

Comparison of myelin-sensitive quantitative MR and histological markers in Alzheimer's disease tissue samples

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Impact

In the context of ADRD, factors other than myelin quantity may affect conventional MWF and MTR, including axonal density. Disentangling the contributions of histological features that affect these measures is important for using them to study white matter in ADRD.

Synopsis

Motivation: The study of white matter in Alzheimer's disease and related dementias (ADRD) is increasingly important, but myelin-dependent MR measures are not well characterized in ADRD.

Goals: We aim to determine histological correlates of quantitative MRI parameters myelin water fraction (MWF), magnetization transfer ratio (MTR), and macromolecular pool fraction (Fsat).

Approach: Using single and multivariable regression analysis, we compared MWF, MTR, and Fsat from ADRD samples to histological measures of myelin content (myelin basic protein), axonal density (neurofilament), and oligodendrocyte count across GM, WM, and WM lesion ROIs.

Results: Myelin content and axonal density independently contributed to variations in MWF, MTR, and Fsat.

Introduction

Accumulating evidence suggests that significant myelin degradation occurs in ADRD¹⁻³. Understanding the effects of histopathological features in ADRD on MR measures of myelin is necessary toward developing myelin-specific MR measures that remain valid in the context of ADRD. This abstract focuses on myelin-sensitive quantitative MRI (qMRI) parameters in ADRD tissue samples and compares them to histological quantities of myelin content, axonal density, and oligodendrocyte count.

MRI Methods

Three ex vivo frozen samples with Alzheimer's pathology were procured from the Michigan ADRC brain bank. The samples were thawed for 48 hours, cut into ~13mm thick sections, and placed into histology cassettes for MR scanning in a 7.0T Varian/Agilent scanner at ~15°C to mitigate degradation. We conducted 2D CPMG myelin water imaging (MWI)^{4,5} and magnetization transfer (MT)^{6,7} scans with 1-mm slices⁸. Myelin water fraction (MWF) was estimated from the MWI data, and MT ratio (MTR) and macromolecular pool fraction (Fsat) from the MT data, per [8].

Histological Methods

Samples were fixed in formalin, embedded in paraffin, and microtomed to obtain contiguous 5 μ m slices corresponding to the center of the original sample. Four histological analyses were performed: luxol fast blue (LFB) staining and myelin basic protein (MBP), neurofilament (NF), and OLIG2 (found in oligodendrocyte cell bodies) immunohistochemistry. We used the blue channel minus the red channel as a measure of LFB optical density, the mean intensity value for the DAB chromogen for MBP and NF, and the number of oligodendrocytes detected per mm^2 for the oligodendrocyte count (OC).

Statistical Methods

48 ROIs (1- mm^2) from histology in gray matter (GM), normal appearing WM (NAWM), and WM lesions (WML) were matched to ROIs on fast spin echo (FSE) images. Parameters from the three qMRI maps were extracted and averaged over each ROI. Linear mixed-effects modeling was used for single and multivariable regression with each qMRI parameter as the dependent variable, MBP, NF, and OC as fixed-effect explanatory variables, and sample ID as a random effect to account for variability across samples.

Results

[Figure 1](#) displays qMRI and histology maps for one sample labeled with GM, NAWM, and WML ROIs. The values for the qMR and histology measures show the expected patterns in GM and NAWM, and the values fall somewhere between the two for WMLs ([Figure 2](#)). By looking at matched ROIs between the qMR and histology images, [Figure 3](#) shows the relationship between qMR measures and the underlying histological profile across the ROIs without assumption about tissue type. Results indicate significant correlation across the compared pairs, except for MWF vs. OC. Given the dependency of the histology measures on fiber and cellular density, we used multivariable regression analysis to determine whether the variation in qMR measures could be explained by MBP alone. Multivariable regression models that included both MBP and NF as independent variables performed better than models using MBP alone, as indicated by significant differences in the maximum likelihood estimates using ANOVA, and lower Akaike information criterion (AIC) values for combined models. In contrast, Fsat was well modeled with MPB alone. OC did not contribute to improving multivariable regression models. These findings are demonstrated in [Figure 4](#), which shows regression plots for mean-adjusted residual qMR data after regressing out MBP. Although MBP and NF are both affected by fiber density, ROIs with low MBP are not always associated with low NF and vice versa ([Figure 5a](#)), and the relation between MBP and NF is distinct in GM vs. WM. The high correlation between Fsat and MTR ([Figure 5c](#)), and deviation in MWF vs. MTR ([Figure 5b](#)), suggests that MWF and MTR provide partially distinct information about the ROIs.

Discussion

The estimated qMRI parameters match literature values^{4,9}. MBP and NF both contributed to qMRI parameter variations seen in the multivariable regression observed across ROIs. This is consistent with previous studies that show that MWF estimates are biased by axonal diameter (g-ratio), and that MTR is correlated to NF, likely due to the non-myelin macromolecules in axons¹⁰⁻¹⁶. Limitations include the low number of samples and ROIs due to challenges in co-registering histology and qMR maps, and lack of measures such as inhomogeneous MTR that are more specific to myelin¹⁷, and will be addressed in future studies.

