BRAIN COMMUNICATIONS

REVIEW ARTICLE

Quantitative magnetization transfer imaging in relapsing-remitting multiple sclerosis: a systematic review and meta-analysis

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Myelin-sensitive MRI such as magnetization transfer imaging has been widely used in multiple sclerosis. The influence of methodology and differences in disease subtype on imaging findings is, however, not well established. Here, we systematically review magnetization transfer brain imaging findings in relapsing-remitting multiple sclerosis. We examine how methodological differences, disease effects and their interaction influence magnetization transfer imaging measures. Articles published before 06/01/2021 were retrieved from online databases (PubMed, EMBASE and Web of Science) with search terms including 'magnetization transfer' and 'brain' for systematic review, according to a pre-defined protocol. Only studies that used human *in vivo* quantitative magnetization transfer imaging in adults with relapsing-remitting multiple sclerosis (with or without healthy controls) were included. Additional data from relapsing-remitting multiple sclerosis subjects acquired in other studies comprising mixed disease subtypes were included in meta-analyses.

Data including sample size, MRI acquisition protocol parameters, treatments and clinical findings were extracted and qualitatively synthesized. Where possible, effect sizes were calculated for meta-analyses to determine magnetization transfer (i) differences between patients and healthy controls; (ii) longitudinal change and (iii) relationships with clinical disability in relapsing-remitting multiple sclerosis. Eighty-six studies met inclusion criteria. MRI acquisition parameters varied widely, and were also underreported. The majority of studies examined the magnetization transfer ratio in white matter, but magnetization transfer metrics, brain regions examined and results were heterogeneous. The analysis demonstrated a risk of bias due to selective reporting and small sample sizes. The pooled random-effects meta-analysis across all brain compartments revealed magnetization transfer ratio was 1.17 per cent units (95% CI −1.42 to −0.91) lower in relapsing-remitting multiple sclerosis than healthy controls (z-value: −8.99, $P < 0.001$, 46 studies). Linear mixed-model analysis did not show a significant longitudinal change in magnetization transfer ratio across all brain regions [$\beta = 0.12$ (−0.56 to 0.80), t-value = 0.35, $P = 0.724$, 14 studies] or normal-appearing white matter alone [$\beta = 0.037$ (−0.14 to 0.22), t-value = 0.41, $P = 0.68$, eight studies]. There was a significant negative association between the magnetization transfer ratio and clinical disability, as assessed by the Expanded Disability Status Scale [$r = -0.32$ (95% CI −0.46 to −0.17); z-value = −4.33, $P < 0.001$, 13 studies]. Evidence suggests that magnetization transfer imaging metrics are sensitive to pathological brain changes in relapsing-remitting multiple sclerosis, although effect sizes were small in comparison to inter-study variability. Recommendations include: better harmonized magnetization transfer acquisition protocols with detailed methodological reporting standards; larger, well-phenotyped cohorts, including healthy controls; and, further exploration of techniques such as magnetization transfer saturation or inhomogeneous magnetization transfer ratio.

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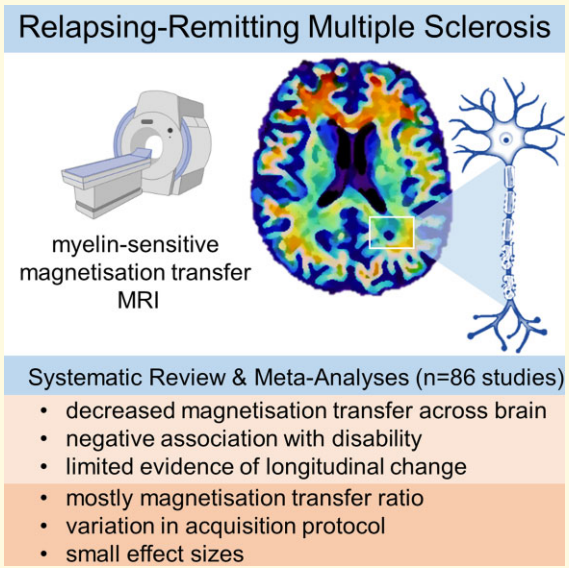
Abbreviations: 9HPT = nine-hole peg test; APLA = anti-phospholipid antibody; BDNF = brain-derived neurotrophic factor CELs = contrast-enhancing lesions; CI = confidence interval; DMTs = disease-modifying therapies; EDSS = Expanded Disability Status Scale; FLAIR = fluid-attenuated inversion recovery; IfN-α/β = interferon-alpha/beta; ihMTR = inhomogeneous magnetization transfer ratio; MS = multiple sclerosis; MTI = magnetization transfer imaging; MSFC = multiple sclerosis functional composite; MTR = magnetization transfer ratio; MTsat = magnetization transfer saturation; NABT = normal-appearing brain tissue; NAGM = normal-appearing grey matter; NAWM = normal-appearing white matter; PASAT = Paced Auditory Serial Addition Test; PPMS = primary progressive multiple sclerosis; qMT = quantitative magnetization transfer; RNFL = retinal nerve fibre layer; ROI = region of interest; RRMS = relapsing-remitting multiple sclerosis; SDMT = Symbol-Digit Modalities Test; SE = spin echo; SPMS = secondary progressive multiple sclerosis; T1-w = T_1-weighted; T2-w = T_2-weighted; T25FW = Timed 25-Foot Walk; TE = echo time; TR = repetition time; WM = white matter

Graphical Abstract



Introduction

Multiple sclerosis: a heterogeneous disease

Multiple sclerosis (MS) is an immune-mediated disease involving widespread focal injury (lesions) to myelin—the fatty sheath which insulates neuronal axons—and nerve fibres within the CNS, accompanied by neuroinflammation.[1] This results in irreversible neurodegeneration.

Demyelination and neuronal damage manifest as heterogeneous clinical disability such as weakness, visual disturbances and cognitive impairment. Acute clinical episodes, or relapses, define the relapsing-remitting MS (RRMS) subtype and are often accompanied by new lesions on MRI. Although diverse in pathological appearance, lesions are indicative of inflammation and demyelination. In RRMS, relapses are interspersed with periods of stability or remission, although the clinical course varies and the choice of effective disease-modifying therapies (DMTs) is currently limited.

Reliable, non-invasive *in vivo* biomarkers are necessary to predict and track disease progression in individuals, and objectively assess the effectiveness of both current and emerging treatments.[2] The relationship between clinical disability and conventional MRI measures of disease burden such as lesion load visible on T_2-weighted (T2-w) imaging[3] and atrophy[4] is, however, weak. This reflects a need for validated quantitative MRI metrics which are more sensitive and specific to disease-related pathological microstructural change in RRMS.

Magnetization transfer imaging

Magnetization transfer imaging (MTI) is sensitive to subtle pathological changes in tissue microstructure which cannot

typically be quantified with conventional MRI.[5,6] MT signal is indirectly derived from protons 'bound' to macromolecules.[7]

Considering a simple two-pool model for hydrogen nuclei in the brain,[8] the so-called 'free' pool of water protons shows relatively unrestricted diffusion and contributes to the bulk source of conventional MRI signal. Hydrogen nuclei in the 'bound' pool, however, are closely coupled to macromolecules (including lipids such as myelin) and have hindered rotational and translational motion, resulting in T2 decays too rapid (~10 μs) for the signal to be detectable at typical echo times (TEs).

MTI exploits the continuous exchange of magnetization between pools to obtain signal indirectly from this 'bound' pool. Since the frequency spectrum of the 'bound' pool is much broader than the 'free' water peak, an applied off-resonance radiofrequency pulse may selectively saturate 'bound' protons. Magnetization exchange between the two pools reduces longitudinal magnetization of the 'free' pool and hence it's signal intensity. Among other factors, the magnitude of this effect depends on the size of the 'bound' pool, which hence provides a surrogate marker of myelin integrity. MTI has therefore been used to study white matter (WM) diseases, including MS.[6,9]

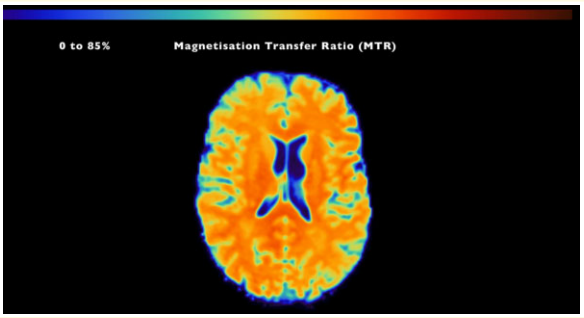
Quantifying magnetization transfer

Magnetization transfer ratio (MTR), calculated as the percentage change in signal with and without a saturation pulse (Video 1), has been widely applied in clinical studies due to relatively brief acquisition and ease of calculation. MTR is, however, susceptible to field inhomogeneities and T1 relaxation effects, and varies widely depending upon specific acquisition parameters [e.g. repetition time (TR), excitation flip angle, sequence type, saturation pulse offset, power, shape and duration].[10] Biological interpretation of MTR, as well as inter-site and inter-study comparisons, are therefore challenging, and present a barrier to clinical translation.

Magnetization transfer saturation (MTsat) inherently corrects for B1 inhomogeneities and T1 relaxation,[11] by approximating the signal amplitude and T1 relaxation at low flip angles with an additional T_1-weighted (T1-w) image.[11,12] MTsat hence addresses some limitations of MTR, within clinically feasible acquisition times and specific absorption rate limits, and the resulting parametric maps have visibly better tissue contrast than MTR (Video 1).[11]

Inhomogeneous MTR (ihMTR) exploits observed asymmetry of the broadened spectral line of the bound pool, thought to be driven by dipolar coupling effects,[13] and compares single frequency saturation at positive and negative frequency offsets with simultaneous saturation at two frequencies ($\pm$).[14,15] While not yet fully understood, ihMTR[15] is thought to be particularly sensitive to highly restricted protons in lipid chains and therefore more specific to the phospholipid bilayer of myelin than other MTI methods.

Fully quantitative MTI [quantitative magnetization transfer (qMT)] approaches using multi-compartmental models describe MT effects most rigorously by systematically varying the saturation offset and power. Important derived parameters include the fractional pool size ratio (F or PSR), the relative macromolecular content (MMC) and the macromolecular proton fraction (f) which provide indicators of myelin content. Calculation of either F or f requires estimation of the longitudinal relaxation rate, R_1, for each pool.[16] The MT exchange rate from the bound to the free pool (k_f) may also help to gauge myelin status. qMT is time-consuming to acquire, requires complex analysis and tends not to provide whole-brain coverage. qMT application has therefore mostly been limited to small-scale methodological studies.



Video 1 Examples of a MTR and a MTsat parametric map from a person with relapsing-remitting multiple sclerosis. Colour scales are compressed to 0–85% for MTR and 0–6.5% for MTsat for high contrast between white matter (in red) and grey matter/demyelinated lesions (in blue).

Rationale

Previous reviews provide an overview of qMT, MTI[17] and its specific application in MS.[9,18,19] More recently, Weiskopf *et al.*[20] have provided a technical review of the concepts, validation and modelling of quantitative MRI, including qMT. The biophysical models used to describe MT effects in tissue, experimental evidence in brain development, ageing and pathology have also been reviewed.[6] Lazari and Lipp[21] and van der Weijden *et al.*[22] systematically reviewed myelin-sensitive MRI validation, reproducibility and correlation with histology in humans and animal populations. Campbell *et al.*[23] and Mohammadi and Callaghan[24] have addressed incorporation of MTI-derived *g*-ratio measures to determine relative myelin-to-axon thickness.

