple Sclerosis Center and Neuroimmunology Section, Department of Neurology, Washington University School of Medicine, St. Louis, Missouri
2Division of Multiple Sclerosis, Department of Neurological Sciences, University of Nebraska Medical Center, Omaha, Nebraska
3Department of Radiology, Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Missouri
4Biostatistics Shared Resource and Siteman Cancer Center, Washington University School of Medicine, St. Louis, Missouri
5Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri

Correspondence
Anne H. Cross, Department of Neurology, Washington University School of Medicine, Campus Box 8111, 660 S. Euclid Avenue, St. Louis 63110, MO. Tel: +1-314-747-0405; Fax: +1-314-747-1345; E-mail: crossa@wustl.edu

Abstract
Diffusion basis spectrum imaging (DBSI) combines discrete anisotropic diffusion tensors and the spectrum of isotropic diffusion tensors to model the underlying multiple sclerosis (MS) pathologies. We used clinical MS subtypes as a surrogate of underlying pathologies to assess DBSI as a biomarker of pathology in 55 individuals with MS. Restricted isotropic fraction (reflecting cellularity) and fiber fraction (representing apparent axonal density) were the most important DBSI metrics to classify MS using brain white matter lesions. These DBSI metrics outperformed lesion volume. When analyzing the normal-appearing corpus callosum, the most significant DBSI metrics were fiber fraction, radial diffusivity (reflecting myelination), and nonrestricted isotropic fraction (representing edema). This study provides preliminary evidence supporting the ability of DBSI as a potential noninvasive biomarker of MS neuropathology.

Introduction
Multiple sclerosis (MS) is a disease with profound heterogeneity clinically, radiologically, and pathologically. Current clinical subtypes of MS do not fully capture its pathological heterogeneity. Identifying pathologically meaningful subtypes of MS are crucial in tailoring immunotherapies and moving toward a personalized medicine approach in the care of people with MS (PwMS).1

Diffusion basis spectrum imaging (DBSI) models diffusion-weighted MRI signals as a combination of discrete anisotropic diffusion tensors (reflecting the axon and myelin integrity of fibers), and a spectrum of isotropic diffusion tensors (detecting inflammation and tissue loss surrounding axons).2 The accuracy of DBSI in capturing and quantifying white matter (WM) pathologies has been shown in tissue phantoms, spinal cord, and optic nerves of mice with experimental allergic encephalomyelitis, the corpus callosum (CC) of mice with cuprizone-induced demyelination, autopsied human MS spinal cord, and in biopsied human brain tissue.3–7

Here, we assessed the ability of DBSI to provide pathologically meaningful differentiation of MS subtypes in living PwMS.

Methods
This cross-sectional study included 55 PwMS (according to 2010 Revised McDonald Criteria)8 from John L. Trotter MS Center at Washington University in St. Louis. MS clinical subtypes were confirmed by two MS neurologists independently and included relapsing-remitting MS (RRMS), secondary-progressive MS (SPMS), and primary-
progressive MS (PPMS). We used the consensus definition of SPMS which indicates that “in most clinical contexts, SPMS is diagnosed retrospectively by a history of gradual worsening after an initial relapsing disease course, with or without acute exacerbations during the progressive course”.\textsuperscript{9} Patients underwent detailed clinical assessment, and structural and diffusion imaging. Diffusion data were collected using a 3 Tesla TIM Trio (Siemens) scanner with a 32-channel head coil at $2 \times 2 \times 2 \text{ mm}^3$ resolution in the axial plane with repetition time/echo time $= 10{,}000/120 \text{ msec}$, and employing a 99-direction diffusion-weighting scheme (maximum b-value $= 1500 \text{ sec/mm}^2$).

Voxel-wise DBSI metrics were calculated for two sets of regions of interest (ROIs) in each PwMS, one consisting of all brain WM lesions, and the other ROI was the normal-appearing CC (excluding lesions in the CC). Normal-appearing WM often harbors pathology in MS, and the CC was chosen for study due to its large size and ease of identification, axonal coherence, and predilection to be affected in MS. WM lesion ROIs for the whole brain were created on fluid-attenuated inversion recovery (FLAIR) images by automatic segmentation using the lesion prediction algorithm implemented in Lesion Segmentation Tool of Statistical Parametric Mapping (SPM) toolbox in MATLAB.\textsuperscript{10}

To create the ROIs for normal-appearing CC, we first traced CC on high-resolution magnetization-prepared rapid acquisition with gradient echo (MP-RAGE) images using semiautomatic thresholding in Amira 6.0.1. These CC traces were eroded by one voxel to reduce partial volume effects. SPM-detected lesions within CC were excluded to obtain normal-appearing CC ROIs.

