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T1/T2-weighted ratio in multiple sclerosis: A longitudinal study with clinical associations

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ABSTRACT

Background: T1w/T2-w ratio has been proposed as a clinically feasible MRI biomarker to assess tissue integrity in multiple sclerosis. However, no data is available in the earliest stages of the disease and longitudinal studies analysing clinical associations are scarce.
Objective: To describe longitudinal changes in T1-w/T2-w in patients with clinically isolated syndrome (CIS) and multiple sclerosis, and to investigate their clinical associations.
Methods: T1-w/T2-w images were generated and the mean value obtained in the corresponding lesion, normal-appearing grey (NAGM) and white matter (NAWM) masks. By co-registering baseline to follow-up MRI, evolved lesions were assessed; and by placing the mask of new lesions to the baseline study, the pre-lesional tissue integrity was measured.
Results: We included 171 CIS patients and 22 established multiple sclerosis patients. In CIS, evolved lesions showed significant T1-w/T2-w increases compared to baseline (+7.6%, $P < 0.001$). T1-w/T2-w values in new lesions were lower than in pre-lesional tissue (-28.2%, $P < 0.001$), and pre-lesional tissue was already lower than baseline NAWM (-7.8%, $P < 0.001$). In CIS at baseline, higher NAGM T1-w/T2-w was associated with multiple sclerosis diagnosis, and longitudinal decreases in NAGM and NAWM T1-w/T2-w were associated with disease activity. In established multiple sclerosis, T1-w/T2-w was inversely correlated with clinical disability and disease duration.
Conclusion: A decrease in T1-w/T2-w ratio precedes lesion formation. In CIS, higher T1-w/T2-w was associated with multiple sclerosis diagnosis. In established multiple sclerosis, lower T1-w/T2-w values were associated with clinical disability. The possible differential impact of chronic inflammation, iron deposition and demyelination should be considered to interpret these findings.

1. Introduction

Magnetic resonance imaging (MRI) is the most important para-clinical tool in diagnosing and monitoring multiple sclerosis (MS), and a key surrogate endpoint in clinical trials. However, pathophysiological research has shown that conventional MRI has limited sensitivity to the microstructural changes, both at the lesional level and the normal-appearing grey (NAGM) and white matter (NAWM) (Filippi et al., 2019). In clinical routine, new T2-weighted (w) lesions and contrast-enhancing lesions are commonly used for monitoring subclinical disease activity and evaluating the effectiveness of pharmaceutical treatments (Wattjes et al., 2015). However, at least at individual level, these sequences have poor correlation with clinical findings. Several novel MRI techniques have been developed to probe MS related cerebral changes in a more specific and quantitative manner. Magnetization transfer imaging, diffusion tensor imaging, and myelin-water imaging have been described as more specific techniques to unravel the diversity of microstructural tissue changes not visible with conventional MRI. Nevertheless, these techniques have limitations, such as longer acquisition times, poor spatial resolution, lack of harmonization or the need for expertise in image post-processing (Enzinger et al., 2015).

The ratio of T1-w and T2-w image intensities (T1-w/T2-w) from

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standard MRI protocols was initially described as a biomarker of cortical myelin content (Glasser and Van Essen, 2011; Glasser et al., 2014; Shams et al., 2019) although further studies have established T1-w/T2-w as a measure of microstructural integrity (Righart et al., 2017; Arshad et al., 2017; Uddin et al., 2019; Pareto et al., 2020). Two works showed the application of the T1-w/T2-w ratio in cortical pathology in MS (Righart et al., 2017; Nakamura et al., 2017). Both studies have demonstrated an association between reduced T1-w/T2-w values and tissue damage. Furthermore, two other studies have found reduced T1-w/T2-w ratios in the NAWM of MS patients compared to healthy controls (Beer et al., 2016; Cooper et al., 2019). Taking it all into consideration, both low myelin content and/or loss of microstructural integrity may explain most of these findings. However, longitudinal studies with clinical associations are scarce and few data is available in the earliest stages of the disease.

In this study, we set out to assess, in patients with clinically isolated syndrome (CIS) and in a cohort of relapsing remitting MS patients treated with disease modifying drugs, baseline and longitudinal changes in T1-w/T2-w ratio in NAGM and NAWM with clinical associations. We also aimed to investigate the dynamics of T1-w/T2-w ratio in focal lesional tissue to gauge the microstructural changes underlying lesion formation and evolution.