The emergence of methods such as MTsat and ihMTR, which provide more specific measures of tissue microstructure than MTR but can be acquired relatively rapidly across the whole brain, present an opportunity to reassess the use of clinical MTI.[11,15,25] An evaluation of the body of evidence for MTI as a marker of disease from diverse studies would allow a better understanding of the effects of technique and other sources of bias across apparently contradictory results in the literature. Moreover, differences in clinical course,[26] current therapeutic approaches[27–29] and CSF biomarker profiles reflecting dominant pathophysiology[30]

justify specific examination of the different MS subtypes. We believe therefore that a systematic review of myelin-sensitive MTI in RRMS with meta-analyses is warranted.

Purpose

The aim of the present study is thus to systematically review (i) MTI techniques used to assess pathological change in RRMS and (ii) sources of inter-study variability and bias. We then aim to apply meta-analyses to provide consensus on (iii) key cross-sectional and longitudinal pathological findings and (iv) the relationship between MTI and clinical disability in RRMS.

Materials and methods

Approval from an ethics committee was not required for the present review.

Registration and protocol

This review was not registered. The protocol was set *a priori* as described but not registered externally.

Search strategy and eligibility criteria

This review adhered to PRISMA guidelines.[31,32] The search terms were 'magnetisation transfer' or 'magnetization transfer' and 'brain' (with MeSH terms). The online databases searched were PubMed, Embase and Web of Science.

Search and eligibility criteria were in accordance with a protocol that had been defined *a priori*. For inclusion, studies had to be primary human research and had to include people with RRMS. Because the focus of the review was on MTI findings and their correlates in RRMS, studies that included people with other MS subtypes (e.g. primary progressive) or post-mortem imaging data, were excluded from the main analysis. Articles in any language were accepted, with a publishing cut-off date of 06/01/2021.

Exclusion criteria were: inclusion of subjects with non-MS pathology (e.g. brain tumours, traumatic brain injury) where RRMS was not the main focus; paediatric (i.e. <18 years of age) or paediatric-onset MS; solely inclusion of healthy participants (i.e. without MS patients); the full text was not retrievable; only phantom, *in vitro*, preclinical *in vivo* or *ex vivo* data; study published before 1980; an imaging technique other than MTI used; non-brain imaging only; non-quantitative methodology; theoretical or simulation-only papers; a clinical trial protocol, Phase I or Phase II clinical trial; conference proceedings; a review or opinion article; and, any study clearly irrelevant to the current review. Duplicated datasets were not excluded, as these could not be identified reliably from the study publications.

Search procedure

Search results were imported into EndNote. Duplicate publications were automatically removed using the in-built de-duplicator tool, and the remaining duplicates were removed manually. Abstracts were checked by the author (E.N.Y.) and removed when exclusion criteria were met. Full texts were manually retrieved by the author (E.N.Y.) with online searches for article DOIs, PMID or title. If this failed, the abstract was excluded. Full-text articles were screened manually by the author (E.N.Y.) for exclusion criteria and rejected where necessary. The remaining selection was categorized according to the MS subtype. Articles without RRMS cohorts or comprising mixed subtypes were excluded from the main review. MTI data for RRMS patients in excluded studies comprising mixed MS subtypes were, however, included in meta-analyses, where it was possible to identify and analyse these separately.

Data extraction

Data were extracted in detail including demographics, acquisition parameters, MT measure and brain region, statistical methodology, summarized clinical findings and study limitations. Where possible, correlation coefficients, MT mean and standard deviation were extracted to calculate effect sizes for meta-analyses.

Statistical analysis

Descriptive statistics were calculated for demographic data, DMTs and steroid usage, and clinical disability measures. Key study findings and limitations were collated according to the MT technique used and the brain region.

When data were available from a sufficient number of studies, random-effects meta-analyses, with brain region as a nested factor, were performed to determine:

1. differences in MT metrics between patients with RRMS and healthy controls (HCs) (significance level, $\alpha = 0.05$, *metafor* package in RStudio v1.3.1093).
2. putative relationships between clinical disability and MT metrics, in studies with reported correlation coefficients.

Where the number of studies, k, was >2 for a given brain region, follow-up sub-analyses were carried out to determine regional effect sizes, corrected for multiple comparisons [$\alpha = 0.05/(1 + n$ of sub-analyses)]. The Sidik–Jonkman method was used to assess between-study heterogeneity. Means were standardized (Hedges' g, *R meta* package) for compartmental qMT metrics and T1 was converted to R1 to ensure consistent directionality.

To assess longitudinal evolution of MT metrics in RRMS, longitudinal data (>1 time-point) were submitted to a mixed-model linear regression with mean MT as the dependent variable, time-point and brain region as fixed effects, and study as a random effect with within-study subgrouping as a nested factor (e.g. active lesions versus reactivated lesions, placebo versus treatment groups; $\alpha = 0.05$; *lmer*, RStudio).

Marginal means for each brain region were estimated (*ggeffects* R package). Follow-up sub-analyses were performed when $k \geq 3$ for a given brain region, with time-point as a fixed effect and study as a random effect, with subgrouping as a nested factor [$\alpha = 0.05/(1 + n$ of sub-analyses)]. Formal sensitivity analysis was not considered applicable to these data.

Qualitative assessment

Longitudinal change in MT, the relationship between MT and treatment, its association with disability and the dependence on the MT metric used were qualitatively assessed.

Risk of bias

Risk of bias was determined qualitatively with Joanna Briggs Institute (JBI) Critical Appraisal Checklists,[33,34] stratified by study type (case–control, randomized controlled trial, cross-sectional, cohort, case report, case series, or closest match of listed study designs). An overall appraisal was given to each study based on checklist criteria. Funnel plots were used to quantify publication bias across studies included in meta-analyses. The observational nature of the data being examined limited formal evaluation of overall certainty of evidence.

Data availability

Extracted data may be provided upon reasonable request to the corresponding author.

Results

Systematic online literature search results

Initial online database searches yielded 6758 results. Following the removal of duplicates, 3274 studies remained, which was reduced to 780 after abstract screening (Fig. 1). Full articles could not be retrieved for 42 studies and these were excluded. Of the remaining 738 articles, 368 studies met exclusion criteria (Fig. 1), leaving 370 articles for categorization by MS subtype.

As RRMS is the focus of this review, 96 studies that did not include patients with the relapsing-remitting MS subtype were excluded. The remaining selection ($k = 274$) was refined to 86 studies that only recruited participants with RRMS (and HCs, when included), and which form the foundations of this review. MTI data for RRMS patients from a further 38 studies, which had been excluded from the main review due to comprising mixed MS cohorts (as per the predefined study protocol) were additionally included in meta-analyses. An overview of excluded MS studies with mixed MS subtypes may be found in Supplementary Tables 1 and 2.

In adherence to our protocol, we did not include Phase I or II clinical trials. We nevertheless retrospectively examined these studies for potential inclusion in meta-analyses; however, these studies either did not include analysable MT data, or incorporated duplicate data from cohorts that had already been included in the existing analysis.

Sample characteristics

An overview of sample size, sex ratio, age and study centre location is provided in Supplementary Table 3 for RRMS cohort studies ($k = 86$). Fifty-seven (44%) included a HC group. Disease duration and Expanded Disability Status Scale (EDSS) score for each study (when reported) is shown in Supplementary Table 4.

Sample size

The median number of patients with analysed MT data was 19 (range: 1–858, $k = 86$) compared with 14 HCs (range: 2–56, $k = 57$, Supplementary Table 3).

Sex

The median female-to-male ratio for analysed MT data was two for RRMS patients ($k = 61$) and 1.43 for HCs ($k = 51$, Supplementary Table 3).

Age

The mean age of people with RRMS was 37.15 years (5.63 SD, $k = 77$). Where mean age was only reported for recruited patients, this was still included; median age was not included. The mean age of HCs was 35.70 years (4.90 SD, $k = 47$) (Supplementary Table 3).

Location

The majority of studies were European ($k = 41/86$) or North American ($k = 30$), with a minority of Asian ($k = 7$, including Iran and Jordan) and international ($k = 8$) studies (or >3 test centres, Supplementary Table 3). The top three study locations were London ($k = 8$),[35–42] Milan ($k = 8$)[43–50] and Lausanne ($k = 6$).[51–56]

Disease duration

The mean disease duration across studies was 6.23 years (4.19 SD, range 0.2–20.8 years, $k = 50/86$ reported as mean, Supplementary Table 4).

Clinical disability

The majority of studies ($k = 73/86$) used EDSS as a measure of disability with median baseline score of 1.5 ($k = 64$, Supplementary Table 4).

Additional clinical correlates included the multiple sclerosis functional composite (MSFC, $k = 11$)[37–39,51,52,56–61] or its subcomponents, i.e. the Paced Auditory Serial Addition Test (PASAT), nine-hole peg test (9HPT) or the Timed 25-Foot Walk (T25FW, $k = 5$),[53,62–65] the Symbol-Digit Modalities Test (SDMT), Stroop test, Wechsler Abbreviated Scale of Intelligence, Adult Memory and Information Processing Battery, Hospital Anxiety and

Depression Scale,[41] Hamilton Depression and Anxiety Rating Scales, Mini-Mental State Examination and the Standard Raven Progressive Matrices.[65]

DMTs and steroid usage

Intra-study and inter-study heterogeneity were apparent in treatment with DMTs and steroids (Table 1 and Supplementary Table 5 for summaries; Supplementary Table 3 for detailed descriptions). Homogeneous DMTs were prescribed across the cohort in 11 studies (Supplementary Table 5); comprising fingolimod,[66] dimethyl fumarate,[67,68] subcutaneous interferon (IfN)-β1a,[58,69] or IfN-β1b,[70–72] intramuscular IfN-β1a[73,74] and subcutaneous glatiramer acetate.[75] Patients in four further studies were either untreated or received homogeneous DMTs which were IfN-α,[76] IfN-β[38,39] and glatiramer acetate.[77]

Patients in five studies were treatment-naïve (and not receiving steroid treatment for a minimum of 14 days before imaging),[37,45,46,78,79] and only the placebo arm of a clinical trial was included in one study.[80] Eleven studies allowed steroid treatment for relapses or did not specify usage, but were otherwise treatment-naïve.[40,43,44,48,50,57,65,81–84] Many studies did not report DMT or steroid usage ($k = 28$ and $k = 56$, Supplementary Table 5 and Table 1, respectively) or did not specify DMTs ($k = 5$).[59,85–88] However, studies that reported steroid usage typically had a washout period of at least 10 days before MR imaging took place.

MTI acquisition protocol parameters

MTI protocols varied across studies (see Supplementary Results); there was heterogeneity in MR system field strength (Fig. 2A), acquisition sequence design, image contrast, image resolution and MT pulse design, including MT pulse offset frequency (Fig. 2B). Sequence parameter details were often, however, unreported.

