T2 MRI spectra detect myelin differences between types of multiple sclerosis lesions

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Running head: T2 MRI spectra of myelin in MS lesions
Number of characters in the title = 84 (with spaces); in the running head = 38;
Number of words in the abstract = 241; in the body of manuscript = 3097
Number of figures = 4; color figures = 1; tables = 1.

Abstract

Objective: Lesions with different types of pathology are found at autopsy in multiple sclerosis (MS). The goal of this study was to determine lesion characteristic in MRI using clinical protocols.

Methods: We imaged fresh brain specimens from 21 MS patients using 1.5T scanners and applied image texture analysis. Through staining of tissue sections for inflammation (microglia and microphages) and myelin (proteolipid protein), MS lesions were classified into 5 categories: pre-active, active, chronic active, chronic inactive, and remyelinated. Based on standard T2-weighted images, texture analysis was performed using a local spatial frequency-based approach that provided multi-scale MR spectra at each voxel.

Results: Following linear mixed-effect modeling, we found that normalized MRI texture heterogeneity was greater in active, chronic active, and chronic inactive lesions that had complete demyelination than in normal appearing white matter (NAWM; $p < 0.01$). In combination, these 3 lesion types as a group were more heterogeneous than pre-active ($p = 0.02$) lesions and NAWM ($p < 0.01$), both with apparently intact myelin. Furthermore, the distribution of remyelinated spectra was similar to that of NAWM but different from demyelinated lesions ($p < 0.01$).

Interpretation: While the 5 defined tissue categories not completely distinguishable at 1.5T, we differentiated demyelination from remyelination and discovered that changes in myelin content drove the severity of texture abnormality. Assessing local patterns of image spectra in clinical MRI may become a useful approach in determining the efficacy of remyelination strategies in MS and other demyelinating disorders (241).

Introduction

Heterogeneity of white matter lesions has been repeatedly found in postmortem neuropathology studies of multiple sclerosis (MS) [1, 2]. Some lesions include features of demyelination and inflammation, others show inflammation but still intact myelin integrity, and yet others demonstrate significant remyelination resembling the structural regularity of normal appearing white matter (NAWM). The various pathological lesions may reflect distinct degrees of tissue injury and repair, and the ability to determine lesion characteristics using MRI is critical as it not only enhances our understanding of disease progression but also helps promote the discovery of new therapies for MS patients.

Identifying lesion pathology in MS has been an ongoing challenge with traditional MRI approaches, highlighting the need for alternative strategies. As shown in previous studies, both demyelinated and remyelinated lesions appear similar in T2-weighted MRI[3], and a spectrum of pathological processes can be observed in a single snap shot of a lesion, providing additional complexity for subclinical evaluation and clinical correlates[4-6]. On the other hand, there is also an intrinsic relationship between tissue pathology and MR images. Biophysically, the content and regularity of lesion structure determine the homogeneity of image voxels[7], and lesions with greater tissue damage should give rise to more heterogeneous organization of MRI signal intensity[8]. Based on this relationship, we can estimate characteristics of tissue microstructure by assessing the local spatial pattern of MRI signal intensity, namely, MRI texture analysis, using mathematical algorithms.

Over the last decades, MRI texture analysis has been used to evaluate the severity of tissue pathology in a variety of neurological diseases including MS. Using standard MR images, texture analysis demonstrates the ability to differentiate normal from abnormal MS tissue, and to distinguish active from inactive MS lesions in the brain[9-11]. Recently, using a new local spatial frequency-based algorithm[12-14], MRI texture analysis detected differences of demyelination, axonal injury and inflammation between focal and diffuse MS abnormalities in fixed postmortem brain samples[15]. In the current study, we adapted this localized texture analysis method to determine whether different types of MS lesions show different texture spectra in the MRI of unfixed postmortem brains, and whether the texture signature relates to the myelin content in lesions as validated by histology.