2. Subjects/Materials and methods

2.1. Patients and clinical assessments

We analyzed two cohorts described as *CIS cohort* and *MS cohort*. First, we assessed previously acquired data from a prospective cohort of patients with CIS (*CIS cohort*) who underwent brain MRI for diagnosis and for monitoring disease evolution (Tintore et al., 2015). CIS was defined as an episode suggestive of CNS inflammatory demyelination (McDonald et al., 2001). At baseline, the demographic data, previous history of neurological abnormalities, the CIS topography, and disability according to the Expanded Disability Status Scale (EDSS) score were recorded. The patients were evaluated on a regular basis (every 3 to 6 months) to assess both the EDSS score and the occurrence of relapses. A lumbar puncture to assess the presence of IgG oligoclonal bands (OB) was performed within the first 3 months of disease onset. Baseline MRI was obtained within the first 3 to 5 months after the onset of symptoms and the second, at 12 months after clinical onset. After excluding alternative diagnosis, all CIS patients were analyzed, regardless of the presence or absence of brain lesions that met criteria for MS at first relapse (e.g, CIS that are already clinically definite MS patients at baseline, and also isolated myelitis or optic neuritis with a normal brain MRI). Fulfillment of McDonald 2017 diagnostic criteria was assessed at baseline (Thompson et al., 2018). From this first cohort, the clinical data used (EDSS and relapses) were analyzed up to 3 years of follow-up. In the second cohort (*MS cohort*), which consisted of established relapsing remitting MS patients starting on disease-modifying treatment, a baseline brain MRI was acquired at treatment onset, and a follow-up brain MRI 12 months afterwards. In the *MS cohort,* baseline and 1-year follow-up clinical data was retrospectively collected, including EDSS and MS relapses.

The study was approved by the local ethical committee and a written informed consent was signed by the participating patients.

2.2. MRI acquisition

All patients from the *CIS cohort* and the *MS cohort* underwent brain MRI at baseline and follow-up on the same 3 T magnet (Tim Trio; Siemens, Erlangen, Germany) with a 12-channel phased array head coil. The MRI protocol included the following sequences: 1) sagittal T1-w 3D MPRAGE (TR = 2,300 ms, TE = 2.98 ms, TI = 900 ms, voxel size = 1.0 × 1.0 × 1.2 mm^3), 2) transverse proton density (PD)- and T2-w fast spin-echo (TR = 3,080 ms/TE = 21–91 ms, voxel size = 0.78 × 0.78 × 3.0 mm^3), and 3) transverse fast FLAIR (TR = 9,000 ms, TE = 87 ms, TI = 2,500 ms, flip angle = 120°, voxel size = 0.49 × 0.49 × 3.0 mm^3).

2.3. MRI qualitative analysis

To define any MRI abnormalities, to fulfill McDonald 2017 diagnostic criteria and to exclude alternative diagnosis, all MRI scans were analyzed by an expert neuroradiologist. This analysis included the number and location of hyperintense lesions on T2-FLAIR scans, the presence of gadolinium-enhancing lesions and the number of new T2-w lesions. A normal brain MRI was defined as displaying no WM abnormalities $\geq$ 3 mm suggestive of MS (Filippi et al., 20192019).

2.4. Processing of the T1-w/T2-w ratio imaging data

T1-w/T2-w images were generated as proposed by Ganzetti *et al.*, (Ganzetti et al., 2014) with the MRTool - Multimodal Mapping extension (v. 1.2, Swiss Federal Institute of Technology, Zurich, Switzerland, http://www.fil.ion.ucl.ac.uk/spm/ext) for Statistical Parametric Mapping (SPM) 12 (University College London, London, UK, http://www.fil.ion.ucl.ac.uk/spm). The processing includes Intensity Non-Uniformity correction and rigid registration of the T2-w images to the T1-w images, with linear calibration of image intensity modes using non-brain tissue masks (eye and temporalis muscle from both the T1-w and T2-w images). The output includes: 1) a normalized, scaled and unbiased T1-w and T1-w/T2-w ratio image, and 2) a normalized unbiased T1-w image, which can be used for tissue segmentation (to allow the evaluation of regional values in T1-w/T2-w in the same space). For an overview of the entire processing pipeline see Fig. 1.

2.5. Brain tissue segmentation and T1-w/T2-w for NAWM and NAGM

Tissue segmentation into GM, WM, and CSF was achieved using the segment tool implemented in SPM12. This procedure was applied to the normalized T1-w image. Lesion masks were subtracted from the WM and GM masks to create NAWM and NAGM masks. Only voxels with a WM or GM probability higher than 0.7 in T1-w were used to define the NAGM and NAWM masks. Mean intensity values on the T1-w/T2-w images were obtained for the NAGM and NAWM masks in each subject. An additional analysis was performed to generate separate masks for deep and cortical GM. Deep GM was segmented with FIRST, (Patenaude et al., 2011) the corresponding mask was created and removed from the NAGM, generating, as a result, the cortical GM masks.

3. Generation of lesion masks and T1-w/T2-w for lesional tissue

3.1. Baseline, follow-up and evolved lesion masks

Baseline and follow-up WM lesion masks were obtained with the Lesion Segmentation Tool (LST) toolbox version 1.2.3. for SPM12, using T2-FLAIR and T1-w images in all cohorts and the corresponding volumes, calculated. In the *CIS cohort* and the *MS cohort*, baseline MRI and lesion mask were co-registered to the follow-up MRI scan to create a mask of the evolved lesional tissue, regardless of their appearance on follow-up scans (see Fig. 2).