Quantitative measures of magnetization transfer

Metrics used

The most frequently used quantitative MT metric was MTR ($k = 75$, Fig. 2C and Supplementary Table 4).[35–63,65–76,78–95,97–103,107–110,112,113,115,117,119] A small number of studies used MTsat ($k = 3$),[11,111,114] ihMTR or quantitative ihMT ($k = 2$),[88,119] or qMT ($k = 16$).[36,64,77,86,87,93,94,96,104–106,108,112,116,118,119] qMT parameters included the $R1_{free}$ ($k = 7$)[77,94,104–106,116,118] or $T1_{free}$ ($k = 5$)[36,86,87,96,112] including under saturation ($T1_{sat}$, $k = 2$),[86,108] $T2_{free}$ ($k = 4$)[77,94,116,118] and $T2_{bound}$ ($k = 5$),[36,77,94,116,118] k_f ($k = 8$)[64,77,87,96,105,106,112,116] including under saturation (k_{sat}, $k = 2$),[86,108] the equilibrium magnetization of the 'bound' pool and the non-ideal inversion of the 'free' pool signal (M0f and Sf, respectively, $k = 2$),[105,106] f ($k = 3$),[36,94,118] and F ($k = 2$).[64,77,93,94,104–106,116]

MT values across the brain

Studies varied as to the brain tissues in which MT was evaluated (Fig. 2D and Supplementary Table 4). Metrics were most often investigated in WM ($k = 55$)[11,35–38,40,43,45,46,48,51–55,58,60,64,66–68,70–72,74,77–79,81–90,93,94,96–98,100,102,105,106,108,110,112,114,115,117–119] and lesions ($k = 58$),[11,35,36,42,43,45,46,49–54,58,59,61,65–75,77,79,80,82–88,90,91,93–98,100–102,105–107,110,112,114–116,118,119] followed by grey matter ($k = 30$),[11,36–38,40,44,48,51–55,57,60,64,68,70,74,82,85,89,97,100–102,105,106,109,116,118] whole brain ($k = 19$)[11,43,47,50,59,61,65,69,74–76,80,82,91,92,99,100,102–104,113] and specific regions of interest (ROIs) ($k = 22$).[35,39–41,43,51–53,56,62,63,72,85,88,97,101,105,106,111,116,118,119] However, the definition of tissue categories varied. A distinction was often (but not always) made between 'normal-appearing' tissue and lesional tissue. Certain studies sub-divided tissue type into lobes (e.g. frontal WM) or ROIs (e.g. deep versus cortical grey matter).

MTR in RRMS and HCs

Meta-analysis. Studies that compared MTR cross-sectionally between RRMS patients and HCs ($k = 46$ with available data, $n = 1130$ RRMS patients/886 HC) were submitted to a random-effects meta-analysis, with brain region as a nested factor. Irrespective of brain region, MTR for RRMS patients was on average 1.17 per cent units [95% confidence interval (CI) −1.42 pu to −0.91 pu] lower than controls (z-value: −8.99, $P < 0.001$, Fig. 3). Between-study heterogeneity was high (total $I^2 = 59.7\%$).

Whole-brain MTR. Whole-brain MTR was measured in 19 studies (Supplementary Table 4 and Fig. 2D).[43,47,50,59,61,65,69,74–76,80,82,91,92,99,100,102,103,113] Average MTR in whole brain ($k = 9$) was 35.58%[47,50,59,65,74,75,80,82,91] with wide inter-study variance (range: 25.1%[82] to 48.44%,[59] Fig. 2E). Subgroup meta-analysis showed that whole-brain MTR was significantly lower for patients than HCs with an absolute mean difference of −1.46 pu (95% CI −1.84 to −1.07 pu) ($P < 0.001$, z-value: −7.39, Fig. 4 subgroup, $k = 11$ with sufficient reported data, $n = 288$ RRMS/231 HC) with low between-study heterogeneity ($I^2 = 12.7\%$).

Normal-appearing WM MTR. MTR of WM was investigated in a large number of studies ($k = 48/86$, Fig. 2D and Supplementary Table 4).[35–38,40,42,43,45,46,48,51–55,58,60,66–68,70–72,74,78,79,81–90,94,96–98,100,102,108,110,112,115,117,119] Typically, WM was defined as whole-brain normal-appearing WM (NAWM), with some exceptions such as ROIs of NAWM contra-lateral to lesions of similar size,[66,68,96] 'dirty-appearing' WM[79,112] and NAWM sub-regions[36,40,42,45,46,81,87,88,117,119] (e.g. lobar WM,[51,52,67,115] NAWM close to cortical grey matter,[43] perilesional NAWM[35,96,110]). The mean NAWM MTR across studies was 69% ($k = 32$)[36–38,42,43,45,46,48,58,60,66–68,70–72,74,78,79,82,84–89,94,98,102,110,112,119] (range: 25.95%[60] to 84%,[67] Fig. 2E).

Overall, NAWM MTR was lower in RRMS patients compared with HCs,[37,39,40,43,58,60,70,78,81,83,86–90,112] although

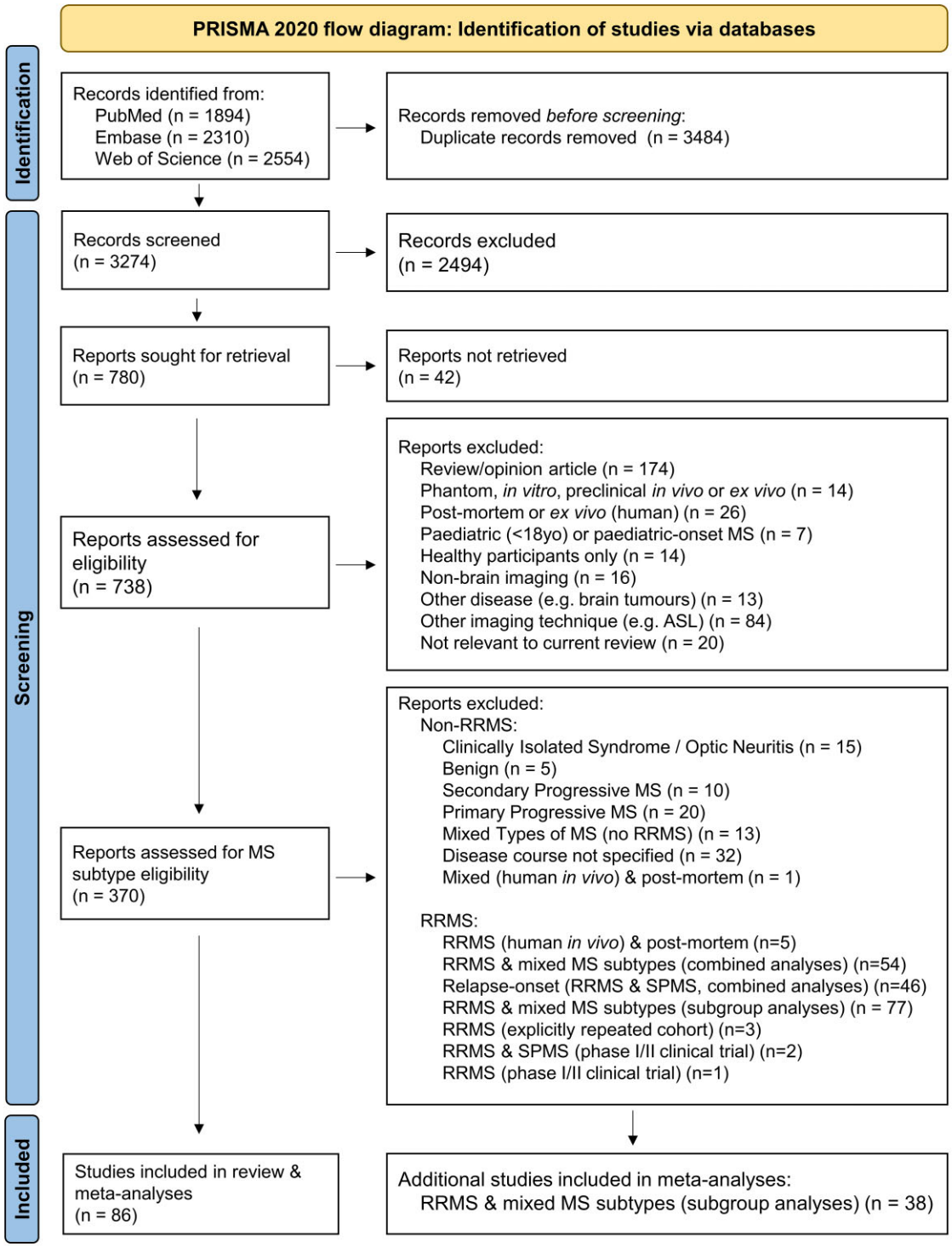


Figure 1 PRISMA 2020 flow diagram for systematic review search process. ASL, arterial spin labelling; MS, multiple sclerosis; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RRMS, relapsing-remitting MS; SPMS, secondary progressive MS. Adapted from: Page *et al.*[32]

some studies found no difference.[36,51,53,54,82,84,94,119] One study reported lower MTR in controls than patients.[97] Random-effects subgroup meta-analysis (Fig. 3) showed MTR of NAWM in RRMS was significantly lower than controls, with an absolute mean difference of −1.25 pu (95% CI −1.57 to −0.92) (z-value −7.55, $P < 0.001$, $k = 31$ with sufficient data, $n = 651$ RRMS/491 HC) and considerable between-study heterogeneity ($I^2 = 52.8\%$).

Table 1 Overview of use of DMTs for patients with relapsing-remitting MS in studies using MTI

DMTs	k	%	Citation
Dimethyl fumarate	4	4.7%	67,68,89,90
Dimethyl fumarate (delayed release)	2	2.3%	91,92
Fingolimod	10	11.6%	49,51–56,66,89,90
Natalizumab	5	5.8%	35,49,89,90,93
Glatiramer acetate	9	10.5%	55,75,77,89,90,92–95
Interferon-β (1a)	13	15.1%	55,58,61,69,73,74,76,90,93,95–98
Interferon-β (1b)/betaferon	5	5.8%	55,70–72,93
Interferon beta (unspecified)	8	9.3%	38,39,51–54,56,94
Pegylated interferon 1a	1	1.2%	99
Laquinomod	1	1.2%	100
Ocrelizumab	1	1.2%	97
Placebo	8	9.3%	35,59,61,85–88
Steroids	**k**	**%**	**Citation**
Methylprednisolone	2	2.3%	71,72
Unspecified	2	2.3%	76,79
None (for indicated time period)	26	30.2%	35,40,41,43–46,48,50,53,54,57,62,65,66,68,73,81–85,94,101–103
Data missing	56	65.1%	11,36–39,42,47,49,51,52,55,56,58–61,63,64,67,69,70,74,75,77,78,80,86–93,95–100,104–119

Studies may be duplicated where treatments were heterogeneous. Study-specific details are given in Supplementary Table 3. DMTs, disease-modifying therapies; *k*, number of studies.