Method

Patients and autopsy

All brain specimens were obtained from the Netherlands Brain Bank (Amsterdam, the Netherlands). Coronal brain sections from 21 patients with MS were obtained after rapid autopsy (mean postmortem delay was 8 hours 24 minutes) (Table). Subjects included 11 females and 10 males, and age at autopsy was 62 ± 11 years [mean ± standard deviation (SD)]; mean disease duration was 23 ± 14 years. Prior to death, all donors gave written informed consent for the use of their tissue and medical records for research purposes. Ethics approval was obtained from the local Institutional Ethics Review Board. Patients with MS as well as other neurological conditions were excluded. Autopsy procedure and tissue sampling followed the MS Center Amsterdam autopsy protocol which has been

previously described[16]. Briefly, for each patient, five 10-mm-thick unfixed coronal hemispheric brain slices were cut and subjected to MR imaging. White matter abnormalities visible on T2-weighted MR imaging as well as NAWM were then sampled.

MRI protocol

Postmortem brain slices were scanned according to a published autopsy protocol[16, 17] using 1.5T whole body MR systems (Sonata or Avanto, Siemens Medical Systems, Erlangen, Germany), with a standard circularly polarized head coil (Sonata) or a 12-channel phased-array head coil (Avanto). From each system, conventional T2-weighted MR images were acquired, centered at the middle of the sample and parallel to the coronal surface. For Sonata, repetition time/echo time (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 individual brain specimens. These tissue blocks were then fixed in 10% formalin and embedded in paraffin. Subsequently, 5-µm-thick sections were cut, mounted onto glass slides (Superfrost, VWR international, Leuven, Belgium), and dried overnight at 37ºC. Sections were deparaffinized in a series of xylene, ethanol, and water. Endogenous peroxidase activity was blocked by incubating the sections in methanol with 0.3% H2O2 for 30 minutes. After this procedure, the sections were rinsed with 0.01 M phosphate-buffered saline

(PBS, pH7.4). Staining and immunohistochemistry were performed on adjacent sections with antibodies against microglia/macrophages (anti-HLA-DR, clone LN3, kindly provided by Dr. Hilgers, Department of Obstetrics and Gynaecology, Amsterdam) for inflammation, proteolipid protein (PLP; Serotec, Oxford, UK) for myelin, and luxol fast blue periodic acid Schiff (LFB-PAS; Pfizer, New York City, NY) for remyelination. Visually, remyelinated lesions were also identified as 'shadow plaques' indicating diffusely reduced myelin stain[3] as compared to NAWM. Staining signals were detected using the EnVision method (DAKOCytomation, Glostrup, Denmark) with 3,3'diaminobenzidine (DAB) as the chromogen.

Scoring, classification, and matching

White matter lesions were scored by an experienced pathological examiner (Dr. Paul van der Valk, Amsterdam) and classified according to established criteria [18, 19]. Specifically, each lesion was classified into one of 5 categories: pre-active (clustered microglia, no demyelination), active (demyelination with influx of inflammatory cells), chronic active (demyelination with inflammation in the border, but not in the center of lesion), chronic inactive (demyelination without inflammation, and with gliosis), and remyelinated (shadow plaques). Furthermore, NAWM areas (no apparent demyelination or inflammation) were examined to control for measurements across samples. Histological sections containing lesions of interest (Fig. 1) were matched to corresponding postmortem T2-weighted MR images as described previously[17]. Lesions and NAWM regions were outlined at matching locations in T2-weighted MRI using ImageJ (v1.47, National Institutes of Health).

MRI texture analysis

The signal intensity patterns of T2-weighted MRI were evaluated using a local spatial frequency-based program as reported previously[15]. In brief, the entire frequency content enclosed in the whole MR image was initially calculated using the classical Fourier transform; then, the spatial distribution of each frequency at individual spatial resolutions of the image was determined by applying the inverse Fourier transform. By repeating this process for all frequencies, one multi-scale spectrum at each voxel was generated, representing the local organizing pattern of tissue structure at different scales. Subsequently, the cosine similarity of these localized spectra between lesion and NAWM voxels was computed in each image. This provided us a measure of tissue heterogeneity summarized from all structural scales localized around each voxel[20].