3.2. New lesions and pre-lesional tissue masks

In the *CIS cohort*, new T2-w lesions visually detected by a trained technician on follow-up MRI and were annotated on T2-proton density (PD) images by using the semiautomatic tool included in Jim 5.0 (http://www.xinapse.com/home.php). New lesion masks were later revised and confirmed by an expert neuroradiologist. For technical reasons, new lesion masks were only available for 52 out of 59 patients with new lesions. T2-PD images and masks for new lesions were then co-registered to baseline MRI scans using SPM12 (first to follow-up and then to

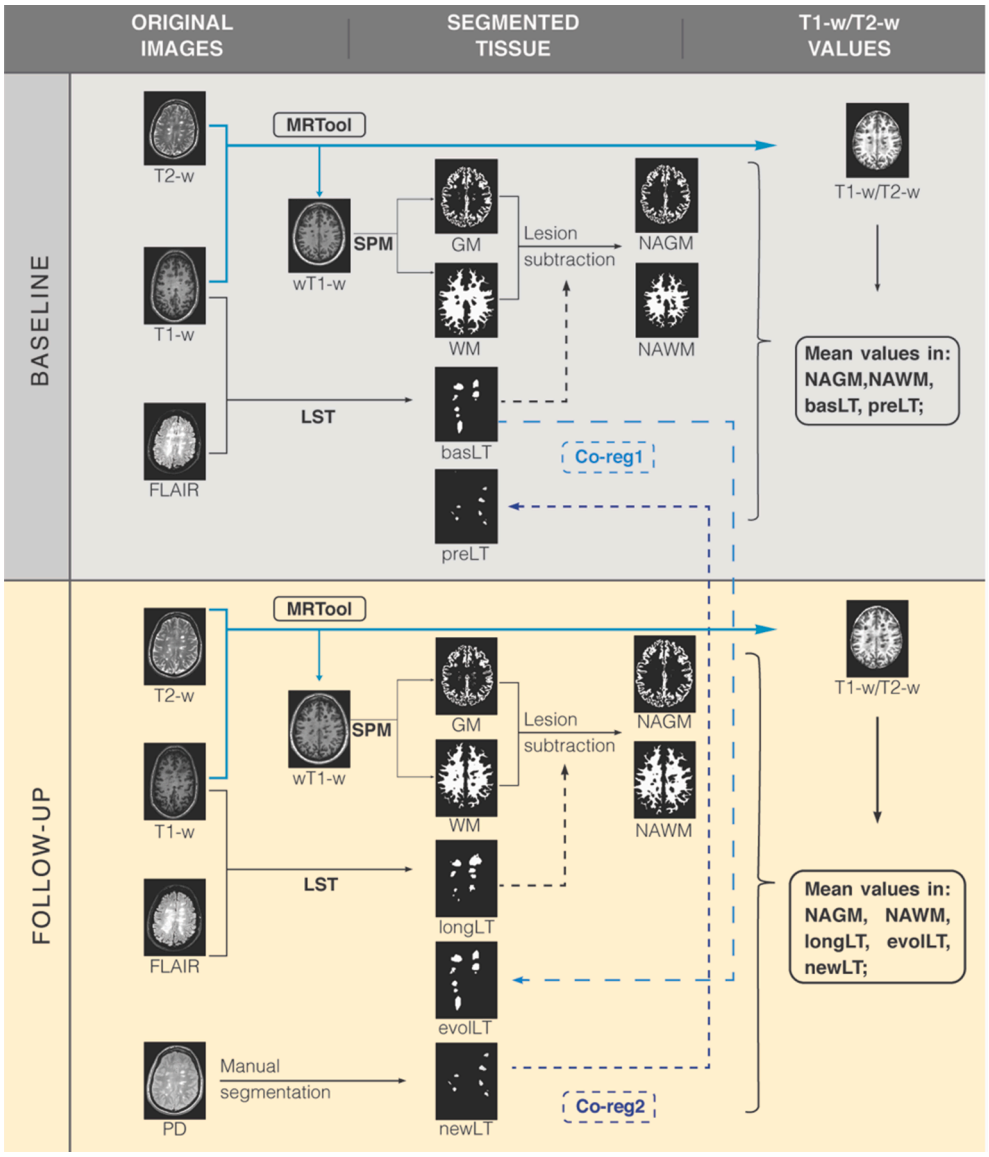

Fig. 1. Overview of the image acquisition and processing pipeline. Flow diagram of steps to generate tissue segmentation and T1-w/T2-w images in clinically isolated syndrome and multiple sclerosis cohorts. See text for further details. Abbreviations: wT1-w = normalized T1-w; LST = lesion segmentation tool; SPM = statistical parametric mapping; GM = grey matter; WM = white matter; NAGM = normal-appearing grey matter; NAWM = normal-appearing white matter; basLT = baseline lesion tissue mask; evolLT = mask of the evolved lesions; newLT = mask for new lesions; preLT = pre-lesional tissue mask; longLT = longitudinal lesion tissue mask; PD = proton density; Co-reg = corregistration.

baseline MPRAGE images) to create a mask of baseline pre-lesional tissue (see Fig. 2).

3.3. T1-w/T2-w Ratio in lesion masks

Since the normalized T1-w/T2-w image from MRTool and the lesion masks were in the native space and differ topographically across the brain, we added an extra step to analyze the corresponding lesion topography. On one side, the raw T1-w images and their corresponding lesion masks, and, on the other side, the calibrated and unbiased T1-w and T1-w/T2-w images, all were registered to the MNI152 T1-w 1 mm brain template from the Montreal Neurological Institute. Mean intensity values on the T1-w/T2-w images were obtained for all lesion masks. An additional exploratory analysis was performed, assessing separately T1-w and T2-w signal intensity at baseline and follow-up lesion masks to analyze whether the numerator or denominator was the main driver of the changes observed.