Grey matter MTR. Twenty-three studies investigated grey matter MTR (Fig. 2D and Supplementary Table 4).[36–38,40,44,48,51,53–55,57,60,68,70,74,82,85,89,97,100–102,109] Mean cerebral normal-appearing grey matter (NAGM) MTR was 31.5% ($k = 9$),[37,38,40,44,48,74,82,102,109] and consistently lower than NAWM MTR[38,40,102] with a wide range (Fig. 2E). Cortical NAGM MTR, for example, was 2.9 per cent units lower when using a balanced steady-state free precession sequence compared with a gradient echo sequence within the same cohort.[85]

Random-effects subgroup meta-analyses showed a significant difference for cerebral and cortical grey matter (Fig. 4, mean difference −0.84 and −0.56 pu, *z*-value −2.81 and −3.25, $k = 14$ and 9, $n = 375/284$ and 234/193 RRMS/HC, respectively, $P < 0.01$ for both) but not deep grey matter (mean difference −0.36, *z*-value: −1.05, $P = 0.294$, $k = 3$, $n = 44$ RRMS/44 HC). However, other studies (which did not report effect sizes) did not find between-group differences in MTR within cerebral[36,54] or cortical NAGM,[51,53] or within the basal ganglia.[51,53] Moreover, sub-regional variation was reported. For example, grey matter MTR in the parieto-occipital lobes, but not other regions, was lower for patients than controls in one study,[40] and voxelwise differences in the left posterior cingulate cortex, right orbitofrontal cortex, bilateral insula and lenticular nuclei were noted elsewhere between patients and controls.[57]

Lesion MTR. Forty-nine studies measured MTR in lesions (Fig. 2D and Supplementary Table 4).[35,36,42,43,45,46,49–54,58,59,61,65–75,79,80,82–88,90,91,93,95,97,98,100–102,107,110,112,115,119] MTR was nearly always lower in WM lesions than in NAWM ($k = 23$, Fig. 2E),[36,42,43,53,60,66,67,70–72,79,83–86,88,94,96–98,110,112,115] 'dirty-appearing' WM[79] and HC WM ($k = 4$).[53,58,84,119] Cortical lesion MTR was also lower than cortical NAGM.[85] However, there was some regional heterogeneity. WM lesion MTR (and ihMTR) was not significantly lower than NAWM in the corpus callosum[88] nor when several NAWM ROIs were combined.[119]

There was clear variation in MTR across lesions (Fig. 2E), partially dependent on lesion characteristics,[53,107] which varied across the literature. In particular, MTR in T1-w 'black holes' was lower than in T1-w-isointense, T2-w visible lesions[67,102] although not always significantly.[42] There was not typically a significant difference between MTR in contrast-enhancing lesions (CELs) such as nodular-enhancing CELs, and non-CELs,[107] 'pure T2-w lesions' or T1 'black holes'.[67] However, ring-enhancing CELs showed lower MTR than densely enhancing[87] or nodular-enhancing CELs.[84] In addition, interdependency between lesion volume and MTR was reported,[43,53] although results are mixed.[80]

MTR in other sub-regions. Seventeen studies measured MTR in other sub-regions of the brain (Fig. 2D and Supplementary Table 4)[35,39–41,43,51–53,56,62,63,72,85,88,97,101,119] including the thalami,[39–41,51,53,85,88,101,119] putamen,[40,51,53,85,88,101] caudate nuclei,[40,51,53,85,101] corpus callosum,[40,63,88,119] internal capsule,[40,43,88,119] globus pallidus,[51,53,85,101] cerebellum,[52,56] hippocampi,[41,85] cerebral corticospinal tract,[62] accumbens,[85] amygdala,[85] cingulate cortex[41] and parietal cortex.[41]

A random-effects meta-analysis with brain sub-region as a nested factor showed no significant difference in baseline MTR between patients and controls [absolute mean difference −3.31 pu (95% CI −8.65 to 2.03), *z*-value = −1.23, $P = 0.215$, $k = 7$, $n = 161$ RRMS/142 HC, Supplementary Fig. 1]. Although between-study variance was low ($I^2 = 0.07\%$), total model variance was high ($I^2 = 98.9\%$) due to high variation in brain region (Fig. 2E).

Since the number of studies examining MTR for most individual brain regions was low ($k < 3$), follow-up subgroup random-effects meta-analyses were only performed for the thalamus ($k = 6$) and putamen ($k = 3$). There was no significant difference in baseline thalamic MTR between RRMS patients and HCs [mean difference −3.97 pu (95% CI −10.07 to 2.12), *z*-value = −1.28, $P = 0.202$, $n = 132$ RRMS/113 HC, Supplementary Fig. 1] and high between-study variance ($I^2 = 99.2\%$). One additional study also found no difference in thalamic MTR between patients and controls (no effect size reported).[51] Similarly, for the putamen, there was no difference between patients and controls [mean difference −5.77 pu (−17.10 to 5.56), *z*-value = −1.0, $P = 0.318$, $n = 77$ RRMS/61 HC] and heterogeneity was high ($I^2 = 99.6\%$). High between-study heterogeneity may be explained by differences in MT sequences used.[85]

Longitudinal MTR change and therapeutic response

Fourteen studies ($n = 563$ RRMS) assessed longitudinal change in mean MTR in one or more brain regions, with a maximum of 3 years follow-up. A linear mixed-model revealed that time did not have a significant effect on MTR when all brain regions were considered [$\beta = 0.12$ (−0.56 to 0.80), t-value = 0.35, $P = 0.724$, Supplementary Table 6 and Fig. 2].

Longitudinal change in whole-brain MTR. Ten studies examined the longitudinal evolution of whole-brain MTR[59,61,69,74–76,80,91,92,99,100] of which five reported sufficient data to estimate longitudinal change in normal-appearing brain tissue (NABT) MTR.[59,74,75,80,91] A linear mixed-model showed that time did not significantly predict NABT MTR [$\beta = -0.117$ (−0.21 to −0.02), t-value = −2.65, $P = 0.019$, $n = 278$ RRMS, Supplementary Table 7].

Nevertheless, individual studies reported small (e.g. <1% absolute change over 2 years[47]) but significant longitudinal decline in whole-brain MTR.[59,76] A slower (non-significant) MTR decline (e.g. ∼0.02% every 2 months over 14 months[80]) and inter-subject variation were also reported.[69,76] Additionally, longitudinal stagnation or increase in MTR with treatment compared with longitudinal decreases in MTR in placebo arms was evident in large, placebo-controlled cohorts over 2 years,[91,100] suggesting MTR as a putative therapeutic endpoint. However, one study reported no deterioration in whole-brain MTR with glatiramer acetate treatment but lacked validation against a placebo arm.[75]

Longitudinal change in NAWM MTR. Sixteen studies examined the longitudinal evolution of NAWM MTR.[38,45,46,53,54,58,66,71,74,78,83,84,96,98,100,120] Eight studies ($n = 100$ RRMS) reported appropriate data for a linear mixed-model to assess longitudinal change; NAWM did not change significantly over time [$\beta = 0.037$ (−0.14 to 0.22), t-value = 0.41, $P = 0.68$, Supplementary Table 8].[45,46,58,66,74,84,96,120]

In studies that reported a significant change over time, and in line with a previous report,[98] absolute change in NAWM MTR was small (<1.5% up to 36 months) with reported estimates of an annual decline of 0.1% in early RRMS, possibly preceding clinical onset by years.[38] However, others found no change in NAWM MTR over 2 years in an early MS cohort with minimal disability, after controlling for age and gender.[53] Alternatives to the arithmetic mean such as histogram peak location may, nevertheless, reveal changes over 12–32 months.[78]

Longitudinal change in grey matter MTR. A linear mixed-model of all brain regions suggests no effect of time on NAGM MTR but there were insufficient data for follow-up analyses (see 'Longitudinal MTR change and therapeutic response' section). In the literature, however, MTR in grey matter decreases gradually (∼0.18 pu annually, compared with 0.01 pu in controls),[38] although perhaps faster than NAWM MTR in RRMS.[38] However, over 2 years, such a gradual decline is not statistically significant.[53] The longitudinal rate of grey matter change is unaffected by anti-phospholipid antibody (APLA) status,[74] or treatment with IfN-β[38] or laquinomod,[100] although the latter may slow decline initially.

Longitudinal change in sub-regional MTR. There was no evidence of longitudinal change in MTR when all brain regions were considered (see 'Longitudinal MTR change and therapeutic response' section). Since there were few studies examining each brain sub-region (Supplementary Fig. 2), no further meta-analyses of longitudinal change in MTR within brain sub-regions were constructed. However, no significant longitudinal change in MTR has been found in the thalamus, putamen, pallidum or caudate over 2 years.[53] Separately, despite a significant change in thalamic MTR (−0.13 pu/year) over 2 years, this was not significantly different from the rate of change in control thalamic MTR,[39] and did not differ between those patients who were or were not treated with IfN-β.

Longitudinal change in lesion MTR. A linear mixed-model showed that lesion MTR did not change significantly longitudinally [$\beta = 0.255$ (−0.52 to 1.02), t-value = 0.67, $P = 0.51$, $k = 11$, $n = 223$ RRMS, Supplementary Table 9].[45,46,59,66,74,75,80,84,96,98,120] However, MTR longitudinal evolution depends on lesion characteristics[53] and may be subtle[69] (Supplementary Figs 2 and 3). MTR of active CELs varies from month-to-month before and after enhancement,[45,46,71,83,93,96] while MTR of GM lesions,[53] 'slowly expanding' lesions,[49] T1-w hypointense[75] and T2-w hyperintense[75,80] lesions may remain relatively stable over several years, irrespective of relapses.[80]

Increases in lesion MTR may also occur,[84] such as within non-expanding lesions, although this may be accompanied by changes in T1[49] and/or lesion load[61]. MTR increases may be seen with treatment (e.g. fingolimod[66] over 2 years) although not always (e.g. laquinomod[100]). Steroids can increase CEL MTR[46,71] although certain DMTs, including delayed-release dimethyl fumarate[91] or IfN β-1b[71,73] do not appear to alter CEL MTR. Furthermore, CELs do not tend to recover to NAWM MTR values,[46,72,98] and their longitudinal evolution may be predicted by the change in MTR of the first-month post-enhancement.[46] MTR in reactivated CELs also may deviate from NAWM MTR to a greater extent than new CELs.[96]

MTR fluctuations in lesions have been partially ascribed to low reproducibility, changes in interstitial water due to acute inflammation, or perhaps remyelination.[68] Yet, when mixed lesion types are considered, a longitudinal global MTR decrease is typical.[53,54]

Clinical correlates of MTR

Thirteen studies reported correlation coefficients between MTR and EDSS permitting a meta-analysis (with the brain region as a nested factor) to be performed. There was a significant negative association between EDSS and MTR across