In addition to assessing the overall tissue heterogeneity, we also calculated the averaged spectrum per frequency in a region of interest (ROI) and identified spectral patterns that best distinguished the myelin content across lesion subtypes. 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, to obtain the normalized T2 texture heterogeneity that was used in subsequent analyses. In addition, an equivalent range of lesion sizes was chosen for assessment between scanners before outcome analyses to compensate for resolution differences. Lesions that were smaller than 2x2 voxels or neighboring CSF boundaries or vessel walls were excluded to avoid partial volume effect in MRI.

Statistics

A linear mixed-effect model was used to assess differences in normalized T2 texture heterogeneity between tissue types from all scans. Different lesion types and NAWM were coded separately. In this model, the type of tissue pathology was considered the fixed effect, and variances between patients and between specimens within patients were defined as random effects. Then, to determine whether normalized T2 texture heterogeneity was different between tissue types with different myelin states, active, chronic active, and chronic inactive lesions were combined as one group, which was compared with the following 3 tissue groups: pre-active lesions, remyelinated lesions, and NAWM. Bonferroni correction was used to account for multiple comparisons after each fitting of the model; $p \leq 0.05$ was set as significance. Subsequently, to identify whether and how specific frequency resolutions explained tissue heterogeneity, a multivariate analysis was used to evaluate spectral differences between the latter 4 tissue groups; likelihood-ratio test was performed with settings allowing for the presence of different covariance matrices between groups. All statistical analyses were conducted using Stata (StataCorp LP, College Station, Texas, USA; version 12.0).

Results

Lesion and sample demographics

We matched 119 tissue blocks that included 70 lesions and 49 NAWM regions from 21 MS patients; 9 of these patients were examined at the Sonata scanner while 12 were scanned with the Avanto system. For each patient, an average of 3 lesions (range 1 - 7)

and 2 NAWM areas (range 1 - 5) were examined. The size of the evaluated lesions ranged from 1.1x1.1 mm^2 to 25.2x25.2 mm^2. Based on staining properties for myelin and inflammation, 9 lesions were classified as pre-active, 24 as active, 23 as chronic active, 11 as chronic inactive, and 3 as remyelinated (Table). All lesions were located in the supratentorial white matter. At least one NAWM area was analyzed in each tissue slice.

Increased texture heterogeneity in the subtype of MS lesions as compared to NAWM

Based on the mixed-effect model, we found that normalized T2 texture heterogeneity was different between tissue types ($p < 0.01$). Specifically, it was significantly higher in active ($p < 0.001$), chronic active ($p < 0.001$), and chronic inactive ($p = 0.001$) lesions than in NAWM. Moreover, normalized T2 texture heterogeneity was higher in chronic active lesions ($p = 0.01$) than in pre-active lesions. There was no difference in normalized T2 texture heterogeneity between active, chronic active, and chronic inactive lesions ($p = 1.0$), and there was also no difference between NAWM and pre-active or remyelinated lesions ($p = 0.39$ and 1.00 respectively; Fig. 2).

Greater texture heterogeneity in MS lesions with greater demyelination

Based on our customized design that enabled further stratification of MS lesions according to their myelin states, we assessed the structural property of the following 4 tissue types: 1) intact myelin (NAWM); 2) intact myelin with microglia clustering (pre-active lesions); 3) demyelination with or without inflammation or gliosis (active, chronic active, and chronic inactive lesions); and 4) remyelinated lesions. After correcting for variances between specimen and ROIs, we found that normalized T2 texture

heterogeneity of demyelinated lesions (active, chronic active, and chronic inactive lesions) was greater than that of NAWM ($p = 0.000$) and pre-active lesions ($p = 0.02$), but not significantly greater than remyelinated lesions in this measurement ($p = 0.56$; Fig. 3).