3.4. Statistical analysis

Statistical analysis was performed by using IBM SPSS Statistics 26 for Mac (IBM, Armonk, USA). Kolmogorov-Smirnov test was used to assess normality. Unpaired parametric data using unpaired *t*-test and unpaired non-parametric data using independent- samples Mann-Whitney *U* test. Paired analyses were compared with paired *t*-test for parametric data and related samples Wilcoxon signed rank test for non-parametric data. For multigroup comparisons, repeated measures analysis of variance (ANOVA) was performed to compare T1-w/T2-w values between masks and times, followed by Bonferroni multiple comparisons to identify between which masks and times the differences occur.

In the CIS cohort, to evaluate differences between patients with or without suggestive MS diagnosis at CIS onset (abnormal MRI, positive OB and 2017 McDonald criteria fulfillment) and with or without inflammatory disease (new lesions and new relapses) in T1-w/T2-w values, we performed a general linear model with adjustment for age and sex. Bivariate correlations were analyzed with Pearson correlation for parametric data and Spearman's rank correlation coefficient for non-parametric data. Partial correlations were assessed using covariates of interest (age and sex). A two-tailed $P < 0.05$ was considered statistically significant.

4. Data availability

Requests for access to the data reported in this article will be

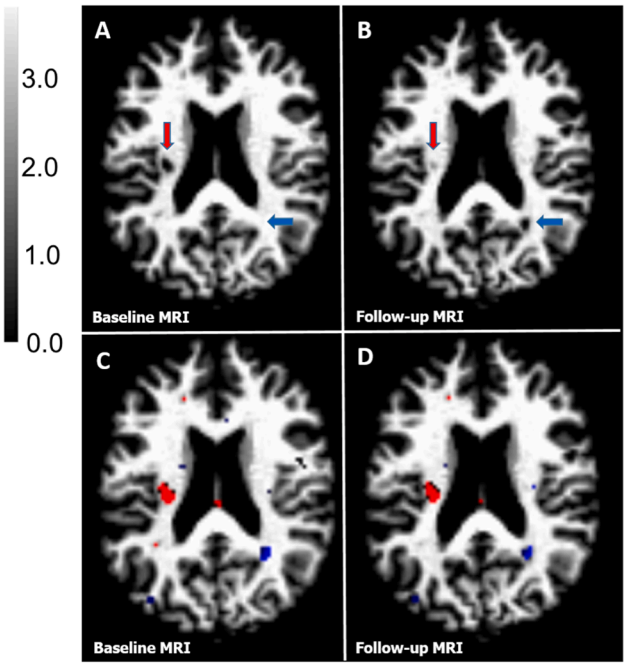

Fig. 2. Example of lesion evolution. Baseline (A and C) and follow-up (B and D) T1-w/T2-w images from a 33-year-old male patient who presented a spinal cord clinically isolated syndrome, with new brain lesions at 12 months scan. The red arrows show an example of a lesion that reduces in size and increases in signal in the T1-w/T2-w image. The blue arrows show an example of a new lesion (in B) and its corresponding pre-lesional tissue (in A). Overlaid Masks of all lesions: red (in C) represents baseline lesion tissue co-registered to follow-up, where evolution lesion tissue is also colored in red (in D); blue represents new lesion tissue in D and pre-lesional tissue in C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

considered by the corresponding author.

5. Results

5.1. Demographic, clinical and radiological characteristics

A total of 171 CIS patients and 22 treated MS patients were included in the study. All clinical, demographic and MRI data is summarized in Table 1.

5.2. T1-w/T2-w In NAGM, NAWM and lesional tissue

In the entire *CIS cohort* with valid data at baseline and follow-up (168 patients), no significant longitudinal T1-w/T2-w changes in NAGM (0.96 ± 0.08 vs 0.97 ± 0.08, $P = 0.64$) and NAWM (1.52 ± 0.16 vs 1.53 ± 0.16, $P = 0.69$) were observed. In the subgroup of patients with abnormal MRI at baseline (117 patients), T1-w/T2-w in baseline lesional tissue was lower compared to T1-w/T2-w ratio in baseline NAWM (0.85 ± 0.1 vs 1.52 ± 0.15, $P < 0.001$) and NAGM (0.85 ± 0.1 vs 0.97 ± 0.08, $P < 0.001$). T1-w/T2-w ratio in evolved lesional tissue was significantly higher (+7.6%) than baseline lesional tissue (0.91 ± 0.15 vs 0.85 ± 0.1, $P < 0.001$). In the subgroup of patients with new lesions (52 patients), T1-w/T2-w in new lesions tissue was lower than in pre-lesional tissue (-28.2%, 1.02 ± 0.24 vs 1.42 ± 0.24, $P < 0.001$) (Fig. 3). Interestingly, T1-w/T2-w in pre-lesional tissue was significantly lower than baseline NAWM (-7.8%, 1.42 ± 0.25 vs 1.54 ± 0.18, $P < 0.001$) (Fig. 3) but higher than baseline lesional tissue (+65.1%, 1.42 ± 0.25 vs 0.86 ± 0.1, $P < 0.001$) (Fig. 3).

