Addressing the enigma of invisible pathology in MS: a multimodal approach to characterize diffusely abnormal and normal-appearing white matter

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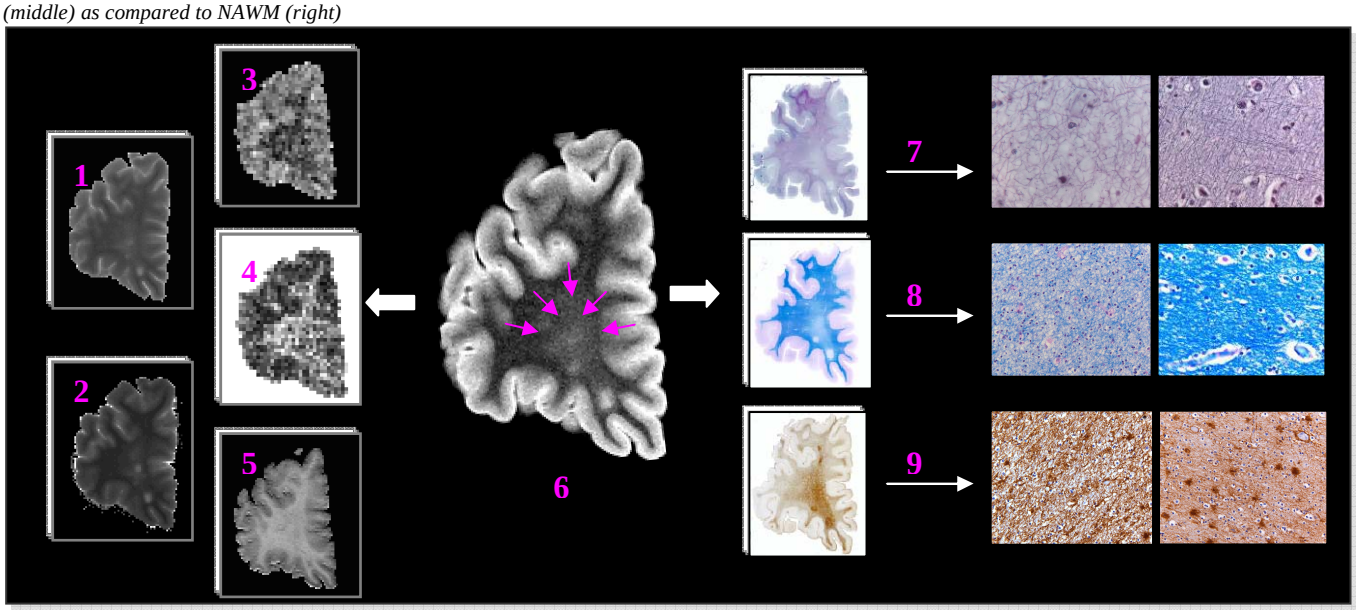
Objective:
Pathology beyond MR visible lesions in the NAWM (normal-appearing white matter) and DAWM (diffusely abnormal white matter; not generally scored in clinical practice) in multiple sclerosis (MS) brains are both suspected to contribute to the clinico-radiological dissociation (Figure 6).
As the histopathological correlates of DAWM are unknown, the aim of this study was to radiologically and histopathologically characterize tissue changes in DAWM (detected on T2-weighted MRI). Furthermore, we investigated whether NAWM is normal in histopathological terms, and whether quantitative MRI may be better equipped to reveal subtle pathology in NAWM, as qualitative MRI does not.

Materials and Methods:
17 formalin-fixed, coronally cut brain slices from 10 chronic MS patients (7 females) were examined both with qualitative MRI at two different field-strengths: (1) dual-echo T2 spin-echo at 1.5T (TR/TE/NEX: 2755ms/45ms/90ms/2; FoV: 80x128mm; matrix size: 160x256; slice thickness: 3mm) (Figure 6), (2) and a mainly proton density [PD] weighted sequence at 4.7T (FSE3D; TR/TE/NEX: 4000ms/9ms/2; echo train length: 8; partition thickness: 0.39mm; 64 partitions per slab; FoV:100x100mm; matrix size 256x256). Furthermore, we performed quantitative MRI at 1.5T: magnetization transfer ratio (MTR) using Flash3D with a 7.68 ms Gaussian pre-pulse of frequency offset 1500 Hz and equivalent flip angle 500°; diffusion tensor imaging (DTI) using a STEAM sequence with 6 diffusion encoding directions and b=750 s mm^{-2}; to obtain apparent diffusion coefficient (ADC) and fractional anisotropy (FA); T1-mapping using a Flash3D sequence with a range of flip angles (2-25°) and B1 correction; and T2-mapping using a CPMG sequence (Figure 1-5). In NAWM and DAWM (1.5T), a total of 31 regions of interest (ROIs) were placed. These ROIs were compared with corresponding ROIs on the 4.7T PD and on histological slices (10µm) (Figure 7-9). Quantitative MRI measurements were correlated with histopathological findings using a commonly adopted correlation approach. Histopathology included assessments for axonal- and myelin density, astrogliosis, microglial reactivity, acute axonal pathology, and blood-brain barrier leakage.

Fig. 1-5:Quantitative MRI: 1:T2-map; 2: T1-map; 3:ADC; 4: FA; 5: MTR
Fig. 6: Qualitative MRT at 1.5T; arrows: DAWM
Fig. 7-9: Histopathology: 7: Bodian silver stain (left), showing axonal loss in DAWM (middle) as compared to NAWM (right); 8: Luxol fast blue stain (left), showing myelin pallor in DAWM (middle), as compared to NAWM (right); 9: Stain for glial fibrillary acidic protein (left), showing fibrillary gliosis in DAWM (middle) as compared to NAWM (right)



Results:
DAWM at 1.5T was also diffusely abnormal at high-field (4.7T). This suggests that DAWM is not simply a confluent, multifocal lesional pattern, but may reflect a separate pathological process. This was confirmed by histopathological findings, showing that axonal loss, myelin pallor and gliosis was greater in DAWM and differed significantly ($p \leq 0.001$) from NAWM. Correspondingly, significant differences ($p \leq 0.001$) of FA, T2 and T1 maps ($p=0.003$) between NAWM and DAWM could be detected (mean FA: 0.75 in NAWM versus 0.52 in DAWM; mean T1(ms): 205 in NAWM, versus 381 in DAWM; mean T2 (ms): 50.6 in NAWM, versus 77.3 in DAWM).
Correlations were detected between T1 and axon counts ($r=-0.48$, $p=0.01$) and gliosis ($r=0.5$,$p=0.01$), as well as between T2 and axon counts ($r=-0.64$, $p=0.01$) and myelin (0.65, $p=0.01$), and between FA and axons ($r=0.50$, $p=0.01$) and myelin ($r=0.55$, $p=0.01$).

Conclusion:
Tissue damage in DAWM is more extensive than that in NAWM and represents a separate pathological process, which reflects the effects of cumulative ongoing pathology in the MS brain, including (secondary) axonal degeneration. Quantitative MR methods are specific in terms of different underlying pathologies and were correlated to histopathological findings.
Inclusion of those subtle tissue changes in measurements of disease burden may result in a more accurate estimation of disease progression than evaluation of the lesion load alone, and it may even influence future treatment decisions.

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