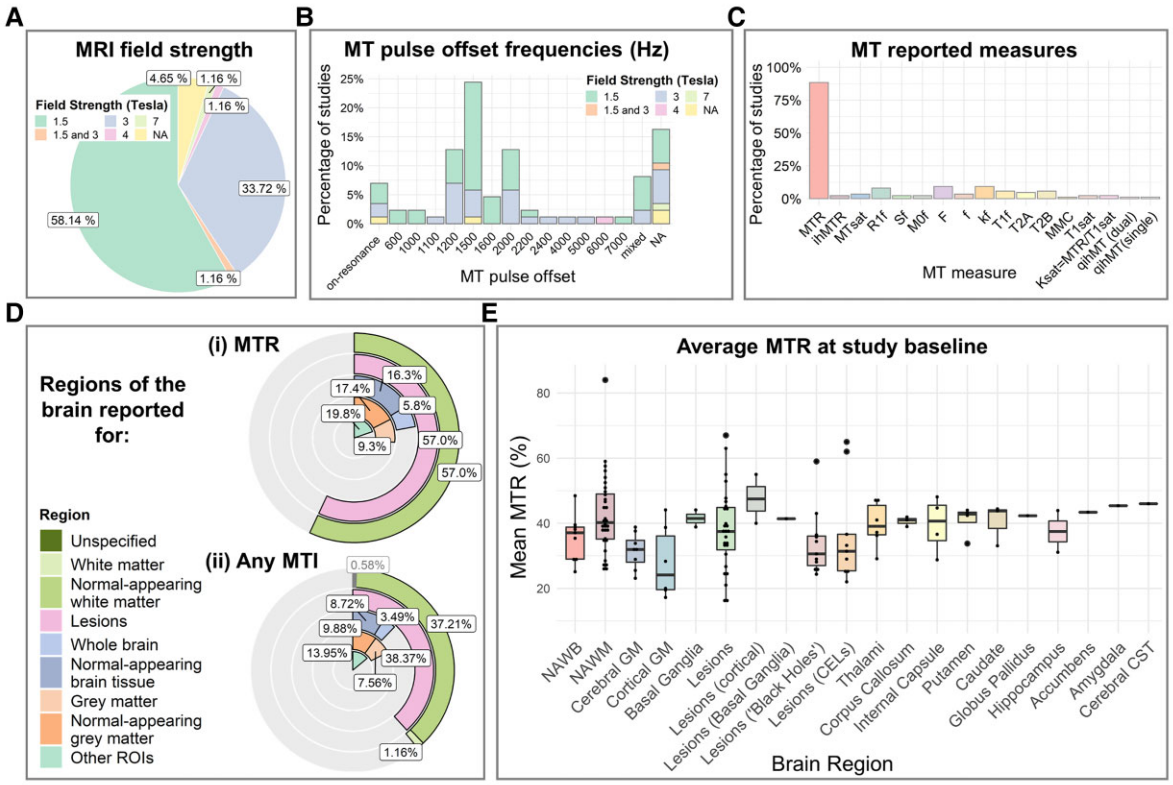


Figure 2 MRI characteristics of studies which used MTI in relapsing-remitting MS ($k = 86$). Plots summarise **A** field strength of the MR system, **B** pulse offset frequencies of the MT pulse, **C** MT metrics used across studies, **D** brain regions in which (i) MTR or (ii) any MTI metric was reported, and **E** the average MTR across brain regions at study baseline. CELs, contrast-enhancing lesions; CST, corticospinal tract; GM, grey matter; MMC, macromolecular content; MT, magnetization transfer; MTR, MT ratio; ihMTR, inhomogeneous MTR; MTsat, MT saturation; qihMT, quantitative inhomogeneous MT; NAWB, normal-appearing whole brain; NAWM, normal-appearing white matter; ROIs, regions of interest.

all brain regions; $r = -0.32$ [95% CI −0.46 to −0.17] (z-value = −4.33, $P < 0.001$, $k = 13$, $n = 438$, Fig. 5) and between-study heterogeneity was low (total $I^2 = 0\%$). Across individual studies, sub-regional results were mixed but in general, suggest that there is no association between EDSS and MTR.[85,88]

Whole-brain MTR and clinical correlates. In terms of whole-brain MTR clinical correlates, there is some evidence that NABT MTR correlates with EDSS[65] (Fig. 5) but not retinal nerve fibre layer (RNFL) thickness or low letter contrast acuity.[82] NABT MTR may predict longitudinal memory decline and, in combination with brain parenchymal fraction and 2-year change in ventricular fraction, information processing speed over 7 years.[59] No such association was found between NABT MTR and verbal fluency.[59] However, this study was limited by the lack of comparative longitudinal control data. Furthermore, longitudinal evolution of NABT MTR does not appear to depend on APLA status of patients.[74]

NAWM MTR and clinical correlates. Many studies examined the relationship between clinical disability and NAWM MTR (Supplementary Table 4), yet only three studies reported effect sizes. A subgroup meta-analysis for NAWM showed a negative association between EDSS and NAWM MTR [$P < 0.05$, $r = -0.42$ (95% CI −0.79 to −0.04), $n =$ 122 RRMS, Fig. 5] with low between-study variance ($I^2 =$ 0%). However, the small number of studies ($k = 4$) limits the generalisability of this finding, particularly given under-reporting of non-significant effect sizes. Indeed, all studies ($k = 10/86$) which examined the association between NAWM MTR and EDSS found no association,[37,38,58,60,75,78,85,115,119] although one study reported a significant correlation between baseline NAWM MTR and change in EDSS over 18 months (but not baseline EDSS).[48]

Evidence of relationships between NAWM MTR and other clinical measures was mixed. For example, NAWM MTR was associated with MSFC z-score at 24-month follow-up but not baseline[58] while, separately, there was no relationship between MSFC z-scores and NAWM MTR[60] or 2-year change in NAWM MTR.[38] Associations may also be region- and model-dependent; for example, temporal lobe MTR was one of several significant predictors of MSFC and SDMT (an attention test) scores, independently, in regression models.[51]

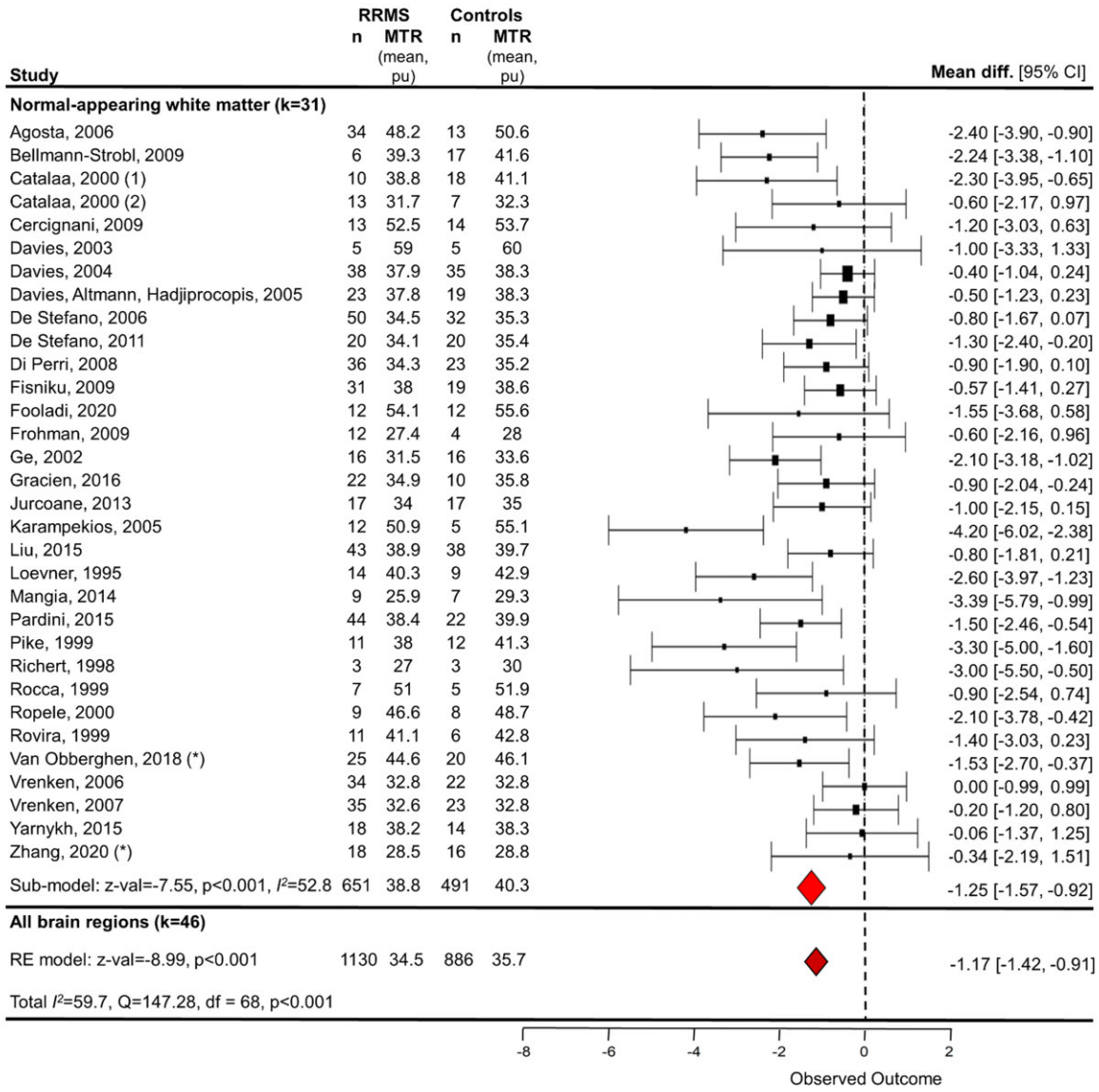


Figure 3 Random-effects meta-analysis of the difference in mean MTR in between relapsing-remitting MS patients and control subjects in NAWM and all brain tissue types. Study baseline data were used. One study (Catalaa[78]) was included twice as separate protocols and cohorts were used. A random-effects model with brain region as a nested factor showed that mean MTR was 1.17 per cent units [z-value = −8.99, $P < 0.001$, 46 studies (including grey matter and whole brain studies in Fig. 4), 1130 RRMS/886 HC] lower for people with RRMS than HCs across all brain tissue types. A random-effects model for NAWM alone showed that mean MTR was 1.25 per cent units (z-value = −7.55, $P <$ 0.001, 31 studies/$n = 32$; 651 RRMS/491 HC) lower for people with RRMS than HCs. NAWM, normal-appearing white matter; RE, random-effects; RRMS, relapsing-remitting multiple sclerosis. *Averaged over sub-regions.

In terms of other biomarker correlates, WM MTR was weakly associated with serum neurofilament—a marker of neuronal injury—in RRMS (although not in control subjects), adding to evidence validating MT imaging as a biomarker of myelin integrity.[55] NAWM MTR does not however appear to be related to RNFL thickness or low contrast letter acuity.[82]

Grey matter MTR and disability. Eight studies examined the relationship between grey matter MTR and EDSS (Supplementary Table 4) with some demonstrating significant associations[37,109] and others finding no such relationship.[38,57,60,85,89] One study found an association between baseline grey matter MTR and change in EDSS, but not baseline EDSS.[48] A follow-up subgroup random-effects meta-analysis showed no significant association between-study baseline (cortical or cerebral) grey matter MTR and EDSS [$P = 0.675$, $r = -0.10$ (95% CI −0.57 to 0.37), $n =$ 82 RRMS, Fig. 5] and low between-study heterogeneity ($I^2 = 0\%$), but the number of studies was small ($k = 3$).