For analysis, we applied recursive partitioning,\textsuperscript{11} a non-parametric decision tree-based regression and classification method, to use DBSI metrics to classify PwMS. Recursive partitioning involves constructing a decision tree by dividing a dataset into subsets according to descriptors, or rules that discriminate between different subsets while trying to maximize the homogeneity within subsequent subsets. In other words, as the splits occur, the nodes become more homogeneous. The median values for the following DBSI-derived metrics were included in the recursive partitioning:

I Anisotropic components:

a Radial diffusivity (representing myelin integrity of residual axons)

b Axial diffusivity (representing residual axon integrity)

c Fiber fraction (representing apparent axonal density)

II Isotropic components:

a Restricted fraction (reflecting cellularity)

b Nonrestricted fraction (reflecting extra-axonal environment associated with tissue loss, inflammation, and cerebrospinal fluid partial volume).

We also included T2-weighted lesion volume (standardized using median absolute deviation) in the recursive partitioning of WM lesion ROIs.

The Institutional Review Board at Washington University approved the study.

Results

Of the 55 subjects in the study (22 RRMS, 16 SPMS, and 17 PPMS), females comprised a larger proportion among all subtypes as expected (Table 1). RRMS patients were on average approximately 14 and 11 years younger than SPMS and PPMS patients, respectively. Despite a shorter disease duration, PPMS subjects had similar median expanded disability status scale (EDSS) score (6) to those with SPMS.

Figure 1A shows the recursive partitioning tree using DBSI metrics for brain WM lesions. The most significant splits were based on DBSI-derived restricted isotropic fraction and fiber fraction. RRMS subtype was predicted by relatively higher values for both restricted isotropic fraction and fiber fraction. Four final classes (terminal nodes) were identified, with two predicted to contain individuals with PPMS, one predicted to contain RRMS, and one predicted as SPMS. Of the nine subjects predicted to have SPMS, seven also had SPMS as a clinical designation. Figure 1B shows the confusion matrix of the classifier using DBSI metrics of WM lesions versus predefined clinical subtypes. Based on DBSI metrics of WM lesions, 35 PwMS (64%) were predicted to have the same subtype as their predefined clinical subtypes. Notably, WM lesion volume, as well as other DBSI-derived metrics, did not improve the classification.

Recursive partitioning using DBSI metrics of normal-appearing CC showed that the most significant (first and second) splits were based on DBSI-derived fiber fraction (Fig. 1C). At the top split, lower fiber fraction (consistent with lower axon density) predicted a subgroup of PwMS as having SPMS. Lower but important splits were based on nonrestricted fraction and radial diffusivity. Of note, DBSI-derived radial diffusivity helped predict SPMS versus RRMS. Those with higher radial diffusivity were predicted as having SPMS versus RRMS (consistent with more severe demyelination in SPMS). Five final nodes were identified including two classes predicted as having RRMS, two as having SPMS, and one as having PPMS. DBSI of normal-appearing CC predicted 37 PwMS (67%) to have the same subtype as their predefined clinical subtypes (Fig. 1D).

Discussion

Prior histopathological studies of relapsing and progressive MS central nervous system (CNS) tissues have
types in our study, although prior studies have often
been more osteosclerotic in nature than T2-weighted lesion volume at discerning clinical sub-
types. It is noteworthy that DBSI metrics performed better
when using brain WM lesions to predict MS clinical sub-
types. The most important DBSI metrics in brain lesion.

We found that restricted isotropic fraction and fiber frac-
tion of WM lesions were the most important DBSI metrics
when using brain WM lesions to predict MS clinical sub-
types. It is noteworthy that DBSI metrics performed better
than T2-weighted lesion volume at discerning clinical sub-
types in our study, although prior studies have often
reported higher T2 lesion loads to be associated with SPMS
versus RRMS. Out of 22 subjects designated as RRMS clini-
cally, 15 were predicted to have RRMS by DBSI metrics, 6
were predicted to have PPMS and only one was predicted
to have SPMS. These results suggest that RRMS WM
lesions in our subjects were more similar to PPMS than
SPMS in terms of axon density and cellularity.