Conclusion

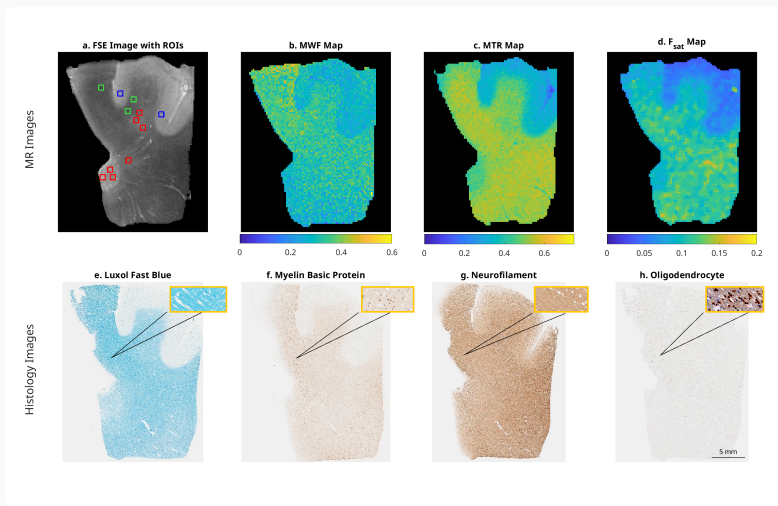
Variations in MBP and NF seem to contribute to the variation seen in the qMRI maps of ADRD tissue samples with WMLs, indicating that myelin content, as well as axonal density, independently contribute to variations in MWF, MTR, and Fsat signals. Knowing which histological parameters affect MR metrics in these samples may aid in understanding the impact of WM degradation on ADRD.

Acknowledgements

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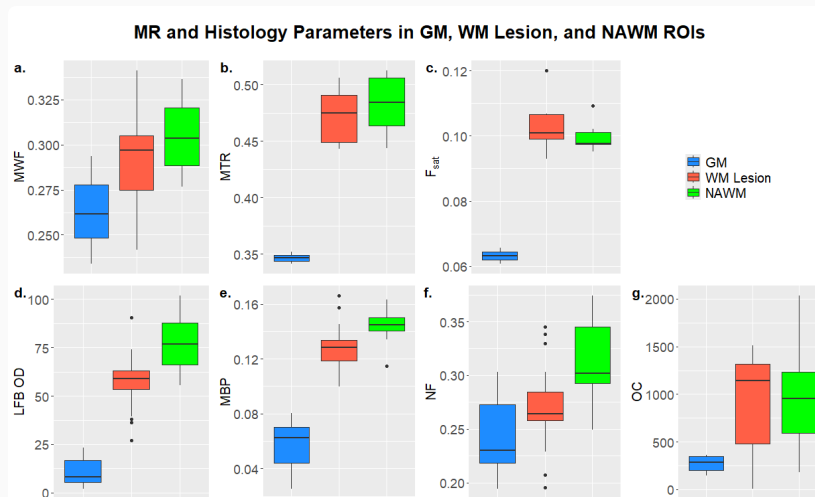
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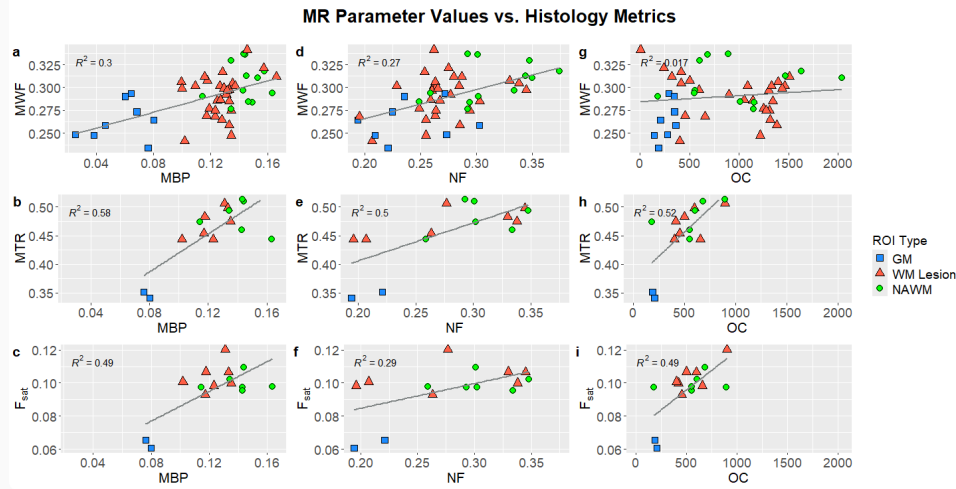
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Figure 1: qMRI maps and histology images for a human brain tissue sample with AD pathology. (a) Fast spin echo image ($TR=4s$, $TE_{eff}=40ms$, $ESP = 10ms$, segments = 32, echo train length= 8) with ROIs; green=NAWM, red=WML, blue=GM. (b) MWF calculated with a 20ms cutoff. (c) MTR calculated with the average of the -10kHz and 10kHz MT off-resonance images at an $8.8\mu T$ power level. (d) Fsat map. (e) Luxol fast blue stain. (f-h) Immunohistochemistry for MBP, NF, and OLIG2 (insets from 100x300 micron WM area).