T1-w/T2-w values were higher in deep GM as compared to cortical GM, both at baseline (cortical GM: 0.96 ± 0.08 vs deep GM 0,99 ± 0,09, $P < 0.001$) and follow-up (cortical GM: 0.97 ± 0.08 vs deep GM 0,99 ± 0,09, $P < 0.001$) in the CIS cohort. In the lesional tissue, a significantly higher percentage reduction in T2-w compared with the increase in T1-w signal intensity was observed (percentual change in T2-w: −2.89% ± 10.96 vs T1-w: +1.70% ± 4.03; $P < 0.001$) in CIS patients.

In the *MS cohort*, no longitudinal changes in T1-w/T2-w values in NAGM and NAWM were observed after 12 months of therapy: NAGM (baseline vs follow-up: 0.97 ± 0.13 vs 0.93 ± 0.1; $P = 0.13$) and NAWM (1.49 ± 0.21 vs 1.44 ± 0.19; $P = 0.24$). T1-w/T2-w in baseline lesional tissue was lower compared to baseline NAGM (0.78 ± 0.14 vs 0.96 ± 0.12, $P < 0.001$) and NAWM (0.78 ± 0.14 vs 1.47 ± 0.21, $P < 0.001$). Baseline lesional tissue T1-w/T2-w was not different from evolved lesional tissue (0.78 ± 0.14 vs 0.80 ± 0.17, $P = 0.67$).

Table 1
Demographics, clinical and MRI characteristics.

Cohort	CIS	MS	p
	(n = 171)	**(n = 22)**	
Female, n (%)	110 (64.3)	14 (63.6)	0.949
Age, mean (SD)	33.2 (7.5)	36.5 (6.8)	0.052*
Disease duration, years, mean (SD)	0.3 (0.12)	8.9 (7.7)	<0.001*
Follow-up time, years, mean (SD)	3.2 (1.7)	2.4 (1.0)	0.119*
MRI interval time, months, mean (SD)	8.9 (4.5)	13.0 (6.0)	<0.001*
CIS topography, n (%)			
Optic nerve	67 (39.2)	NA	
Brainstem	44 (25.7)	NA	
Spinal cord	45 (26.3)	NA	
Other	15 (8.8)	NA	
EDSS, median (range)			
Baseline	1.5 (0–4.5)	1.5 (0–8.5)	0.012£
1 year follow-up	1.0 (0–3.5)	1.5 (0–8.5)	<0.001£
3 years follow-up	1.0 (0–3.5)	NA	
Positive OB, n (%)	82/151 (54.3)	17/19 (89.5)	0.003
Baseline brain MRI			0.002
Normal, n (%)	54 (31.6)	0 (0)	
Abnormal, n (%)	117 (68.4)	22 (1 0 0)	
Gadolinium T1 enhancement, n (%)	40/130 (30.8)	9/22 (40.9)	0.347
Lesion volume, mL, mean (SD)			
Baseline	4.0 (9.3)	15.3 (26.8)	0.094*
Follow-up	3.2 (5.9)	14.5 (24.7)	0.063*
Baseline McDonald 2017			
Fulfillment, n (%)	69 (40.4)	22 (1 0 0)	<0.001
New relapses			
1-year follow-up, n/n (%)	26/171 (15.2)	1 (4.5)	0.323#
3 years follow-up, n/n (%)	37/145 (25.5)	NA	
New lesions (12 months), n (%)	59 (34.5)	4 (18.2)	0.007
Patients on DMT, n (%)	52 (30.4)	22 (1 0 0)	<0.001

Notes: Data is presented as mean ± SD (standard deviation), median (range) or n (%). Age in years at baseline MRI. Disease duration means time from the first relapse to baseline MRI. Follow-up time means time from first assessment to the last visit in CIS cohort, and in MS cohort the time from baseline assessment at DMT starting to the last visit. CIS = clinical isolated syndrome; MS = multiple sclerosis; EDSS = Expanded Disability Status Scale; OB = oligoclonal bands; MRI = magnetic resonance imaging; DMT = disease-modifying treatment. Chi-square test; # Fisher's exact test; * Student's *t*-test; £ Mann-Whitney's test.

5.3. T1-w/T2-w And clinical outcomes

In the *CIS cohort* at baseline, a higher NAGM T1-w/T2-w ratio was observed in patients with an abnormal brain MRI, positive OB and fulfilment of 2017 McDonald criteria. In NAWM, only a trend for a higher T1-w/T2-w was observed in patients fulfilling 2017 McDonald criteria (Table 2). Over follow-up, larger decreases in T1-w/T2-w values in both NAGM and NAWM were observed in patients fulfilling 2017 McDonald criteria, presence of OB, and an abnormal brain MRI at baseline, although these differences did not reach statistical