When analyzing DBSI metrics of normal-appearing CC,
the most significant factor to predict MS subtype was
fiber fraction, with radial diffusivity and nonrestricted iso-
tropic fraction also of importance. Based on the known
pathology of SPMS, it was not unexpected that those pre-
dicted to be SPMS had the lowest fiber fractions (repre-
senting apparent axonal density) within normal-appearing
CC.14 Compared to RRMS, SPMS was also predicted by
greater apparent demyelination based on higher DBSI-
derived radial diffusivity, also not unexpected.15 A higher
nonrestricted isotropic fraction in normal-appearing CC
predicted RRMS over PPMS. This is possibly due to more
edema in the RRMS group, about 15% of whom had

| Characteristics                          | RRMS(n = 22) | SPMS(n = 16) | PPMS(n = 17) |
|-----------------------------------------|--------------|--------------|--------------|
| Sex, n (%)                              |              |              |              |
| Female                                  | 15 (68)      | 13 (81)      | 12 (71)      |
| Male                                    | 7 (32)       | 3 (19)       | 5 (29)       |
| Age (years), mean ± SD                  | 43.0 ± 10.7  | 56.7 ± 7.5   | 54.1 ± 8.2   |
| Disease duration from symptoms onset (years), mean ± SD | 10.4 ± 8.4   | 27.2 ± 11.8  | 12.4 ± 5.9   |
| Expanded disability status scale score, median (range) | 3 (1.5–6)    | 6 (2.5–6.5)  | 6 (3–7.5)    |
| T2 lesion volume (mL), median (interquartile range) | 9.5 (4.4–17.9) | 20.2 (6.1–32.7) | 8.0 (5.2–14.2) |
| DBSI-derived metrics in brain white matter lesions |              |              |              |
| Fiber fraction, median (interquartile range) | 0.40 (0.38–0.42) | 0.39 (0.37–0.47) | 0.37 (0.32–0.41) |
| Isotropic restricted fraction, median (interquartile range) | 0.07 (0.07–0.08) | 0.06 (0.04–0.07) | 0.07 (0.05–0.08) |
| Isotropic nonrestricted fraction, median (interquartile range) | 0.43 (0.40–0.47) | 0.46 (0.39–0.50) | 0.48 (0.43–0.53) |
| Radial diffusivity (µm²/msec), median (interquartile range) | 0.70 (0.65–0.74) | 0.68 (0.64–0.71) | 0.70 (0.66–0.74) |
| Axial diffusivity (µm²/msec), median (interquartile range) | 1.84 (1.74–1.97) | 1.92 (1.83–2.02) | 1.99 (1.79–2.11) |
| DBSI-derived metrics in normal-appearing corpus callosum |              |              |              |
| Fiber fraction, median (interquartile range) | 0.61 (0.58–0.66) | 0.60 (0.54–0.68) | 0.61 (0.58–0.66) |
| Isotropic restricted fraction, median (interquartile range) | 0.19 (0.18–0.21) | 0.16 (0.12–0.19) | 0.20 (0.17–0.23) |
| Isotropic nonrestricted fraction, median (interquartile range) | 0.04 (0.00–0.10) | 0.04 (0.02–0.15) | 0.05 (0.03–0.07) |
| Radial diffusivity (µm²/msec), median (interquartile range) | 0.31 (0.20–0.35) | 0.30 (0.20–0.34) | 0.33 (0.29–0.38) |
| Axial diffusivity (µm²/msec), median (interquartile range) | 2.25 (2.12–2.36) | 2.13 (2.02–2.24) | 2.11 (2.06–2.25) |

RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary-progressive multiple sclerosis; PPMS, primary-progressive multiple sclerosis; DBSI, diffusion basis spectrum imaging.
recently showed that findings from DBSI metrics were consistent with pathology findings in a biopsied inflammatory demyelinating WM brain lesion.\(^7\) While the majority of the MS participants in our study did not have active disease, we cannot completely rule out if active inflammation in some might have confounded the findings with our limited sample size. DBSI of the spinal cord was not a part of the present studies. We are working to overcome technical challenges in applying DBSI to the human spinal cord and hope to include it in future studies. Gray matter lesions are particularly common in progressive forms of MS, but DBSI metrics of gray matter were not included due to the lower image resolution in diffusion-weighted images. Our study was cross-sectional. Longitudinal studies, which are underway, will be valuable to evaluate the meaning of DBSI metrics in relation to the pathological heterogeneity of MS over time. It would also be interesting to compare DBSI with other diffusion models. Last but not the least, our findings need to be validated in external independent datasets, including more subjects with concurrent biopsies.

Overall, this study provides preliminary evidence supporting the ability of DBSI as a potential noninvasive biomarker of MS neuropathology. Future studies are needed to evaluate the utility of DBSI as a potential outcome measure in trials of remyelinating and neuroprotective agents.