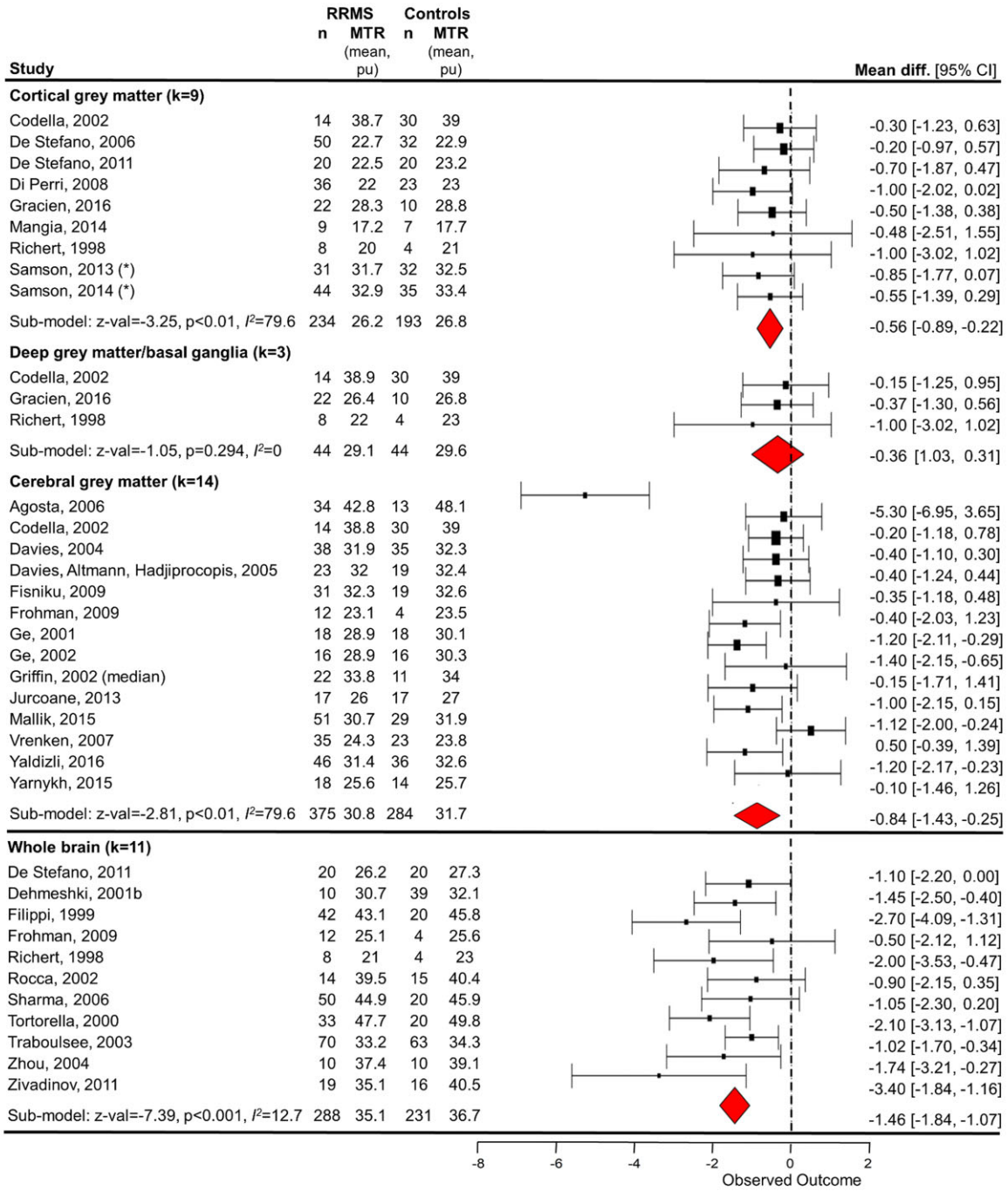


Figure 4 Random-effects meta-analysis of the difference in mean MTR between relapsing-remitting MS patients and control subjects in grey matter and whole brain. Random-effects models of study baseline data showed that mean MTR was lower for people with RRMS than HCs in whole brain (mean difference −1.46, z = −7.39, $P < 0.001$ uncorrected, 11 studies, 288 RRMS/231 HC), cortical grey matter (−0.56, z-value = −3.25, $P = 0.001$, nine studies, 234 RRMS/193 HC), and cerebral grey matter (−0.84, z-value = −2.81, $P = 0.005$, 14 studies, 375 RRMS/284 HC), but not deep grey matter/basal ganglia (−0.36, z-value = −1.05, $P = 0.294$, three studies, 44 RRMS/44 HC). See Fig. 3 for estimate across all brain tissue types, including NAWM. GM, grey matter; NAWM, normal-appearing white matter; RE, random-effects; RRMS, relapsing-remitting multiple sclerosis; WB, whole brain. *Averaged over sub-regions.

Four studies examined the relationship between grey matter MTR and the MSFC.[37,38,57,60] MSFC *z*-score did not correlate with cerebral NAGM,[37] cortical NAGM[60] or voxels of NAGM for which the MTR differed from controls.[57] Furthermore, neither change in MSFC nor its cognitive component correlated with change in MTR in NAGM over 2 years.[38]

Regarding other clinical variables, NAGM MTR was significantly correlated with age[85] as well as RNFL thickness of eyes affected by optic neuritis.[82] Female subjects may also have higher NAGM MTR[37] although this was not a consistent finding.[85] In addition, NAGM MTR correlates with T1 and myelin water fraction.[97] On the other hand, grey matter MTR did not correlate with low contrast letter acuity,[82] RNFL of eyes unaffected by optic neuritis,[82] serum neurofilament levels,[55] immune cell brain-derived neurotrophic factor (BDNF) secretion,[102] APLA status,[74] fatigue[44] or disease duration.[37,57,85] Change in NAGM MTR was not associated with relapse rate, baseline T2 lesion volume or change in T2 lesion volume over 2 years[38] nor APLA status over 3 years.[74]

MTR in other sub-regions and disability. MTR within other sub-regions such as the internal capsule,[43,88] cerebral corticospinal tract,[62] caudate, pallidum, putamen, accumbens, hippocampus and amygdala[85] and corpus callosum[88] was not associated with EDSS. There was a negative association between thalamic MTR and EDSS averaged over 2 years,[39] although 2-year change in thalamic MTR was not associated with EDSS at follow-up,[39] possibly reflecting a lack of change in thalamic MTR over 2 years.[53]

Regarding other clinical correlates, no relationship was found between thalamic MTR or rate of change of MTR over 2 years and MSFC.[39] Nevertheless, the walk component of the MSFC was negatively associated with thalamic MTR.[39] In the cerebral corticospinal tract, MTR was associated with walk velocity and Two Minute Walk Test but not Pyramidal Functional Systems Score, gender or symptom duration, but perhaps slightly dependent on age.[62] MTR of the corpus callosum was positively associated with PASAT (the cognitive component of the MSFC) score, although possibly mediated by lesion load.[63] Cognitively impaired RRMS patients may also have marginally reduced MTR in the corpus callosum compared with unimpaired patients.[63] There may be an influence of age on MTR in the basal ganglia, thalamus and hippocampus.[85] Finally, MTR in an area of the cerebellum thought to be involved in movement trajectories was associated with performance on the MSFC arm component.[56]

Clinical and other imaging correlates of lesion MTR. In lesions, any relationship between clinical disability and MTR is at most weak.[85,119,35,51,58,85,101,115] Only two studies reported a correlation coefficient (Fig. 5) for an association with EDSS and hence a meta-analysis was not performed for lesion MTR alone.

This relationship may depend on lesion type, characteristics[52] and location.[85] For example, cortical, but not WM, lesion MTR was related to EDSS, after adjusting for demographic factors.[85] Furthermore, when lesions were grouped according to their inflammatory and neurodegenerative characteristics, lesions with low MTR were found to predict attention deficits (SDMT) and general disability (MSFC), when combined with age and depression score.[52]

The timescale of the study, disease duration[85] and treatment of confounding variables may affect the strength of association. A longitudinal relationship between MTR in lesions and clinical disability developed with longer disease duration in one study when not present at baseline.[58] Lesion MTR, when combined with T2-w lesion and NAWM measures, was also related to longitudinal change in deambulation (MSFC T25FW).[53] However, baseline T2-w lesion MTR was not a significant predictor of change in memory, verbal fluency or information processing speed over 7 years.[59]

More generally, the association between MTR and clinical disability may depend on which clinical measure(s) are used. For example, lesion MTR was not significantly different between cognitively impaired and unimpaired patients, when assessed by an extensive battery of neuropsychological tests.[65] Similarly, MTR within (mixed-type) lesions did not correlate with motor tasks (finger tapping rate or 9HPT),[50] and was not a significant predictor in regression models to predict general clinical disability (MSFC), attention (SDMT) or fatigue (Fatigue Scale for Motor and Cognitive functions).[51]

Some studies indicate associations between MTR as a measure of myelin integrity and other imaging markers of disease in MS. Weak evidence suggests that the uptake of radiotracer ^{18}F-PBR111, which binds to the 18-kD translocator protein, is greater in around 60% of T2-w fluid-attenuated inversion recovery (FLAIR) hyperintense regions compared with non-lesional regions with high MTR.[35] Higher uptake of ^{18}F-PBR111 is suggestive of a pathological increase in macrophages and microglia. Single-subject MR spectroscopy has shown elevated choline and lactate/lipids suggestive of demyelination and injury to cell membranes, alongside decreases in N-acetyl compounds, creatine and myoinositol indicating axonal loss and increased glial cell infiltration, and decreased MTR compared with NAWM in a tumefactive CEL.[72] MTR in lesions is strongly associated with other imaging metrics such as MMC,[93] and k_f[87,93,112] and, to a lesser extent, quantitative T1[93,97,112] and myelin water fraction.[97] Lesion MTR is negatively correlated with relative activation on functional MRI in motor areas suggestive of functional adaptations to loss of myelin integrity, although perhaps confounded by lesion volume.[50] MTR correlates weakly with diffusion-weighted imaging metrics including fractional anisotropy[110] in large T2-w lesions and mean diffusivity[115] in chronic lesions, but not significantly with susceptibility-weighted phase imaging values, despite a negative trend.[115] Additionally, T2-w and T1-w 'black hole' lesion volume, as well as 2-year change in T2-w lesion volume may predict lesion MTR 13 years later, although uncorrected for baseline lesion MTR.[61]

Nevertheless, as a general trend across the RRMS literature, MTR within lesions does not tend to correlate with other disease biomarkers. T2-w lesion MTR is not significantly associated with age,[85,115] time since diagnosis,[101] visual contrast acuity or RNFL thickness,[82] immune cell BDNF secretion,[102] or APLA status (±).[74] MTR in CELs was not associated with

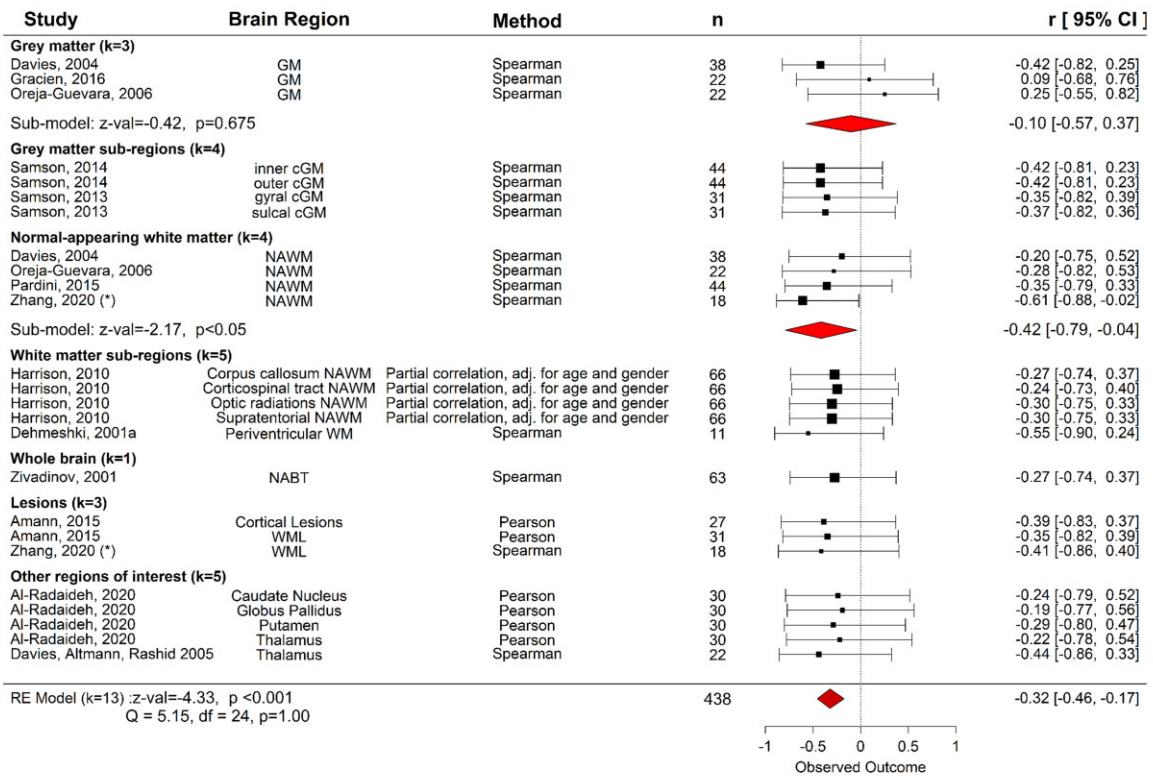


Figure 5 Meta-analysis of association between MTR and clinical disability in relapsing-remitting MS. Clinical disability was defined as EDSS score. A multi-level random-effects model with brain region as a nested factor within each study showed a significant negative association ($r = -0.32$, z-value $= -4.33$, $P < 0.001$, 13 studies, 438 RRMS) between MTR and EDSS across all brain regions. Studies which did not report a correlation coefficient were not included. Random-effects sub-analyses showed a significant correlation between EDSS and NAWM MTR ($r = -0.42$, z-value $= -2.17$, $P = 0.030$, four studies, 122 RRMS), and not grey matter ($r = -0.10$, z-value $= -0.42$, $P = 0.675$, three studies, 82 RRMS). Sub-analyses were not performed when the number of studies, $k < 3$. *MTR values were averaged over sub-regions of NAWM. GM, grey matter; NABT, normal-appearing brain tissue; NAWM, normal-appearing white matter; WML, white matter lesions; RE, random effects; CI, confidence interval.