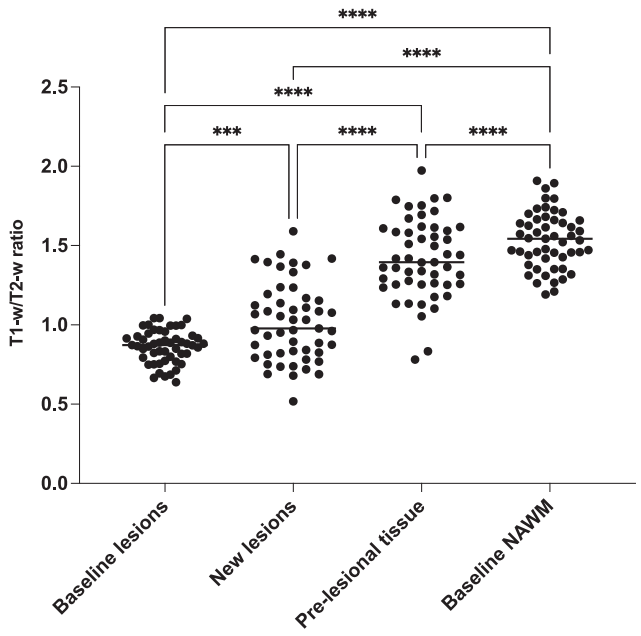

Fig. 3. T1-w/T2-w mean values in different tissues and time points in the CIS cohort. Multigroup comparisons with repeated measures analysis of variance (ANOVA), followed by Bonferroni multiple comparisons. All masks in this graph showed a statistically significant difference among them ($P < 0.001$). NAWM = normal-appearing white matter.

significance. Patients presenting disease activity during the follow-up (new T2-w lesions at 12 months MRI, new relapses one and three years after CIS) showed a decrease in T1-w/T2-w values in NAGM and NAWM, while in stable patients an increase in T1-w/T2-w values was observed **(**Table 2**).** For cortical and deep GM, these same associations are demonstrated in Supplementary Table 1. Longitudinal changes in T1-w/T2-w values in lesional tissue were not associated with clinical activity during the follow-up (data not shown). After age and sex adjustment, no significant correlations were observed between baseline EDSS and baseline T1-w/T2-w values in NAGM (r = 0.015, $P = 0.87$), NAWM (r = 0.032, $P = 0.74$) or baseline lesional tissue (r = 0.094, $P = 0.32$).

In the *MS cohort*, after adjusting for age and sex, disease duration and baseline EDSS were inversely correlated with baseline T1-w/T2-w in NAGM (disease duration: r = -0.557, $P = 0.031$; and; baseline EDSS: r = -0.505, $P = 0.044$) and NAWM (disease duration: r = -0.522, $P = 0.046$; baseline EDSS: r = -0.517, $P = 0.049$).

Partial correlations between T1-w/T2-w at the different compartments and clinical variables (EDSS, disease duration and lesion volume) are presented as supplementary data (Supplementary Table 2), for both cohorts and time-points.

6. Discussion

In this study, we investigated changes in T1-w/T2-w within NAGM, NAWM, and focal lesional tissue in CIS and MS patients. To the best of our knowledge, this is the first study to investigate longitudinal associations with clinical outcomes concerning T1-w/T2-w at all tissue levels. In CIS patients, we found that T1-w/T2-w changes occurring in lesions seem to indicate tissue damage and recovery and that alterations in T1-w/T2-w precede lesion formation. In NAGM and NAWM, discordant associations have been found in the *CIS cohort*: higher baseline T1-w/T2-w values were associated with a diagnosis of MS but, in contrast, longitudinal decreases were associated with worse outcomes. In established MS, lower T1-w/T2-w ratio in NAGM and NAWM was associated with longer disease duration and higher EDSS and remained unchanged after one year of therapy.

Table 2
T1w/T2-w and clinical outcomes in the *CIS cohort.*

	NAGM T1-w/ T2-w	p value	NAWM T1-w/ T2-w	p value
Baseline				
Brain MRI				
Abormal	0.97 (0.01)	<0.001	1.51 (0.01)	0.729
Normal	0.92 (0.01)		1.50 (0.02)	
Oligoclonal Bands				
Positive	0.97 (0.01)	0.013	1.52 (0.02)	0.263
Negative	0.94 (0.01)		1.50 (0.02)	
2017 McDonald criteria				
Fulfillment	0.98 (0.01)	0.001	1.53 (0.02)	0.080
Non-fulfillment	0.94 (0.01)		1.49 (0.01)	
Change over follow-up				
Brain MRI				
Abormal	−0.18 (0.58)	0.061	0.08 (0.63)	0.249
Normal	1.78 (0.86)		1.38 (0.93)	
Oligoclonal Bands				
Positive	−0.56 (0.71)	0.137	−0.40 (0.72)	0.261
Negative	0.98 (0.75)		0.79 (0.77)	
2017 McDonald criteria				
Fulfillment	−0.39 (0.80)	0.180	−0.32 (0.87)	0.248
Non-fulfillment	0.97 (0.61)		0.94 (0.66)	
New lesions at 12 *m*				
Yes	−1.04 (0.79)	0.019	−0.85 (0.86)	0.048
No	1.35 (0.62)		1.32 (0.67)	
New relapses at 12 *m*				
Yes	−2.54 (1.17)	0.005	−2.00 (1.25)	0.026
No	1.08 (0.52)		1.08 (0.56)	
New relapses at 36 *m*				
Yes	1.69 (1.02)	0.016	−1.73 (1.08)	0.032
No	1.25 (0.64)		1.05 (0.68)	

Notes: Data is presented as mean/SD (standard deviation) and changes as percentage. The associations were assessed by using general linear model (adjusted for age and sex). Differences were considered statistically significant at $P < 0.05$. Abbreviations: CIS = clinical isolated syndrome; NAGM = normal-appearing grey matter; MRI = magnetic resonance imaging; OB = oligoclonal bands; NAWM = normal-appearing white matter.

