CHAPTER 3.2

Multi-scale MRI spectrum detects differences in myelin integrity between MS lesion types

Yunyan Zhang MD, PhD\textsuperscript{1,2,3}, Laura Jonkman MSc\textsuperscript{4}, Antoine Klauser PhD\textsuperscript{4}, Frederik Barkhof MD, PhD\textsuperscript{5}, V. Wee Yong PhD\textsuperscript{2,3}, Luanne M. Metz MD FRCPC\textsuperscript{2,3}, Jeroen J. G. Geurts PhD\textsuperscript{4}

Departments of \textsuperscript{1}Radiology and \textsuperscript{2}Clinical Neurosciences, and \textsuperscript{3}Hotchkiss Brain Institute, University of Calgary, Alberta, Canada, and Departments of \textsuperscript{4}Anatomy and Neurosciences, and \textsuperscript{5}Radiology and Nuclear Medicine, VU University Medical Center, Amsterdam, the Netherlands

*Multiple Sclerosis Journal (2015)*
ABSTRACT

Background: Lesions with different extents of myelin pathology are found at autopsy in multiple sclerosis (MS), but the difference is not discernable in MRI.

Objectives: To determine whether analysis of the local spectrum in MRI is sensitive to lesion differences in myelin integrity.

Methods: We imaged fresh brain slices from 21 MS patients using 1.5T scanners. White matter lesions were identified in T2-weighted MRI, matched to corresponding specimens, and then classified into 5 categories in histology: pre-active (intact myelin); active, chronic active, chronic inactive (complete demyelination); and remyelinated lesions. Voxel-based frequency spectrum was calculated in T2 MRI to characterize lesion structure (image texture).

Results: MRI texture heterogeneity resulting from all spectral scales was greater in completely demyelinated lesions than in myelin-preserved lesions (p = 0.02) and normal appearing white matter (p < 0.01). Moreover, the spectral distribution pattern over low frequency scales differentiated demyelinated lesions from remyelinated and pre-active lesions (p < 0.01), where different lesion types also showed distinct texture scales.

Conclusion: Using multi-scale spectral analysis, it may be possible for standard MRI to evaluate myelin integrity in MS lesions. This can be critical for monitoring disease activity and assessing remyelination therapies for MS patients.
INTRODUCTION

Pathological heterogeneity of white matter lesions has been repeatedly shown in postmortem studies of multiple sclerosis (MS).\textsuperscript{1,2} Differences in the degree of inflammation and demyelination are associated with distinct consequences of axonal damage, the major contributor of progressive disability in patients.\textsuperscript{3} On the other hand, the process of repair known as remyelination can restore the lost structure and protect axons from further degeneration.\textsuperscript{4,5} Using clinical MRI, however, it is difficult to determine de- or re-myelination in MS lesions.\textsuperscript{6}

Advanced MRI has shown considerable promise in assessing myelin structure in white matter based on the difference in MRI relaxation time between myelin and water protons. However, the interpretation of myelin-water interaction can be challenged in the presence of interfering pathologies such as degraded myelin and inflammation in MS lesions.\textsuperscript{7,8} Other studies demonstrate the promise of radial diffusivity to estimate myelin pathology, but this measure can be also confounded by the coexistence of inflammation and crossing fibers in white matter.\textsuperscript{9,10} A prior study suggested the utility of discrimination functions that combine T1-, T2-weighted MRI and magnetization transfer imaging in predicting myelin status in an animal model; nonetheless, using either MRI contrast alone was not successful.\textsuperscript{11}