Similar spectral patterns between pre-active and remyelinated lesions and NAWM

Besides assessing texture heterogeneity summarized from all frequency resolutions, we also examined the distribution pattern of texture spectra spanning from low to high frequencies in each tissue. Using a multivariate test for multiple groups, we discovered significant differences between demyelinated and myelinated spectra ($p < 0.01$), especially over frequencies 0.105 to 0.126 Hz/mm, which corresponded to lesion diameters of 5 to 7 mm. In contrast, there was no significant difference between NAWM, pre-active and remyelinated spectra in any frequency that corresponded to the range of lesion sizes included in this study, and the distribution pattern of these myelinated spectra appeared similar to each other, in contrast to the demyelinated spectra (Fig. 4).

Discussion

In this validation study, we used unfixed brain specimens to show that different types of MS lesions generated distinct MRI texture signatures. In particular, the myelin content in these lesions appeared to drive the pattern of their MRI texture. As hypothesized, lesions with demyelination (with or without inflammation) were more heterogeneous than lesions with intact myelin and only inflammation. Moreover, the spectral distribution of remyelinated lesions was similar to that of apparently intact myelin in NAWM, but not of demyelinated lesions, suggesting the possibility of using multi-scale texture spectra to

evaluate myelin repair. As our results were based on clinical MRI protocols applied to unfixed brain tissue, this approach may be used *in vivo* after further validation.

Pathological variance in 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 spectrum of pathological processes is often seen[1]. To accurately evaluate lesion activity, MS lesions are often divided into several subtypes based on their inflammatory and demyelinating properties[18, 19]. Using this standard, we classified MS lesions identified in this study into 5 categories: active, chronic active, chronic inactive, pre-active, and remyelinated lesions. In addition, instead of simply determining the existence of myelin loss in active, chronic active, and chronic inactive lesions, we defined these lesions as being completely demyelinated; lesions with any evidence of preserved myelin were excluded. This specific setting provided us a unique opportunity for additional grading of lesion severity.

Consistent with pathological characteristics, we found that lesions with greater tissue damage caused more heterogeneity of normalized texture in standard T2-weighted MRI. Previously, using fixed brain samples with MS, T2 texture heterogeneity at 7T was found to increase from NAWM to diffusively abnormal WM and then to focal MS lesions, corresponding to increasingly greater demyelination, axonal loss, and at a lesser degree, inflammation[15]. In the present study, with further classification of lesion pathology, we demonstrated that normalized T2 texture heterogeneity tended to be different between different categories of MS lesions. In particular, active and chronic lesions containing

both demyelination and inflammation showed the worst texture heterogeneity, while pre-active lesions with only inflammation had the least heterogeneity.

Subsequently, through mixed-effect modeling of recombined lesion groups, we discovered that the integrity of myelin drove the heterogeneity of MRI texture in MS lesions. In this study, based on our classification criteria, MS lesions with absolutely no myelin (active and chronic lesions), apparently intact myelin (pre-active lesions), and reformed myelin (shadow plaques) were evaluated. We identified that lesions with apparently healthy myelin had the least normalized T2 texture heterogeneity, similar to that seen in NAWM; those with no myelin had the strongest heterogeneity; and remyelinated lesions demonstrated an intermediate heterogeneity. Conversely, the active, chronic active, and chronic inactive lesions that had variable degrees of inflammation but equally no myelin did not show differences in normalized T2 texture heterogeneity, and the heterogeneity of pre-active lesions was not significantly greater than that of NAWM, both with apparently intact myelin. These results suggest the importance of myelin integrity to MRI texture heterogeneity and is consistent with previous findings using 7T imaging of fixed brain samples, showing that myelin density stained with luxol fast blue was independently correlated with the heterogeneity of T2 MRI texture in MS brain[15].