anti-CD3 plus anti-CD28 stimulated BDNF secretion, despite a negative trend.[102] MTR in T1-w 'black holes' is not associated with RNFL thickness or visual contrast acuity.[82] There is some evidence that APLA+ patients show greater reduction in MTR in T1 'black holes' compared with APLA-patients over 3 years, but this may be driven by lesion volume changes.[74] Evidence for associations between lesion MTR and disease duration or gender is mixed, and may depend upon acquisition parameters and lesion type.[85,115]

Magnetization transfer saturation

Three studies used MTsat (Fig. 2C),[11,111,114] beginning with Helms *et al.*[11] who showed that, on a whole-brain histogram, the WM MTsat mode appeared visually reduced in a RRMS patient compared with controls. Furthermore, compared with NAWM, MTsat in a CEL and non-enhancing lesions was visually lower on a parametric map.[11]

Saccenti *et al.*[114] confirmed that MTsat was significantly lower in WM 'plaques' and periplaques than NAWM. Yet, MTsat did not correlate with EDSS or disease duration in plaque, periplaque or NAWM ROIs.[114] MTsat may additionally correlate with radial diffusivity, T1w/T2w ratio and synthetic MR-derived myelin volume fraction, although this was stronger in plaques than NAWM.[114]

Finally, Kamagata *et al.*[111] used MTsat as a surrogate for myelin volume fraction to calculate the tract-averaged MR *g*-ratio within WM in a small RRMS cohort.[111] The *g*-ratio was increased (indicating myelin degradation and/or axonal loss) compared with HCs, in motor somatosensory, visual and limbic regions. Subnetwork *g*-ratio strongly negatively correlated with WM lesion volume, but not with disease duration or EDSS, although the latter was correlated with *g*-ratio connectome nodal strength mainly in motor, visual and limbic regions.

Inhomogeneous MTR

Two studies employed ihMTR as a measure of myelin status in RRMS.[88,119] ihMTR was reduced in lesions and NAWM compared with control WM, and reduced in lesions compared with NAWM.[119] Within sub-regions, single-slice ihMTR was lower for patients in the thalamus, frontal, temporal and occipital lobes compared with controls, but not

different in the corpus callosum, internal capsule or putamen.[88] ihMTR varied across WM tracts, but was highest in the internal and external capsule and lowest in the genu of the corpus callosum.[88,119] ihMTR in WM lesions, but not NAWM, was negatively associated with EDSS.[119] However, when sub-regions were considered, EDSS was significantly associated with ihMTR (but not MTR) in frontal and temporal NAWM, the corpus callosum, internal capsule and the thalami.[88]

Quantitative magnetization transfer

qMT metrics examined varied across studies (see 'Quantitative measures of magnetization transfer: metrics used' section). Sled and Pike[116] first modelled the compartmental MT signal in RRMS in two lesions on a single-slice proton density-weighted image for a RRMS patient. Compared with frontal WM, lesions had reduced k_f, F, $R1_{free}$ and $T2_{bound}$ and increased $T2_{free}$. Parameter estimates were higher for the newer lesion compared with the older lesion for k_f, F and $R1_{free}$, but lower for $T2_{free}$ and $T2_{bound}$. Indeed, other studies also show lower k_f and k_{sat} lesions than NAWM and HC WM, while $T1_{free}$ and $T1_{sat}$ present the inverse pattern.[86,87,112] Up to 4 months before the appearance of new or reactivating CELs, k_f may even decrease while $T1_{free}$ increases.[96] However, changes are subtle, and month-by-month change may be less predictable for reactivating CELs.

Increasing lesion severity coincides with decreasing k_f[87,96,112,116] and k_{sat},[86] while conversely $T1_{free}$[87,112] and $T1_{sat}$[86] are elevated in acute, compared with mild, lesions. However, dense CELs have higher k_f but lower $T1_{free}$ values than ring CELs.[87] F[106], f[36,118], $R1_{free}$[106,94] and T_{2bound},[36,94] are also reduced in lesions compared with NAWM and control WM, with reduced F and R_{1free} in T2 hyperintense lesions visible on selective inversion recovery-derived parametric maps.[104,105] Finally, MMC is reduced in CELs but may recover post-enhancement.[93] The relationship between pathology and qMT-derived metrics is evidently complex, but may still differentiate between lesions with similar MTR, particularly when lesions are T1-w isointense.[112]

Differences between NAWM and control WM qMT are, however, subtle. Some studies report differences for qihMT,[119] $T1_{free}$,[112] F[94] and k_f,[87,94,112] while others show no differences for k_f,[64,116] F,[64] f,[36] $T2_{bound}$,[36] $T1_{free}$,[87] $R1_{free}$[94] or qMT.[119] Nine studies were submitted to a random-effects meta-analysis to compare qMT in NAWM and WM.[36,86,87,94,112,116,118] There was a significant difference between patients and controls across all qMT metrics [standardized mean difference −0.60 (95% CI −0.95 to −0.25), z-value: −3.51, $P < 0.005$, $n = 87$ RRMS/98 HCs, Fig. 6]. Additional follow-up models for metrics where $k \geq 3$, however, showed no significant difference for $R1_{free}$, $R2_{bound}$, f and k_f ($\alpha = 0.0125$, Fig. 6) despite a trend for k_f. Other brain regions were not assessed due to limited data.

In cortical grey matter, k_f, F, $R1_{free}$ and $T2_{bound}$ appear lower and $T2_{free}$ higher than in lesions and frontal WM.[116] RRMS patients have lower k_f than controls in cortical grey matter but F does not differ, except for patients with high disability.[64] No differences between patients and controls were found in cerebral or cerebellar grey matter for f, $T1_{free}$ or $T2_{bound}$.[36] In deep grey matter, f was lower for patients than controls.[118] However, differences in methodology can results in over- or underestimation of f in certain ROIs (e.g. thalami).[118]

Few studies have examined the relationship between qMT and clinical disability in RRMS. Cortical grey matter k_f may be negatively associated with EDSS and Choice Reaction Time, but not SDMT or PASAT.[64] Associations between EDSS and both qMT and qihMT in lesions, but not NAWM have also been reported.[119] Combining qMT parameters, and including covariates such as lesion load and age may improve models[94] but collinearity (e.g. between f and $T2_{bound}$ or k_f and $T1_{free}$) may be problematic if used in the same model.[36,112]

Risk of bias

Seven studies (8.1%) were given an '*excellent*' rating based on JBI Critical Appraisal Checklist criteria (Supplementary Table 10). The majority of studies rated '*good*' or '*ok*' ($k = 33$, 38.4% each) and 13 studies (15.1%) were given a '*poor*' rating. The latter result, however, was partly driven by methodological 'proof of principle' studies for which there was no specific checklist.

Overall, the main sources of bias, where relevant, were inadequate examination of confounding factors, poor standardization and reliability of MTI outcomes, inappropriate statistical analyses, particularly concerning no correction for multiple comparisons, poor matching of cases and controls, and a lack of detail regarding setting/site description. Funnel plots also suggest that case–control studies with high precision are lacking, particularly for analyses of grey matter (Supplementary Fig. 4). Similarly, there appears to be a bias towards small, less powerful studies which examined the relationship between clinical disability and MTI in WM (Supplementary Fig. 5). In contrast, studies that used compartmental models had relatively high precision, particularly R1 and MTsat (Supplementary Fig. 6).

Discussion

Our search demonstrated a broad literature of MS-specific MTI studies, a considerable number of which were excluded due to the lack of distinctions between MS subtypes or grouped subtypes in analyses and results. Eighty-six studies used MTI to investigate cerebral RRMS pathology, the vast majority (87%) of which used MTR. We also incorporated in meta-analyses additional RRMS data from a further 38 studies which included mixed MS subtypes.

Common findings

Lesion MT was found to be lower than in NAWM. MT was also generally reduced in non-lesional brain for patients compared with HCs, indicative of subtle loss in microstructural integrity. Conversely, smaller sub-regions (e.g. thalamus, putamen) did not show such differences. The