Given the intimate relationship between MRI and the underlying tissue structure, quantitative analysis of the organizing pattern of image voxels (MRI texture) may be useful. MRI-histology studies show that the primary substrate of white matter in T1- and T2-weighted MRI is myelin,\textsuperscript{12,13} and statistical analysis of the texture in T2-weighted MRI demonstrates 95% accuracy in classifying myelin content in mouse brain.\textsuperscript{14} Using spatial frequency-based methods, researchers show that coarse MRI texture differentiates persistent lesions from recovering ones in MS and predict patients with or without disability progression in 2 years.\textsuperscript{15} Using 7T T2-weighted MRI, voxel-based spectral analysis identifies difference in pathology between MS lesions and diffusely abnormal and normal appearing white matter (NAWM) as confirmed in formalin-fixed MS brains.\textsuperscript{17} Nonetheless, the sensitivity of frequency-based analysis to pathological differences between subtypes of MS lesions is unknown. In this study, we aimed to test whether MS lesions with different myelin content is differentiable using localized frequency spectrum in T2-weighted MRI, and determine what spectral features relate to de- and re-myelination in fresh brain specimens of MS patients.

METHODES

Patient and sample profile

Unfixed brains from 21 MS patients were obtained from the Netherlands Brain Bank after autopsy. All donors gave written informed consent prior to death for use of tissue and medical records for research. Patients with other neurological diseases besides MS were excluded. Ethics approval was obtained from the local Institutional Ethics Review Board. Autopsy and tissue sampling followed a published protocol.\textsuperscript{18} In brief, five 10-mm-thick coronal brain slices were
cut per subject. After imaging, photographs of these brain slices in T2-weighted/FLAIR MRI were printed. Then, hyperintense white matter lesions were marked on the printed MRI photographs, matched to corresponding brain slices, and cut from each slice with inclusion of anatomical landmarks (Fig. 1). These tissue blocks were also photographed before being cut from the surrounding brain for accurate MRI matching.\textsuperscript{19}

**Figure 1** | An example of lesion matching between MRI and postmortem specimens. Individual lesions are highlighted with boxes, where boxes with the same color indicate the same lesion examined in MRI and histology at corresponding locations.

**MRI protocol**

Postmortem brain slices were scanned using 1.5T MR systems (Sonata or Avanto, Siemens Medical Systems, Erlangen, Germany) as previously reported.\textsuperscript{19} Standard T2-weighted MR images were acquired centered at the middle of the brain slice and paralleled to the coronal surface. For Sonata, TR/TE = 2500/85 ms; in-plane resolution = 0.5x0.5 mm; matrix size = 384x512; and slice thickness = 4 mm. For Avanto, TR/TE = 2755/90 ms; in-plane resolution = 0.8x0.8 mm; matrix size = 256x256; and slice thickness = 3 mm.

**Histology and immunohistochemistry**

After MRI, selected white matter areas were sampled from corresponding brain specimens. Tissue blocks were fixed in 10% formalin and embedded in paraffin. 5-µm-thick sections were
cut, mounted onto glass slides, and dried overnight at 37°C. Sections were de-paraffinized in a series of xylene, ethanol, and water, and rinsed with 0.01 M phosphate-buffered saline. Staining and immunohistochemistry were performed on adjacent sections with antibodies against microglia/macrophages (anti-HLA-DR, clone LN3; local product) for inflammation and proteolipid protein (PLP; Serotec, Oxford, UK) for myelin. Remyelinated lesions were identified as ‘shadow plaques’ with diffusely reduced myelin stain in luxol fast blue periodic acid Schiff (LFB-PAS; Pfizer, NY, USA) as compared to NAWM. Demyelinated lesions were defined as complete loss of myelin stain.

**Scoring, classification, and matching**

White matter lesions were scored by an experienced neuropathologist and classified according to established criteria. Each lesion was classified into one of 5 categories: pre-active (clustered microglia with intact myelin), active (demyelination with influx of inflammatory cells), chronic active (demyelination with inflammation at lesion border, but not the center), chronic inactive (demyelination without inflammation, with gliosis), and remyelinated (shadow plaques; Fig. 2). NAWM areas (no apparent demyelination or inflammation) were also examined for control. Histology sections were co-registered to corresponding MR images with the assistance of landmarks, labels, and photographs. For exact matching, digital images of histology sections were copied onto areas of their origin in T2-weighted MRI as detailed in previous reports. Overall, this was a MRI-guided approach and regions of interest (ROI) were outlined initially in T2-weighted MRI (ImageJ; NIH, USA) before histological matching.