Given the difference in normalized T2 texture heterogeneity between lesions with different myelin integrity, we further examined the spectral distribution patterns of myelinated and demyelinated lesion voxels to explore how individual frequency resolutions related to myelin integrity. In contrast to normalized T2 texture heterogeneity

that reflected the joint abnormality from all structural scales, the distribution of multi-resolution spectrum revealed the heterogeneity of tissue structure at each scale associated with individual frequencies (Fig. 4). In this study, we identified a marked increase in the spectral amplitude of demyelinated lesions, in contrast to the decrease in myelinated spectra over frequencies 0.07 to 0.126 Hz/mm. Between myelinated lesions that shared similar spectral distribution, we found that the spectral amplitude of remyelinated voxels remained to be higher than that of pre-active lesion voxels, which was also higher than of NAWM voxels. This may be explained by the microstructural property of reformed myelin in remyelinated lesions, where the myelin sheath is thinner and segments are shorter between nodes than that of intact myelin[21]. While this data is still preliminary, it supports the investigation of local spectral distributions of T2 MRI texture for assessing remyelination.

The identification of a measure of remyelination is critical for monitoring disease evolution and therapeutic efficacy in demyelinating disease. In MS, remyelination occurs naturally[22, 23], and promoting remyelination is thought to be a promising means to improve prognosis[24]. However, identifying remyelination *in vivo* has been a major challenge. In the literature, several myelin-relevant MRI techniques are being developed and have demonstrated promising results[25-27], but the feasibility of using these methods in clinical studies needs further verification. Moreover, myelin-sensitive MRI approaches may not be able to differentiate myelin from myelin debris[5, 28], an inherent and abundant product of demyelination. In this study, we found that mathematical modeling of tissue

MRI texture may be an alternative approach for measuring myelin integrity using standard MRI. This is in agreement with experimental findings shown previously[29].

In this study, we used T2-weighted images acquired from different scanners with different imaging protocols. After normalization with the texture of NAWM, we found that combining results from different MRI datasets is feasible, as validated by histology. In multi-center clinical trials, the use of different scanners is common. This often leads to significant variances in image inhomogeneity that in turn can create enormous obstacles for quantitative MRI analyses[30, 31]. As attested in this study, the use of an internal reference may be an option. In this way, both scanner inhomogeneity and anatomical variances between patients could be minimized. In addition, all of the brain specimens were imaged within 11 hours of postmortem process, and this makes the interpretation of our data possible in the context of in vivo imaging. As T2-weighted MRI is routinely acquired in the standard care of patients, this approach may be directly tested in a clinical setting without compromise of care delivery.

There are some limitations in this study that need to be addressed in the future. The number of lesions per category is relatively small, which may have limited the statistical power of our results. Nonetheless, we found significant differences between lesions with different degrees of myelin integrity and identified consistent spectral patterns between myelinated voxels after normalization. Moreover, to evaluate lesion states, we focused primarily on inflammation and demyelination. While this is a standard approach and has been used in several established studies[18, 19], we seek to evaluate additional aspects of MS

pathology including axonal integrity. We also plan to use a large sample size to confirm our findings in this study.

Conclusion

Using non-fixed postmortem brain specimens, we verified the feasibility of using an image processing approach, local spatial-frequency analysis, to discern MS lesion types in standard T2 MR images. We discovered that changes in myelin integrity are sufficient to produce differences in T2 MRI texture heterogeneity between MS lesions. Moreover, lesions with remyelination seemed to have regained a degree of structural regularity but still had greater heterogeneity than the NAWM. These findings suggest the possibility of determining myelin integrity in MS lesions using localized texture analysis in clinical MRI. With further validation, this approach may become a useful method of evaluating remyelination therapies.

Acknowledgement

We thank the patient donors and their families for supporting this research, the Netherland Brain Bank for providing these valuable brain specimens, and Professor Paul van der Valk (Neuropathologist, Amsterdam) for assessing lesion pathology. We appreciate the funding support from the Natural Sciences and Engineering Council of Canada, the MS Society of Canada, and Alberta Innovates – Health Solutions;, and the funding support from the Dutch MS Research Foundation (grant number 09-358d MS).