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Author Contributions

A.H.C., S-K.S., K.T., R.T.N, P.S., and R.S. contributed to the conception and design of the study. A.S., P.S., D.P.,
A.G., A.H.C., S-K.S, and R.T.N. contributed to acquisition of the data. K.T. and A.S. contributed to statistical analysis. A.S., K.T., P.S., R.T.N., D.P., R.S., A.H.C., and S-K.S. contributed to interpretation of the data. A.S. wrote the first draft of the manuscript. A.S., K.T., P.S., R.T.N., D.P., A.G., R.S., A.H.C., and S-K.S. reviewed the manuscript and provided revisions for intellectual content.

Conflict of Interest

Afsaneh Shirani is funded through a clinician scientist development award from the National Multiple Sclerosis Society (USA), and a clinical research training scholarship from the American Academy of Neurology. Peng Sun: nothing to disclose. Kathryn Trinkaus: nothing to disclose. Dana Perantie: nothing to disclose. Ajit George: nothing to disclose. Robert Naismith has received honoraria for consulting for Alkermes, Biogen, Celgene, Novartis, TG Therapeutics; and for speaking for EMD Serono, Genzyme, Genentech, and Novartis. Robert Schmidt: nothing to disclose. Sheng-Kwei Song is currently funded by NIH U01EY025500, R01NS047592, P01NS059560, and NMSS RG-1701-26617. Anne Cross was funded in part by the Manny & Rosalyn Rosenthal – Dr. John L Trotter MS Center Chair in Neuroimmunology of Barnes-Jewish Hospital Foundation. She has received honoraria for consulting for Biogen, Celgene, EMD-Serono, Genzyme, Genentech, Novartis, and TG Therapeutics. Washington University may receive royalty income based on a technology licensed by Washington University to DxGPS LLC. That technology is evaluated in this research.

References

1. De Jager PL. Identifying patient subtypes in multiple sclerosis and tailoring immunotherapy: challenges for the future. Ther Adv Neurol Disord 2009;2:8–19.
2. Cross AH, Song SK. A new imaging modality to non-invasively assess multiple sclerosis pathology. J Neuroimmunol 2017;304:81–85.
3. Wang Y, Sun P, Wang Q, et al. Differentiation and quantification of inflammation, demyelination and axonal injury or loss in multiple sclerosis. Brain 2015;138:1223–1238.
4. Lin TH, Chiang CW, Perez-Torres CJ, et al. Diffusion MRI quantifies early axonal loss in the presence of nerve swelling. J Neuroinflammation 2017;14:78. https://doi.org/10.1186/s12974-017-0852-3.
5. Wang Y, Wang Q, Haldar JP, et al. Quantification of increased cellularity during inflammatory demyelination. Brain 2011;134:3587–3598.
6. Wang X, Cusick MF, Wang Y, et al. Diffusion basis spectrum imaging detects and distinguishes coexisting subclinical inflammation, demyelination and axonal injury in experimental autoimmune encephalomyelitis mice. NMR Biomed 2014;27:843–852.
7. Shirani A, Sun P, Schmidt RE, et al. Histopathological correlation of diffusion basis spectrum imaging metrics of a biopsy-proven inflammatory demyelinating brain lesion: A brief report. Mult Scler 2018; doi: 10.1177/1352458518786072. [Epub ahead of print].
8. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69:292–302.
9. Lublin FD, Reingold SC, Cohen JA, et. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology 2014;83:278–286.
10. Egger C, Opfer R, Wang C, et al. MRI FLAIR lesion segmentation in multiple sclerosis: Does automated segmentation hold up with manual annotation? Neuroimage Clin 2017;13:264–270.
11. Strobl C, Malley J, Tutz G. An introduction to recursive partitioning: rationale, application and characteristics of classification and regression trees, bagging and random forests. Psychol Methods 2009;14:323–348.
12. Kutzelnigg A, Lucchinetti CF, Stadelmann C, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain 2005;128(Pt 11):2705–2712.
13. Revesz T, Kidd D, Thompson AJ, et al. A comparison of the pathology of primary and secondary progressive multiple sclerosis. Brain 1994;117(Pt 4):759–765.
14. Bjartmar C, Wujek JR, Trapp BD. Axonal loss in the pathology of MS: consequences for understanding the progressive phase of the disease. J Neurol Sci 2003;206:165–1671.
15. Patrikios P, Stadelmann C, Kutzelnigg A, et al. Remyelination is extensive in a subset of multiple sclerosis patients. Brain 2006;129(Pt 12):3165–3172.

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