**MRI texture analysis**

The signal intensity patterns of T2-weighted MRI were evaluated using a newly developed algorithm. As it can detect series of frequency spectra, one spectrum per voxel (localized), and each with various frequency resolutions (multi-scale), this algorithm is also called local, multi-scale spectral analysis. To calculate the spectra, the entire frequency content in an image was initially calculated using the classical Fourier transform. For each frequency, its content is populated back to the image domain through inverse Fourier transform such that each voxel is allocated a share of that frequency. The amount of share per voxel is based on the regularity of tissue structure around the voxel at that frequency. By repeating this process for all frequencies, a spectrum of frequencies is formed per voxel (supplemental Fig. 1), which provides a signature of tissue composition at individual voxels. Subsequently, the similarity of these spectra between lesion and reference (NAWM) voxels was computed. This became a measure of overall tissue heterogeneity resulted from all frequency scales specific to a lesion voxel: large value represents severe tissue damage, such as complete demyelination.

In addition to assessing overall tissue heterogeneity, we also evaluated individual frequency scales in a spectrum. As different frequencies reflect different coarseness in texture (Fig. 2), this analysis allowed us to identify specific frequencies and spectral patterns that best identified the myelin integrity in lesion subtypes. Moreover, to facilitate inter-scanner comparison, the image dimension of Sonata scans was adjusted to 256x256 to match that of Avanto scans, and lesion texture heterogeneity was normalized by the mean texture of NAWM at each scanner.
The normalized T2 texture heterogeneity was used for analyses where applicable. In addition, an equivalent range of lesion size was chosen between scanners before outcome assessment to compensate for resolution differences. Lesions smaller than 2x2 voxels or neighboring CSF boundaries or vessel walls were excluded to avoid partial volume effect in MRI.

Figure 2 | T2-weighted MRI, frequency-specific texture map, and histological stains organized in columns 1 to 4. Example lesions are highlighted in T2-weighted MRI and texture maps (boxes and blown-outs with arrows). Corresponding histology at these lesion areas are shown in stained images for myelin (proteolipid protein, brown; 3rd column) and for inflammation (microglia and microphages, aggregates of brown with box highlights; 4th column). The texture maps of pre-active (A) and remyelinated (E) lesions are generated at a frequency scale of 0.54 Hz/mm, higher than the frequency scale of 0.32 Hz/mm for texture maps of active (B), chronic active (C), and chronic inactive (D) lesions. Higher frequency scale indicates greater regularity in myelinated lesions. Each texture map highlights the spatial distribution of the specific frequency used to generate the map, where colors represent the amplitude of this frequency: ascending from dark blue, light blue, to gray (see color bar at bottom left).
Statistics
A linear mixed-effect model was used to assess differences in normalized T2 texture heterogeneity between lesion types. In this model, each ROI was coded separately with consideration of both inter-patient and inter-specimen variances. Using a similar approach, the heterogeneity difference between lesions with different myelin states was assessed, where p ≤ 0.05 was set as significance, with Bonferroni correction to account for multiple comparisons. Subsequently, a multivariate analysis was performed to identify frequencies that separate myelin states. All statistical analyses were done using Stata (StataCorp LP, College Station, Texas, USA).

Results
Sample demographics
We matched 119 tissue blocks from 21 patients. Subject age at autopsy was 62 ± 11 years [mean ± standard deviation (SD)]; mean disease duration was 23 ± 14 years; and mean postmortem delay was 8 hours 24 minutes (Table 1). Nine patients were imaged with Sonata and 12 with Avanto scanner. From each patient, an average of 3 lesions (range 1 - 7) and 2 NAWM areas (range 1 - 5) were examined, totaling 70 lesions and 49 NAWM regions from all patients. Nine lesions were classified as pre-active, 24 active, 23 chronic active, 11 chronic inactive, and 3 remyelinated lesions. Examined lesion size ranged from 1.1x1.1 mm$^2$ to 25.2x25.2 mm$^2$. At least one NAWM area was analyzed in each tissue slice for control.