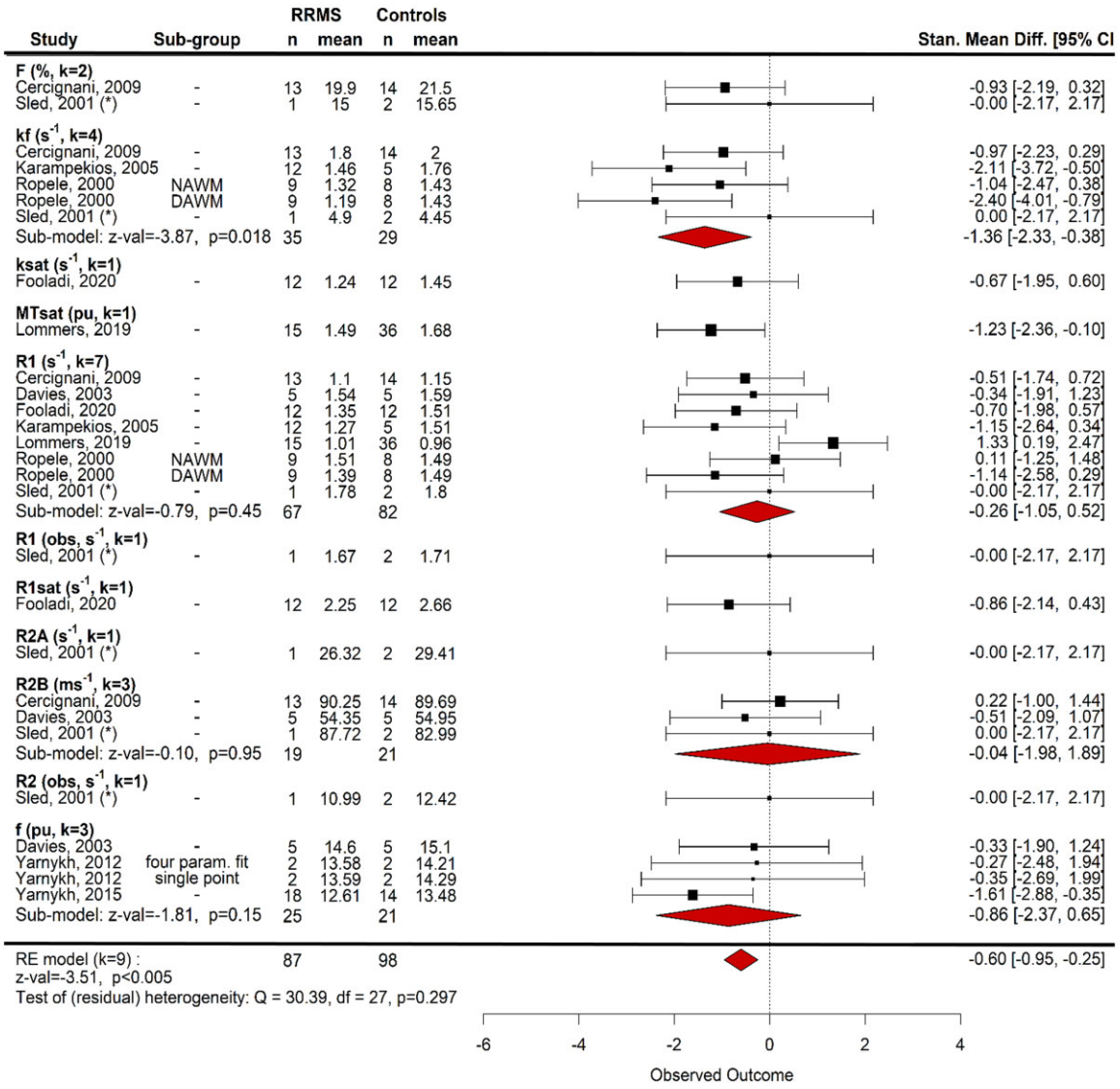


Figure 6 Random-effects meta-analysis of magnetization transfer compartmental model parameters in WM. Metric was a nested factor within study and subgroup (e.g. DAWM versus NAWM) was nested within metric. T1 and T2 were converted to R1 and R2, respectively, for comparability. For people with RRMS, compartmental model metrics were significantly lower than HCs (standardized mean difference −0.60, z-value = −3.51, $P = 0.002$, nine studies, 87 RRMS/98 HC). Random-effects models for individuals metrics were not significant after correction for multiple comparisons, despite a trend for the forward exchange rate, k_f (standardized mean difference −1.36, z-value = −3.87, $P = 0.018$, four studies). R1 (−0.26, z-value = −0.79, $P = 0.45$, seven studies), R2B (−0.04, z-value = −0.10, $P = 0.95$, three studies) and f (−0.86, z-value = 1.81, $P = 0.15$, three studies) did not differ between patients and HCs. DAWM, dirty-appearing white matter; NAWM, normal-appearing white matter; Stand Mean Diff, standardized mean difference. (*) frontal white matter; $\alpha = 0.05$ for omnibus test and $\alpha = 0.05/4 = 0.0125$ for subgroups.

absolute sensitivity of MT metrics to pathological changes in the brain of people with MS is modest; the difference in MTR between patients with RRMS and HCs is estimated to be small (∼0.5–2%) compared with inter-study variability. Meta-analyses did not support a significant annual longitudinal decline in MT in RRMS despite qualitative evidence to the contrary and a trend in NABT. In lesions, MT is inclined to fluctuate over time.

Although associations between MT measures and clinical disability in RRMS were apparent, relationships were weak, and confounded by factors such as age. This association may be limited by the lack of longitudinal data over sufficient time periods for divergence in disability to become apparent.

Studies examining longitudinal change and clinical correlates were limited to MTR; we did not identify any such studies using other techniques, such as MTsat, ihMTR or qMT.

Sample characteristics

Overall, patient sample sizes across the RRMS MTI literature were small, with a median of <20 subjects, and many

studies were statistically underpowered. Research with a technical or proof-of-concept focus tended to include a single subject or handful of participants (e.g.[11,42,105,106,116,118]). Conversely, international clinical trials recruited much larger cohorts (e.g.[91,92]), but at the expense of standardized, well-documented MTI protocols.

Comparisons between MS and (typically) age-matched HC subjects featured in a number of studies, albeit often with smaller control than patient groups. Such well-matched control data are important to account for confounding variables such as age,[85] and may additionally provide reference measures to help improve comparability of MT metrics across studies and centres.

Treatment effects are a further potential confound of MT microstructure measures, and inter- and intra-study heterogeneity was apparent in DMT and steroid usage which is an additional source of variability. Although some studies control for treatment effects, greater consistency is required in studies whose primary focus is imaging biomarker validation.

Imaging acquisition protocols

Systematic comparison of MTI in RRMS demonstrates substantial heterogeneity of MTI acquisition protocols. There was wide variation in magnetic field strength, pulse sequence, image weighting, excitation flip angle, TR and TE. With the rapid evolution of MRI hardware and techniques, such sources of variation are inevitable and well-recognized in the quantitative MRI literature. The nature of MT acquisition, however, makes MT measurements particularly sensitive to these factors. For example, simulations suggest that the difference between grey and WM MTR at 3 T at an offset frequency of 1.5 kHz is around 43% larger than at 1.5 T.[117] Use of proprietary hardware and pulse sequences allows broader access of MTI to research groups with limited MRI pulse programming expertise, but typically fixes, restricts and even conceals important pulse sequence parameters.

MT measurements are especially sensitive to characteristics of the MT pulse. Quantification typically assumes selective saturation of the 'bound' pool with minimal direct saturation of the 'free' water pool. The extent to which this is achieved *in vivo* and the resulting tissue-type contrast, however, depends on the complex relationship between tissue properties, hardware, sequence parameters and MT pulse design features including the offset frequency, power, pulse duration and shape.[98] In particular, our finding of the wide variance in NAWM MTR in RRMS cohorts is suggestive of sequence parameter dependence. Early experiments with relatively low offsets (e.g.[110,113]) are likely to have a greater direct saturation effect. Improved harmonization and standardization of MT protocols between centres would help to minimize these sources of variability.

The majority of large-scale MT studies in RRMS to date have used MTR, which is relatively easy to acquire and analyse. Importantly, however, MTR signal is markedly dependent on T1 and B1 effects in addition to magnetization transfer processes, which limits its specificity as a microstructural imaging marker of myelin integrity.

qMT provides the most accurate modelling of MT processes and is helpful for probing microstructure in healthy and pathological tissue; however, prolonged acquisition is needed at multiple pulse powers and offset frequencies with adequate spatial resolution. Whole-brain coverage is therefore not currently feasible for clinical imaging in patients.

Emerging MT methods such as MTsat and ihMTR provide potentially more robust and specific measures of myelin integrity than MTR within clinically feasible acquisition times.[11,121] Histological validation in felines has shown that MTsat is sensitive to demyelination,[122] and, in mice, ihMTR signal is more specific to myelin than MTR.[121] Both techniques, however, require further validation with histology and study in larger patient and HC cohorts.

Tissue types and definitions

The substantial variation observed in MTR values for different tissue types is likely due not only to varying acquisition parameters discussed above, but also how tissue type is defined, and variations in methods by which the regions are segmented from structural imaging. For example, individual studies examine different combinations of WM, NAWM, cortical and deep grey matter structures, atlas-based ROIs, and whole-brain analyses. Moreover, a number of different 'lesion types' are recognized in RRMS, as defined by their signal characteristics; for example, T2-w or FLAIR hyperintensities, T1-w hypointense lesions or 'black holes', and contrast-enhancing lesions. A clear definition of lesion subtypes is therefore important for the interpretation of their MT characteristics.

Sources of bias and limitations

Study quality, including assessment ratings of application of methods to minimize bias, was variable; the large majority of studies classified as 'good' or 'ok', and those rated 'poor' were largely associated with small methodologically focused papers.

Bias was apparent towards small sample sizes, and also towards studies using MTR compared with other techniques. Overall, high precision case–control studies were lacking and bias was apparent towards small, less well-powered studies correlating clinical disability with MTI measures. Overall, the small number of studies that used compartmental MTI models showed relatively high precision compared with MTR. Inadequate examination of confounding factors, poor standardization and reliability of acquisition methods, flawed statistical analyses, poor matching of cases and controls and lack of detail regarding the research setting were also identified in a significant number of studies.

Across studies, there was a near-universal bias towards European and North American populations, which is likely to reflect the geographical prevalence of MS, the attention given to the disease within healthcare systems, and access to

MRI and research protocols. Importantly, analysis of the location of study centres highlights possible bias due to data duplication from multiple or overlapping analyses of cohorts. This is rarely overtly reported, but may influence the calculation of effect sizes.

With regard to the review process, the literature search procedure was carried out by a single reviewer which may have led to bias in study selection, and influence overall certainty of evidence. Meta-analyses were limited by large inter-study protocol heterogeneity and missing data, and also did not take into account patient or control group demographics. The scope of the present review is also limited to results in RRMS patients. Data from progressive MS subtypes were excluded, but may still provide insights on how MT metrics reflect microstructural damage in MS.

Implications for future studies using MT in RRMS

The findings of this review indicate the potential for MT measures of microstructure as useful disease markers in MS, but equally highlight large variability in quantitative findings compared with modest effect sizes.

Major sources of systematic differences and variance in MTR measured across studies are technical variation in acquisition protocols, and confounding magnetic field homogeneity (B1) and magnetization relaxation processes (notably T1); relaxation processes, in particular, may lead to bidirectional longitudinal fluctuations in MTR. These effects, combined with variability in cohort characteristics and experimental design, contribute to weak association with clinical measures of disease.

Harmonizing MTR acquisition protocols across participating centres will go some way to mitigate this variability, although will not address the confounds of B1 and T1 effects. Signal from more quantitative, clinically applicable MT methods such as MTsat and ihMT is less confounded by these technical features and other tissue characteristics, and hence provide more specific biomarkers of myelin status. These methods, however, require further evaluation, with rigorous validation against tissue reference data, and other biomarkers of MS disease activity and neurodegeneration.

Cohorts which are adequately powered to detect predicted effect sizes are likely to require large multicentre studies of highly characterized patients with defined MS disease subtypes. Further optimization, harmonization and cross-site validation of MTI protocols across multiple MRI platforms, will allow assessment of inter-site variance and potential systematic differences in measures across centres.

Adoption of more consistent definitions and methods for segmenting tissues of interest will also facilitate comparability across sites and studies.

We, therefore, expect that moving towards more quantifiable, harmonized MT protocols in large well-defined and annotated cohorts will provide a more reliable indication of the relationships between MT and clinical features in MS, and hence their potential utility in patient stratification and clinical trial platforms.

Moreover, we suggest that in order for MTI to evolve as a useful imaging tool in MS and other diseases, there is a need to establish consensus standards for image acquisition, analysis and reporting from an international group of experts working across centres, as has been successfully achieved with other quantitative MRI methods such as diffusion and perfusion imaging.[123–125]

Conclusion

This systematic review demonstrates a substantial literature on MTR applied to RRMS. The evidence evaluated suggests that MT imaging can detect subtle disease-related differences. There is, however, large measurement variability due to differences in technique; this dominates over small effect sizes which, in turn, limit clinical and biological interpretation. The implementation of more robust emerging quantitative techniques, and consensus regarding optimized, harmonized protocols in large well-characterized patient cohorts will be required to establish the value of MTI as a useful microstructural marker in RRMS, for translation into wider clinical use.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain Communications* online.

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