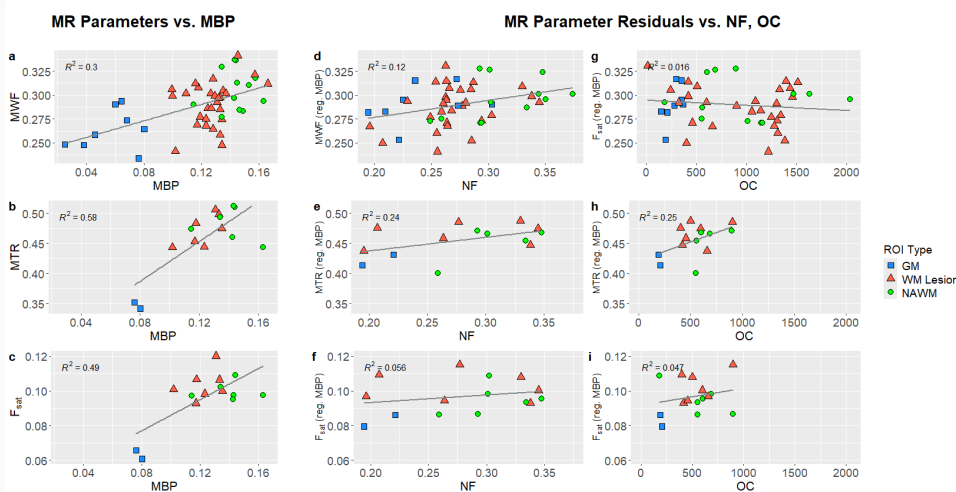
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Figure 2: Box plots (interquartile range, whiskers: 1.5-times interquartile range, outliers as dots) of average mean-adjusted MR (a-c) and histology parameters (d-f) across tissue samples. (g) OC is not mean-adjusted. Parameter values are consistent with literature values [4,9] and are generally highest in NAWM and lowest in GM. The MTR and Fsat boxes are smaller due to a limited number of GM ROIs in these samples, due to having only single-slice MT data.



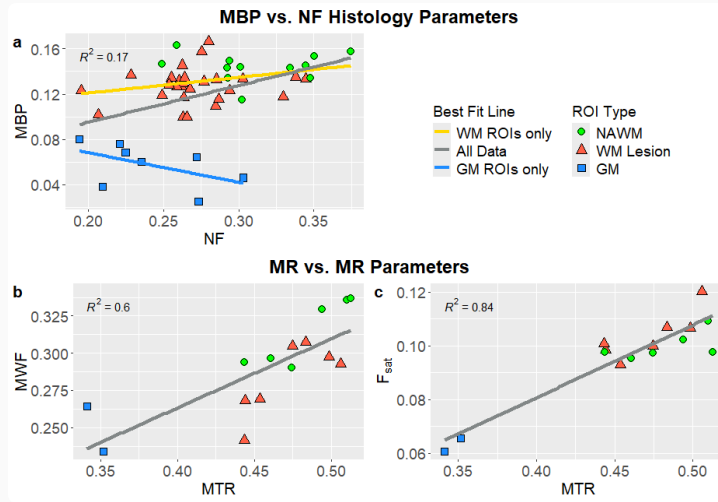
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Figure 3: Single-variable regression analysis between qMRI and histology values across samples. All qMRI parameters correlate with MBP and NF. R^2 values are relatively low; however, the correlations are statistically significant ($p < 0.05$) for all plots except (g). (a-c) MBP plots show a distinct separation between GM and WM ROIs. (d-f) NF plots have GM ROIs with NF content closer to WM values. (g-i) MTR and Fsat correlate with OC.



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Figure 4: Pictorial representation of multivariable analysis. (a-c) qMRI parameters vs. MBP. After regressing for MBP, the residual qMRI values were mean-adjusted and plotted (d-f) vs. NF and (g-i) vs. OC. After the correlation with MBP was removed from the data, MWF still correlated with NF but not OC, MTR correlated with both NF and OC, and Fsat with neither. Lower AIC values indicated a better model with multivariable analysis (MBP and NF) as compared to MBP alone. $\Delta AIC = 5.1$ (MWF) and 4.6 (MTR).



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Figure 5: (a) MBP vs. NF; MBP variance is not well explained by the variation in NF indicating myelin and axonal loss can be independent. The difference in slopes of the fitted lines for GM vs. WM is consistent with more densely packed, thicker axons with more myelin in WM and areas of dense neuronal processes with less myelin in GM. (b) MWF vs MTR and (c) Fsat vs MTR; lesion ROIs deviate from the fitted line in (b) but not in (c), suggesting that MWF and MTR convey different information about WM lesions.