Greatest texture heterogeneity is detected in MS lesions with complete demyelination
Based on the mixed-effect model, we found that normalized texture heterogeneity in T2-weighted MRI was different between tissue subtypes (p < 0.01). The heterogeneity was significantly greater in active (p < 0.001), chronic active (p < 0.001), and chronic inactive (p = 0.001) lesions than in NAWM, and greater in chronic active lesions (p = 0.01) than in pre-active lesions. There was no significant difference in the heterogeneity between active, chronic active, and chronic inactive lesions (p = 1.0), and there was also no difference between intact myelin (NAWM and pre-active lesions) and remyelinated lesions (p = 0.39 and 1.00 respectively; Supplemental Fig. 2) when all frequency scales were considered.

Different texture heterogeneity reflects different myelin states in MS lesions
With further stratification of MS lesions according to their myelin content, we assessed the total texture heterogeneity of the following 4 tissue types: 1) intact myelin (NAWM); 2) intact myelin with inflammation (pre-active lesions); 3) demyelination with or without inflammation or gliosis (active, chronic active, and chronic inactive lesions); and 4) remyelination. After correcting for variances between specimen and ROIs using mixed-effect modeling, we found that normalized T2 texture heterogeneity of demyelinated lesions was significantly greater than NAWM (p = 0.000) and pre-active lesions (p = 0.02), but not than remyelinated lesions (p = 0.56; Fig. 3).
**Significant difference in spectral patterns between de- and re-myelinated lesions**

Besides assessing normalized T2 texture heterogeneity associated with all frequency scales, we also examined the distribution pattern of texture spectra in tissues with different myelin content. With cross-group covariance control, the multivariate test demonstrated significant differences (p < 0.01) between demyelinated and myelinated spectra (NAWM, pre-active and remyelinated lesions), especially over frequencies 0.07 to 0.126 Hz/mm, which corresponded to lesion diameters of 5 to 9 mm. Further, the spectral pattern of remyelinated lesions was similar to that of intact myelin in pre-active lesions and NAWM (Fig. 4), where remyelinated and pre-active lesions also showed the best contrast at finer texture scales than demyelinated lesions (Fig. 2).

**Table 1 | Patient and lesion demographics.**

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Note: M = male; F = female; ND = not known; PPMS = primary progressive multiple sclerosis; SPMS = secondary progressive multiple sclerosis; Scanner ID “1” = Avanto; Scanner ID “2” = Sonata.
FIGURE 3 | Normalized MRI texture heterogeneity grouped by tissue myelin states. Demyelinated lesion group include active, chronic active, and chronic inactive lesions. Both NAWM and pre-active lesions contain intact myelin, but the latter has inflammation in addition. The triangles indicate significant difference in MRI heterogeneity (solid triangle: p<0.01; empty triangle: p<0.05). Shown in plots are mean and standard errors of the heterogeneity.

FIGURE 4 | The distribution pattern of multi-scale spectra in tissue groups with different myelin states. Over low and intermediate frequency range, the amplitude of each frequency averaged from all voxels per group is displayed. Between frequencies 0.07 and 0.126 Hz/mm (shaded area), the amplitude shows a sharp decrease followed by an increase in all of the spectra from remyelinated lesions, pre-active lesions, and NAWM, in contrast to an opposite distribution pattern in demyelinated lesions (p < 0.01). Shown in plots are mean and standard error of spectral amplitude from individual image voxels.
Discussion

Using fresh brain specimens we show that distinct texture signatures in diagnostic MRI are associated with different types of MS tissue, particularly the myelin content in MS lesions. As pathological changes are uneven in space, localized analysis of image spectra provides a valuable means to study tissue structure. Particularly, the distribution pattern and scale-specific texture of multi-scale spectra may have the potential to distinguish de- and re-myelination.