In both cohorts, cross-sectional analysis at baseline revealed lower T1-w/T2-w values in lesional tissue compared to normal-appearing tissue, what may probably reflect differences in both myelin concentration and tissue integrity. This finding is in line with histopathological evidence showing that the pathological abnormalities affecting lesions consist of major demyelination and severe tissue destruction (Bitsch et al., 2001; Barkhof et al., 2003; Laule et al., 2006). In contrast, abnormalities affecting normal-appearing tissue mainly consist of axonal damage and loss and microglial activation, (Kutzelnigg et al., 2005; Howell et al., 2010) but do not include major demyelination. In other words, focal damage in lesional tissue may have a higher myelin-specific nature, which could contribute to the main pathological substrate of the lowest T1-w/T2-w values found in our study, while the normal-appearing tissue show a non-myelin-specific nature of damage. This gradient is in line with a study that demonstrated a significant reduction of T1-w/T2-w ratio in cortical and WM lesions, compared with homologous non-lesioned areas (Granberg et al., 20172017).

In the CIS cohort, a significant increase in T1-w/T2-w comparing evolved lesional tissue with baseline lesional tissue was observed. This same finding was not observed in the MS cohort, where T1-w/T2-w values in lesional tissue did not change over follow-up. Besides edema resolution (probably driving more T2-w signal decreasing), we assume that increasing T1-w/T2-w values in CIS lesions depend, to some extent, on the level of remyelination in the lesion itself and might somehow indicate tissue recovery. The mechanisms and targets of demyelination in *MS* may be different in the distinct subgroups or stages of the disease,

(Lucchinetti et al., 2000) or in the different types of lesions (Faizy et al., 2016). An important difference between our cohorts is disease duration. A study showed that remyelination is a frequent event in early MS stages, but in chronic lesions, the extent of remyelination was limited in most patients (Goldschmidt et al., 2009). Thus, myelin dynamics in *MS* depends on individual remyelination potential, justifying differences between the cohorts studied. Therapy effects are other potential variables for changes in ratio, which are not evaluated in this study.

Interestingly, we have showed that T1-w/T2-w ratio in pre-lesional tissue was already decreased when compared to baseline NAWM. These results suggest that changes in NAWM of patients with MS occur before lesions become evident on conventional MRI scans. Comparable findings have been identified in previous studies with other methods, such as MRI frequency shifts using susceptibility-weighted imaging (Wiggermann et al., 2013); magnetization transfer ratio (MTR) (Filippi et al., 1998; Goodkin et al., 1998; Pike et al., 2000) and proton MR spectroscopy (Tartaglia et al., 2002).

We have found unexpected associations in NAGM and NAWM in CIS patients: higher baseline T1-w/T2-w values were associated with a diagnosis of MS but, conversely, longitudinal decreases were associated with concurrent disease activity. Hypercellular content state could justify the unexpected higher T1/T2 ratio (Blystad et al., 2016; Thaler et al., 2017) in pathological CIS patients, decreasing with inflammation resolution. These findings also show the low specificity of this marker. Two studies (Righart et al., 2017; Nakamura et al., 2017) demonstrated correlations between reduced values and tissue damage, although in different topographies and pathological substrates: in demyelinated cortex correlating with myelin density, and in cortical NAGM correlating with dendrite density. Still on histological validation at the cortical level, despite a good sensitivity to detect cortical demyelination, a modest T1-w/T2-w ratio specificity was demonstrated in a recent work (Zheng et al., 2022). In addition to the cerebral cortex, in two other studies focusing on different subcortical regions, (Arshad et al., 2017; Uddin et al., 2019) T1-w/T2-w ratio has been suggested as a general measure of microstructure, which may be more affected by axonal diameter/density than myelin density. These studies used myelin water fraction (MWF) as a validated measure of myelin density, (Laule et al., 2006) showing low concordance between T1-w/T2-w and MWF measurements in cerebral WM and subcortical GM structures. On this basis, these works have suggested that T1-w/T2-w and MWFs appear to be sensitized to different subcortical microstructural properties. Recently, (Pareto et al., 2020) our group demonstrated moderate correlation between MTR and T1-w/T2-w in NAGM, and strong correlation in NAWM and lesions. This study suggests again that, besides myelin integrity, other factors may be playing a role in T1-w/T2-w measures.

More recently, three different articles also showed higher T1-w/T2-w values in different CNS regions in other disorders: Parkinson disease (Du et al., 2019), Huntington disease (Rowley et al., 2018) and in Alzheimer's patients (Pelkmans et al., 2019). Such differences clearly suggest that T1-w/T2-w is not just measuring myelin, and that other factors such as iron content, microglia activation and amyloid deposition may be playing a role. Certainly, in MS and very early CIS patients, other processes such as edema resolution, inflammation, iron uptake and removal, and axonal/dendrite diameter and density may influence these values.

In the *MS cohort*, T1-w/T2-w values were inversely correlated with disease duration and EDSS. Previous non-longitudinal studies showed different results concerning clinical associations between T1-w/T2-w and EDSS. In line with our results, T1-w/T2-w values in NAWM were inversely correlated with EDSS in a study of 244 MS patients (Beer et al., 2016). In contrast, another work did not show any significant associations between T1-w/T2-w and EDSS, although the sample size was much smaller (26 patients) (Granberg et al., 20172017). A recent longitudinal work (Cooper et al., 2021) in CIS and early MS patients showed that T1-w/T2-w in NAWM is associated with increasing lesion volume and disease activity in the first 2 years of disease after first clinical presentation.