For this manuscript, each author has contributed in a number of ways. Y Zhang, L Jonkman, and J Geurts have perceived and designed the study, drafted and edited the manuscript; F Barkhof has invested expertise in the MRI experiments and edited the manuscript; A Klauser, VW Yong, and LM Metz have provided analytical, histological, and clinical expertise and edited the manuscript.

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Figure captions

Figure 1: T2-weighted MRI, texture map, and histology of example MS lesions, organized in columns 1 to 4. Lesions of interest are highlighted both with boxes and blown-outs (arrows) in T2 MR images (1st column) and texture maps (2nd column); corresponding histology at these locations are shown in the staining images of proteolipid protein for myelin (brown, 3rd column) and microglia and microphages for inflammation (aggregates of brown, 4th column). Colors in texture maps represent texture heterogeneity: smallest with dark blue, intermediate with light blue, and strongest with gray. The texture maps of pre-active (A) and remyelinated (E) lesions are generated at the same frequency of 0.54 hz/mm, and texture maps of active (B), chronic active (C), and chronic inactive (D) lesions at 0.32 hz/mm. Higher and lower frequencies correspond to finer and coarser texture.

Figure 2. Normalized T2 MRI texture heterogeneity in the 5 types of MS lesions defined in this study from all samples. There is a trend of increasing heterogeneity from NAWM, to pre-active lesions, and then to active, chronic active, and chronic inactive lesions ($p < 0.01$); normalized T2 texture heterogeneity of remyelinated lesions appears to be higher than pre-active lesions but lower than active and chronic lesions ($p > 0.05$). Plots demonstrate the mean and standard error of normalized heterogeneity. NAWM: normal appearing white matter; ChrActive: chronic active; ChInactive: chronic inactive.

Figure 3. Normalized T2 MRI texture heterogeneity in lesion groups with distinct myelin states. Based on our criteria, lesions with complete loss of myelin (active, chronic active,

and chronic inactive lesions) from both scanners were grouped as demyelinated lesions. Compared with intact myelin (pre-active lesions and NAWM), the normalized T2 heterogeneity of demyelinated lesions is significantly greater (*), and tended to be greater than remyelinated lesions that demonstrate similar heterogeneity to pre-active lesions.

Figure 4. The distribution of multi-scale spectra in different lesion types and normal appearing white matter (NAWM). Over low and intermediate frequencies, while with different amplitude, the spectral distribution shapes are similar between remyelinated lesions, pre-active lesions, and NAWM (left pannel). Conversely, the active and chronic lesions demonstrate markedly different distributions as compared to NAWM (right panel), particularly over low frequencies (dotted lines) showing an opposite spectral pattern to that of NAWM.

Table: Patient and lesion demographics.

Patient ID	Sex	Age, y	Scanner ID	MS Type	MS Duration, y	PMD (h:min)	# lesions	# NAWM
1	M	71	1	ND (chronic)	26	7:00	1	2
2	F	70	1	PPMS	40	6:55	4	3
3	F	57	1	ND (chronic)	21	20:00	3	2
4	M	47	1	SPMS	7	7:15	1	1
5	F	44	1	PPMS	8	10:15	2	1
6	F	76	1	PPMS	19	9:45	1	1
7	F	69	1	ND (chronic)	53	7:30	4	2
8	M	63	1	SPMS	24	7:05	6	4
9	F	81	1	ND (chronic)	64	4:00	2	2
10	F	77	2	ND	24	10:00	5	3
11	M	72	2	ND (chronic)	23	7:55	1	1
12	M	50	2	ND (chronic)	15	5:25	4	2
13	F	66	2	SPMS	22	6:00	1	1
14	M	49	2	SPMS	24	8:00	6	5
15	F	60	2	PPMS	7	8:50	3	2
16	M	54	2	PPMS	12	8:15	7	2
17	F	50	2	SPMS	17	7:35	5	4
18	M	56	2	ND	13	10:10	1	1
19	M	67	2	ND	37	11:00	5	4
20	F	81	2	ND (chronic)	21	6:30	1	2
21	M	54	2	SPMS	ND	7:00	7	4

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.