Pathological heterogeneity of MS lesions is well appreciated in postmortem studies. Even within the same subject, lesions with different degrees of tissue injury are observed. Within each lesion, a range of pathological processes is often seen. To accurately evaluate disease activity, MS lesions are often divided into subtypes based on inflammation and demyelination pathologically. Using this standard, we classified lesion samples into 5 categories. Moreover, as active, chronic active, and chronic inactive lesions were all identified as complete demyelination, they were further assessed as a combined group. This provided a unique opportunity to compare intact myelin, de-, and re-myelination in MRI.

Historically, conventional MRI has not gained wide recognition for assessing myelin structure due to lack of pathological specificity. However, there is evidence indicating a clear relationship between T1- and T2-weighted MRI and myelin content. In unmyelinated areas of the neonate brain, white matter initially shows hypointense on T1- and hyperintense on T2-weighted MRI as compared to the cortex. It is the increase in the membrane components of the myelinating oligodendrocytes that gradually reverses the T1- and T2-weighted MRI signal. Moreover, while signal intensity change occurs early in T1-weighted images, T2-weighted MRI becomes a superior contrast to identify myelin development in children over 12 months, where growing myelin sheath replaces the interstitial water space, leading to increasing hypointensity in T2-weighted MRI. The signal intensity ratio map of T1- to T2-weighted MRI shows consistently greater sensitivity and lower variability to myelin content than magnetization transfer ratio, fractional anisotropy, and FLAIR images. Given that the distribution pattern of MRI voxels dictates the structural property of underlying tissues, assessing the local organization of MRI signal intensity is expected to provide further insight into the integrity of myelin structure.

Image voxels have coherency. Similar voxels with uniform distribution reflects a regular structure, such as intact myelin or remyelinated lesions. Such unique patterns of inter-voxel relationship can be detected using advanced image analysis approaches such as spatial frequency analysis used in this study. Unlike the standard Fourier transform however that only provides the total amount of frequencies in an image, current analysis of multi-scale spectra calculates a range of frequencies at each image voxel. This allows for identifying structural changes at various scales such as those ranging from cellular infiltration to myelin degradation. Tissue damage breaks the uniformity of image voxels, causing slow change in signal intensity and that increases the low frequency content; the opposite applies to tissue repair. With various degrees of tissue injury and repair around a voxel, a range of changes in frequency ensures as detected using our localized spectral analysis. Previously using 7T T2-weighted MRI of formalin-fixed brain samples with MS, texture heterogeneity summarized from all frequency scales shows strong correlation.
with the staining density of myelin in a mixed sample of lesions and non-lesion areas. In the present study, by assessing subtypes of MS lesions using clinical MRI, we show that texture heterogeneity is also sensitive to the difference in myelin integrity between lesions.

It is understandable that besides myelin content, other MS pathologies may also contribute to the pattern of MRI spectra. However, the significant difference in texture heterogeneity between de- and re-myelinated lesions may suggest the relevance of MRI spectrum and myelin integrity (Fig. 3). Moreover, in a prior study when properties of myelin, axons, and inflammation were simultaneously examined in MS brain with or without focal lesions, MRI texture heterogeneity correlated the strongest with myelin density. In the present study, our findings also support the critical role of myelin as compared to inflammation in MRI texture. For example, pre-active lesions containing no apparent pathology but inflammation demonstrate similar texture to healthy myelin in NAWM; likewise, although the extent of inflammation (and gliosis) varies between active, chronic active, and chronic inactive lesions, their MRI texture heterogeneity is similar, and this is consistent with their uniform feature of no myelin. Nonetheless, to confirm our results, we seek to evaluate other types of lesion pathology besides myelin in the future.

In addition to assessing texture heterogeneity, we further investigated at which scales myelin states are different by assessing the distribution pattern of MRI spectra. Unlike overall texture heterogeneity, spectral distribution reveals the organization of tissue structure at each scale (Fig. 4). Given that low frequencies refer to large-scale changes that reflect coarse texture, healthier myelin should generate better texture, thus fewer low frequencies. In this sample, we identified a marked reduction in spectral amplitude over low frequencies 0.07 to 0.126 Hz/mm in remyelinated lesions as compared to demyelinated lesions. Notably, while sharing a similar distribution pattern, the different types of myelinated spectra (NAWM, pre-active lesions, remyelination) differ based on amplitude. The low frequency content in remyelinated spectra remained to be higher than in pre-active lesions, with the latter being higher than in NAWM spectra. This may be explained by the suboptimal nature in tissue microstructure of reformed myelin in remyelinated lesions, where the myelin sheath is thinner and segments are shorter between nodes than that of intact myelin. However, the similarity in distribution between intact and remyelinated spectra may suggest ongoing repair in MS lesions.