Concerning T1-w/T2-w values in cortical and deep GM, ferritin iron has a strong effect on decreasing T2-w signal, but only a weak effect on increasing T1-w signal, (Vymazal et al., 1995) leading to an slight increase in T1-w/T2-w. This could explain the higher T1-w/T2-w in deep GM compared to cortical GM, as the basal ganglia have been shown to accumulate iron.

Compared to previous studies, we could highlight some positive aspects of ours, namely the first complete T1-w/T2-w brain tissue analysis of a large CIS cohort with prospective clinical and radiological evaluation. Concerning our methodology, some limitations should be noted. We did not include healthy controls for comparison. Although T1-w/T2-w has usually been applied to high resolution 3D T1-w and 3D T2-w sequences, we used 2D T2-w sequences with 3 mm slice thickness, which may have introduced a partial volume effect in our T1-w/T2-w. Cerebral atrophy may also influence tissue segmentation, inducing partial volume effects. However, the short duration of disease in the CIS cohort could mitigate this effect, due to minor atrophy observed in this phase. Another limitation is the lack of B1 + residual correction of the T1-w/T2-w maps, something that is not yet widely available, but means that it might be wise to regress measures of head size (i.e. all head tissues, not just brain size in the subject's own physical space) and body mass index (BMI) out of any statistical analyses as covariates of no interest. We have considered age and sex. But it would be very interesting to investigate the role of BMI in future studies. An additional assessment that might be included is the cortical lesional tissue, using MRI sequences as double inversion recovery and phase sensitive inversion recovery, to evaluate T1-w/T2-w in cortical lesion GM. Nevertheless, the total cortical lesion volume is much lower than the total volume of cortical NAGM, thus we may not expect a change in the mean value.

In conclusion, no longitudinal changes in T1-w/T2-w values in NAGM and NAWM were observed over 1 year in both cohorts. In CIS patients, T1-w/T2-w changes occurring in lesional tissue seem to indicate tissue damage and recovery with alterations in T1-w/T2-w ratio predating lesion formation. This finding can be adressed in future studies, with potential clinical applications in predicting the appearance of new lesions. In NAWM and NAGM, higher baseline T1-w/T2-w values in CIS patients were associated with baseline MS diagnosis, whereas longitudinal decreases in T1w/T2w ratio were associated with concurrent disease activity. In established MS, lower T1-w/T2-w was associated with worse clinical outcomes and disease duration. The differential impact of chronic inflammation, iron deposition and demyelination should be considered to interpret all these findings. Future research should take confounding effects such as iron and inflammation into account, through multimodal MRI acquisitions and post-mortem studies, to quantify T1-w/T2-w in healthy and pathological tissue.

Disclosures

M. Boaventura received a grant as ECTRIMS- Clinical Training fellow and has received a speaker honorarium and/or travel expenses for scientific meetings from Biogene, Merck and Roche.

J. Sastre-Garriga: over the last 12 months, J. Sastre-Garriga has engaged in consulting and/or participating as speaker/chair in events organized by Merck, Bayer, Celgene, Sanofi and Biogen, is director of Revista de Neurología and editor for controversies of the Multiple Sclerosis Journal.

A. Garcia-Vidal has nothing to disclose.

A. Vidal-Jordana receives support for contracts Juan Rodes (JR16/00024) from Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III, Spain; and has received speaking honoraria and travel expenses from Novartis, Roche, Mylan, Teva, Biogen and Sanofi.

D. Quartana has received travel expenses for scientific meetings from Biogene and Sanofi.

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A. Rovira serves on scientific advisory boards for Novartis, Sanofi-Genzyme, Icometrix, SyntheticMR, Bayer, Biogen and OLEA Medical, and has received speaker honoraria from Bayer, Sanofi-Genzyme, Bracco, Merck-Serono, Teva Pharmaceutical Industries Ltd, Novartis, Roche and Biogen.

X. Montalban has received speaking honoraria and travel expenses for participation in scientific meetings, has been a steering committee member of clinical trials or participated in advisory boards of clinical trials in the past years with Actelion, Bayer, Biogen, Celgene, Genzyme, Merck, Novartis, Roche, Sanofi-Genzyme, Teva Pharmaceutical, Excemed, MSIF and NMSS.

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CRediT authorship contribution statement

Mateus Boaventura: Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing. **Jaume Sastre-Garriga:** Supervision, Project administration, Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Aran Garcia-Vidal:** Methodology, Writing – original draft, Writing – review & editing. **Angela Vidal-Jordana:** Writing – original draft, Writing – review & editing. **Davide Quartana:** Methodology, Data curation. **René Carvajal:** Methodology, Data curation. **Cristina Auger:** Methodology, Data curation, Writing – review & editing. **Manel Alberich:** Methodology, Data curation. **Mar Tintoré:** Methodology, Writing – original draft, Writing – review & editing. **Àlex Rovira:** Supervision, Project administration, Conceptualization, Methodology, Writing – review & editing. **Xavier Montalban:** Supervision, Project administration, Writing – review & editing. **Deborah Pareto:** Supervision, Project administration, Conceptualization, Methodology, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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