In this study, our results are based on standard T2-weighted MRI acquired with different imaging protocols from 2 scanners. To facilitate interpretation of results from heterogeneous datasets, we applied a normalization approach using intra-image NAWM. Use of internal reference is well adopted in quantitative MRI analysis. This can be particularly useful for multi-center clinical trials, where use of different scanners is common. Moreover, with the assessment of fresh brain specimens, potential variations in MRI outcome due to protein cross-linking after formalin fixation can be minimized. This makes it possible to test such postmortem results with in vivo MRI in future studies. Localized analysis of multi-scale spectra in clinical MRI may be useful for assessing myelin status in MS lesions.

We note some limitations in this study. The number of lesions per category is relatively small, which may have limited our statistical power. Nonetheless, we evaluate multi-scale spectra at...
each voxel, which is summarized per lesion with controlling for intra-subject and intra-sample variances. This may have indirectly increased the diversity of our samples. To classify lesion states, we focused primarily on inflammation and demyelination. While this excludes other processes of lesion pathology such as axonal injury, it is a typical practice and has been used in several publications.\textsuperscript{20, 21} In this study, we have not explicitly evaluated partial demyelination that is common in MS lesions; however, lesions with remyelination are often not fully repaired, as seen in figure 2, and these lesions are in fact partially demyelinated entities. Not surprisingly, an intermediate heterogeneity is identified in such remyelinated lesions. With assessment of individual scales and the distribution of multi-scale spectra besides texture heterogeneity, remyelinated lesions may be differentiable from demyelinated lesions using clinical MRI.

In summary, mathematical analysis of local multi-scale spectra derived from standard MRI may have the ability to differentiate myelin integrity between different types of MS lesions. This ability could have significant clinical implications including monitoring disease activity in clinical practice. Furthermore, with the rising interest in discovering remyelination therapies, this image post-processing method may help screen new treatments thereby to improve the prognosis of patients with MS and other demyelinating disorders.

Acknowledgement

We thank the patient donors and their families for supporting this research and Professor Paul van der Valk (Neuropathologist, Amsterdam) for assessing lesion pathology. We also thank the following agencies for funding this project including the MS Society of Canada (ID: 1609), Natural Sciences and Engineering Council of Canada (ID: 418737-2012), Alberta Innovates – Health Solutions (Zhang), and the Dutch MS Research Foundation (ID: 09-358d MS).
REFERENCES


Supplemental Figure 1 | Demonstration of the calculation method for local, multi-scale spectral analysis. Panel A shows an example MR image. The total frequency content of image A is calculated using fast Fourier transform (FFT) as shown in Panel B, where the small box highlights the location of an individual frequency. Through computation of the inverse FFT (iFFT) of the highlighted frequency, a cartoon distribution of this frequency in the image domain is shown in Panel C, which reflects the share of this one frequency at individual voxel locations of the image (Panel A). Panel D shows 4 stacks of frequencies at 4 cartoon voxels. This is obtained by repeating the iFFT process for each frequency contained in Panel B. Individual colors in each stack of Panel D represent specific frequency resolutions or scales localized at a voxel.

Supplemental Figure 2 | Normalized MRI texture heterogeneity in each type of MS lesions and normal appearing white matter (NAWM). Shown in plots are mean and standard errors of the heterogeneity summarized from all frequency scales in a tissue in arbitrary unit (a.u). The triangles indicate significant difference between lesions (active, chronic active: ChrActive, and chronic inactive: Chlnactive) and NAWM (p < 0.01). No difference between pre-active and remyelinated lesions.